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BALTIC SEAWEED BIOREFINERY

Doctoral Thesis

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ANNOTATION

Seaweeds are one of the world's most underrated biomass resources. They are part of the European Union's 'blue growth' strategy as an alternative resource to meet the growing demand for sustainable biomaterials. The application of the biorefinery concept allows obtaining products with high added value along with the biofuel. The cultivation and use of seaweeds in the Baltic region are in the early stage. In the Thesis, the potential applications of Baltic seaweeds are evaluated by applying the biorefinery concept. During the study, the author develops the research by proposing seaweeds as a feedstock, evaluates the products and technologies potentially applicable in the biorefinery. In order to improve the availability of feedstock, a seaweed cultivation laboratory stand is being set up.

The aim of this work 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 become recognized as a significant type of the biomass.

The Doctoral Thesis is based on seven thematically unified scientific publications published in various scientific journals and are available in scientific information repositories and international databases. The aim of these publications is to identify the seaweed species present in Latvia and to look for the direction for seaweed use.

The introduction of the work introduces with the aim and set tasks, describes the structure of the work and provides an overview of the author's practical and scientific contribution.

The first chapter provides an overview of the scientific literature, previous research and focuses on the properties of seaweed biomass. The obtained results and discussion are given in the third chapter. Conclusions are made at the end of the work.

ANOTĀCIJA

Makroaļģes ir viens no lielākajiem nenovērtētajiem biomasas resursiem pasaulē. Tās ir iekļautas Eiropas Savienības "zilās izaugsmes" stratēģijā kā alternatīvs resurss, lai apmierinātu arvien augošo pieprasījumu pēc ilgtspējīgiem bioloģiskas izcelsmes materiāliem. Biorafinērijas koncepcijas pielietošana ļauj no biomasas iegūt produktus ar augstu pievienoto vērtību un biodegvielu. Baltijas reģionā makroaļģu audzēšana un izmantošana ir sākuma stadijā. Promocijas darbā tiek izvērtēta Baltijas jūras aļģu izmantošanas iespējas pielietojot biorafinērijas koncepciju. Darba gaitā autore attīsta pētījumu apskatot makroaļģes kā izejvielu, izvērtē biorafinērijā potenciāli iegūstamos produktus un to iegūšanas tehnoloģijas. Lai uzlabotu izejvielas pieejamību tiek izveidots laboratorijas stends makroaļģu kultivēšanai.

Šī darba mērķis ir veikt integrētus pētījumus, lai novērtētu Latvijā pieejamo makroaļģu sugu iespējamu pielietojumu biorafinērijā. Šī darba ietvaros tiek veikti pētījumi, lai identificētu potenciālās jūras makroaļģu sugas, noskaidrotu iespējamo biomasas daudzumu un meklētu virzienu jūras aļģu izmantošanai tā, lai tā būtu daļa no valsts ekonomikas un tiktu atzīta par nozīmīgu biomasas veidu.. Pētījuma veikšanai izvēlētas trīs indikatorsugas, kas pārstāv katru no trim galvenajiem aļģu tipiem.

Promocijas darba pamatā ir septiņas tematiski vienotas zinātniskās publikācijas, kas publicētas dažādos zinātniskos žurnālos, un pieejamas zinātniskās informācijas datu krātuvēs un starptautiskajās datubāzēs. Šo publikāciju mērķis ir identificēt Latvijā esošās jūras aļģu sugas un meklēt virzienu jūras aļģu izmantošanai.

Darba ievads iepazīstina ar darba mērķi un izvirzītajiem uzdevumiem, apraksta darba struktūru un sniedz pārskatu par autora praktisko un zinātnisko ieguldījumu.

Pirmā nodaļa sniedz pārskatu par zinātnisko literatūru, iepriekšējiem pētījumiem un koncentrējas uz makroaļģu biomasas īpašībām. Iegūtie rezultāti un diskusija ir doti trešajā nodaļā. Darba beigās tiek izdarīti secinājumi.

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I especially thank Annette Bruhn and her team, who welcomed me in their labs and shared their experiences during my internship at Aarhus University.

I am also very thankful to all my ex-colleagues from IESE for sharing their expertise and right words to motivate me. Special thanks to Raimonda and Antra for technical help and Kaspars for moral support, that helped me to get through the final parts of the Thesis.

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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 [2]. 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 [3].

Traditionally seaweeds have been used for medical purposes, soil improvement, feed supplements, combustion, and insulation material, but mostly as a food source [4]. 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 [5]. In more recent studies seaweeds have been recognised as a source of 3rd generation biofuels [6]. 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 [7]. Recently seaweed cultivation has been recognised as a profitable business, and seaweed cultivation develops on the western regions of the Baltic Sea [8]. In contrast, eastern areas of the Baltic Sea are still considered as unsuitable for seaweed cultivation [9].

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 [10]. 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 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 a 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 (Paper 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 favourable parameters for 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).

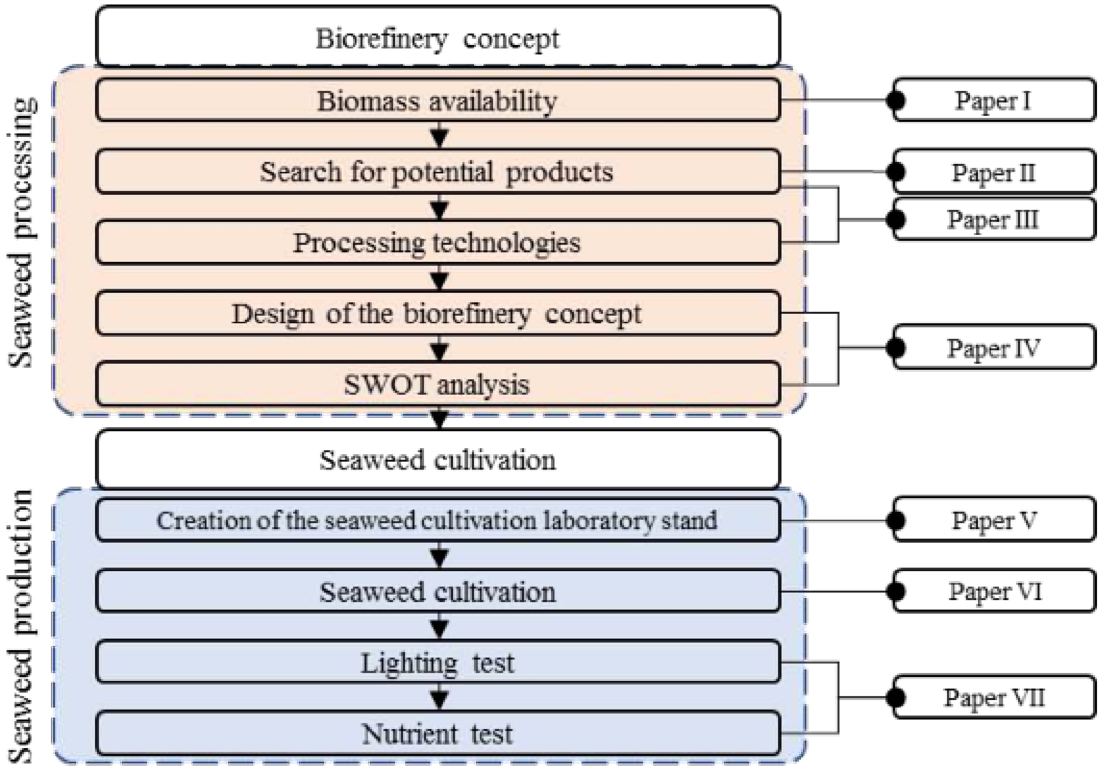

Fig. 1. Overview of the development of applied methodology.

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.

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 seaweed 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).
3. Gruduls, A., Bāliņa, K., Ivanovs, K., Romagnoli, F. Low temperature BMP tests using fish waste from invasive round goby of the Baltic Sea. Agronomy Research, 2018, 16 (2), pp. 398–409. (Indexed in Scopus) (3 citations).
4. Bāliņa, K., Boderskov, T., Bruhn, A., Romagnoli, F. Increase of *Fucus vesiculosus* fertilization success: Testing of different receptacle drying methods to increase spore release. *Energy Procedia*, 2018, 147, pp. 282–287. (Indexed in Scopus).
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).
6. 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).
7. 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).
8. 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).
9. Sabūnas, A., Romagnoli, F., Pastare, L., Bāliņa, K. Laboratory Algae Cultivation and BMP Tests with *Ulva intestinalis* from the Gulf of Riga. *Energy Procedia*, 2017, 113, pp. 277–284 (Indexed in Scopus) (4 citations).
10. Romagnoli, F., Pastare, L., Sabūnas, A., Bāliņa, K., Blumberga, D. Effects of pre-treatment on Biochemical Methane Potential (BMP) testing using Baltic Sea *Fucus vesiculosus* feedstock, *Biomass and Bioenergy*, 2017, 105, pp. 23–31 (Indexed in Scopus) (11 citations).
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).

Monographies

1. Blumberga, D., Gedrovičs, M., Kirsanovs, V., Timma, L., Kļaviņa, K., Kubule, A., Kļaviņš, J., Muižniece, I., Kauls, O., Barisa, A., Bāliņa, K., Lauka, D., Ziemele, J., Kārkliņa, I. Laboratorijas darbu krājums vides inženierzinātņu studentiem. 3.daļa. Rīga: RTU Izdevniecība, 2016. 92 lpp. ISBN 978-9934-10-747-4.
2. Blumberga, D., Veidenbergs, I., Blumberga, A., Dāce, E., Gušča, J., Rošā, M., Romagnoli, F., Pubule, J., Barisa, A., Timma, L., Bāliņa, K., Kļaviņa, K., Kubule, A.,

Lauka, D., Muižniece, I., Kalnbaļķīte, A., Kārkliņa, I., Prodaņuks, T. Biotehonomika: metodiskais materiāls. Rīga: Rīgas Tehniskās universitātes Vides aizsardzības un siltuma sistēmu institūts, 2016. 84 lpp.

Conferences

1. Bāliņa, K., Romagnoli, F., Blumberga, D. Comprehensive Literature Review on Valuable Compounds and Extraction Technologies: the Eastern Baltic Sea Seaweeds *The Conference of Environmental and Climate Technologies CONECT 2020*, May 13–15, 2020, online.
2. Bāliņa, K., Gruduls, A., Romagnoli, F. Lab Scale Cultivation of Baltic Ulva Intestinalis in Different Light and Nutrient Conditions: Effects on Growth and Morphology. *26th European Biomass Conference and Exhibition*, May 14–18, 2018, Denmark, Copenhagen.
3. Bāliņa, K., Romagnoli, F., Blumberga, D. Seaweed Biorefinery Concept for Sustainable Use of Marine Resources. *The Conference of Environmental and Climate Technologies CONECT 2017*, October 12–14, 2017, Riga, Latvia.
4. Bāliņa, K., Romagnoli, F., Pastare, L., Blumberga, D. Use of Macroalgae for Bioenergy Production in Latvia: Review on Potential Availability of Marine Coastline Species. *The Conference of Environmental and Climate Technologies CONECT 2016*, October 12–14, 2016, Riga, Latvia.
5. Bāliņa K., Romagnoli F., Blumberga D. Chemical Composition and Potential Use of Fucus Vesiculosus from Gulf of Riga. *The Conference of Environmental and Climate Technologies CONECT 2015*, October 14–16, 2015, Riga, Latvia.
6. Bāliņa K., Balode M., Blumberga D. Impact of Synthetic Hormone 17α-Ethinylestradiol on Growth of Microalgae Desmodesmus Communis. *6th International Conference "Biosystems Engineering 2015"*, May 7–8, 2015, Tartu, Estonia.
7. Bāliņa K., Romagnoli F., Pastare L., Blumberga D. Use of Macroalgae for Bioenergy Production in Latvia: Review on Potential Availability of Marine Coastline Species. *23rd European Biomass Conference and Exhibition*, June 1–4, 2015, Austria, Vienna.
8. Bāliņa K., Balode M., Putna I. Effects of the Synthetic Hormone 17α-ethinylestradiol on Aquatic Organisms of Different Trophic Levels. Conference *Environmental Science and education in Latvia and Europe* October 24 - 24, 2014, Riga, Latvia.

Supervised and Co-supervised Bachelor and Master's Thesis

1. Agija Līkā. Seaweed growing laboratory testing. Bachelor thesis. RTU, 2016 (In Latvian).
2. Jekaterina Krastiņa. Techno-economic feasibility of seaweed based conversion systems: an LCCA perspective. Master's thesis. RTU, 2017.
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

1.1. Seaweed Biomass in Latvia

Global era of seaweed utilizing for food, fuel and chemicals is just starting. Seaweeds are patiently taking their place in the global energy market as Third generation biofuel [11]. Even though they are described as a remedy for a wide range of diseases, and their nutritional value is aligned to higher plants, their share in globally used products is undeservedly low [12]. Only in recent decades, seaweeds have taken the attention of researchers and society as an opportunity to prevent potential lack of terrestrial resources, to support the rising of global human population and its demands. With this increasing awareness, it starts to raise a question about seaweed cultivation and their use in all regions of the world [13]. There are strategies on how to apply biorefinery principles on seaweed biomass [14]. Seaweed processing pathways and feedstock are described for seaweeds grown in the ocean or ocean-like seawater [15–21]. Application pathways for seaweed biomass from a brackish environment remain unclear. Brackish or low salinity conditions can be a stress for most of the seaweed species, and they cannot even survive in this kind of environment. In contrast, those who have adapted to brackish surrounding water environment has been adjusted their physical characteristics while limiting their size [22–24].

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 [25]. In the Baltic Sea and the Gulf of Riga substrate usually is sandy, muddy and stony, partially covered with seashells. Waves and streams can quickly move the substrate around, not letting seaweed to grow and form seaweed stands [26]. Eventually, detached seaweeds are forming dense seaweed canopies that are able to float far distances. They can continue to grow for so long as waves and currents – respectively affected by wind speed and direction [27] – define their pathway on the surface and finally to the shore. The appearance of seaweed on a beach is not a permanent or regular process; thus, the composition and amount of the biomass washed ashore is not predictable [28]. Although it is a natural coastal process decomposing seaweeds are decreasing the recreational value of beaches, and can be a significant problem during the tourism season [27]. Historically the seaweed waste has been used as a fertilizer on agricultural lands by local inhabitants. According to the EU Bathing Water Directive 2006/7/EC during the beach season in the places where it might interfere with people washed out seaweed biomass is collected by local municipalities [29]. Growing and harvesting of seaweeds remove nutrients from a system, reducing nutrient load and therefore, the possibility of eutrophication [30].

There are three types of seaweeds growing in the Baltic Sea – green (*Chlorophyta*), brown (*Phaeophyceae*) and red (*Rhodophyta*) [25]. The upper area of the littoral zone is in most parts of the Baltic sea dominated by filamentous green seaweeds like *Ulva intestinalis* and

Cladophora sp., with perennial fucoids growing from approximately 1 m depths (Fig. 1.1.) The benthic vegetation of hard substrate bottoms reaches down to a maximum depth of 15 m.

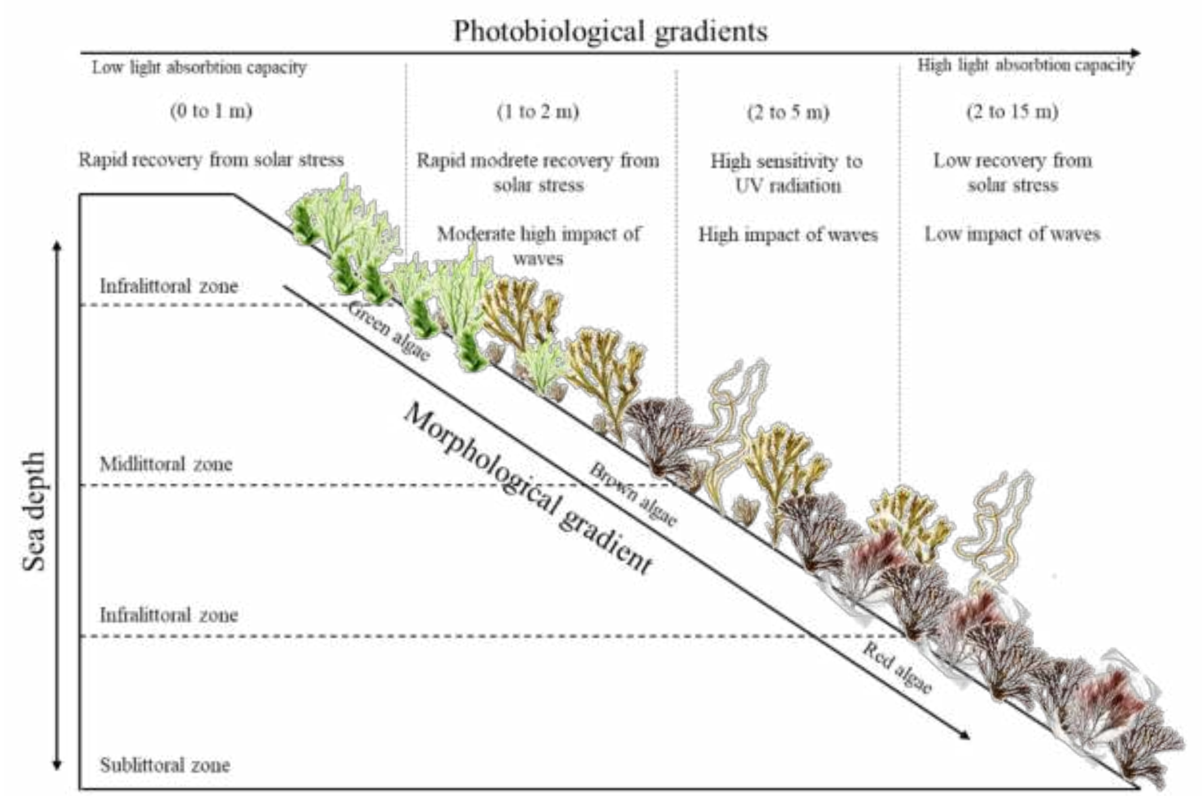

Fig. 1.1. Seaweed distribution in the Baltic Sea depending on depth and light intensity.

Red seaweeds, like *Furcellaria lumbricalis,* inhabit the sublittoral zone. Seaweed growing depth is related to their colour. Different species of seaweeds use a different kind of pigments to produce energy using sunlight. Deeper in water available amount of light reduces. Blue and green light has the wavelength that penetrates deeper in the water, and that is the spectrum red seaweeds use to carry out photosynthesis. Red and yellow light is the most important for green seaweeds, that usually grow close to the water surface. Red seaweeds have a pigment phycobiliprotein that allows to carry out photosynthesis in-depth 0.5–23 m as a result of adaption to low light conditions [31,32].

Following species of seaweeds have been evaluated as abundant in Latvia: 4 brown seaweeds (*Phaeophyceae*), nine red seaweeds (*Rhodophyta*) and four green seaweeds (*Chlorophyta*) species. Most common seaweed species of these genera are *Fucus vesiculosus*, *Furcellaria lumbricalis*, *Ulva intestinalis* [33]. On the coastline of open sea *Furcellaria sp.* is observed more often, but in the Gulf of Riga, the brown seaweeds *Fucus vesiculosus* are more abundant. Green seaweeds, forming filaments on rocks or other substrates are widely distributed on the coastline of Latvia [30].

The distribution of washed-out seaweeds on the Latvian coast of the Baltic Sea is very uneven and difficult to predict, as hydrometeorological conditions are the determining factor for the amount of washed out biomass. Seaweed deposition on the shores of the Baltic Sea is related to the overall wind strength and storm frequency, which contributes to higher wave formation and more robust underwater exposure, as a result of which slower-growing perennial seaweeds can detach from the substrate in larger areas compared to the Gulf of Riga. The maximum deposition of washed-out seaweeds can be found on the coast of the open Baltic Sea, amounting to 228 m^3 / 100 m in autumn, which is most likely related to the increase in wind strength and more frequent autumn storms. In summer, the maximum volume of macrophyte seaweed deposition is only around 17 m^3 / 100 m. The dominance of red seaweeds is pronounced in macrophyte seaweed sediments. The volumes of washed-out seaweed biomass in the Gulf of Riga are smaller than in the open part of the Baltic Sea (max 112 m^3 / 100 m) [34]. The taxonomic composition of washed-out seaweeds indicates a marked predominance of red seaweeds on the coast of the open Baltic Sea and a predominance of brown and green seaweeds on the coast of the Gulf of Riga. The proportion of green seaweeds in washed-out seaweed biomass indicates an increased level of eutrophication in the Gulf of Riga. According to the characteristics of deposition, it is reported that they consist of red seaweeds, brown seaweeds, reed, rushes and other plant fragments, sticks and other alluvial deposits [34].

Study of seasonal dynamics in Melluži beach (Jūrmala) indicate low levels of washed-out seaweeds in spring (April, May, June) and relatively similar in summer and autumn periods. High levels of washed-out seaweeds are reached both in summer when there is a more pronounced accumulation of green seaweeds, and in autumn, when perennial brown seaweeds are detached as a result of wave action. The maximum volumes of washed-out seaweeds on Melluži beach were found during the autumn storms – in October reaching the highest amount 40 m^3 / 100 m. Potential washed out seaweed accumulation sites are considered the coast of the open part of the Baltic Sea – Pāvilosta and Liepāja. On the eastern coast of the Gulf of Riga – the northern pier of Salacgriva and Saulkrasti; on the west coast - Jaunķemeri, Melluži and Lapmežciems [34].

The species of seaweeds washed out on the coast of Latvia are dominated by *Cladophora sp.* and *Ulva sp*. from green seaweeds, *Fucus vesiculosus* from brown seaweeds and *Furcellaria lumbricalis* from red seaweeds [34].

Ulva intestinalis (previously *Enteromorpha intestinalis*) is a filamentous green alga also known as Gut Weed. It occurs worldwide and in the Baltic Sea. Tubular filaments are typically unbranched and may be up to 2 cm in width and 50 cm in length. It is formed of irregular cells arranged in a single layer. In the first stages of life seaweed surface is smooth, but with age surface wrinkles and changes colour from light green to yellow-green. It is abundant in different levels of salinity and very common in brackish water areas, where there is appreciable freshwater runoff and in wet areas of the splash zone [35]. It can grow on different substrates: rocks, mud, sand and even on other seaweeds and seashells. Detached from the substratum, it can continue its growth in floating masses [36].

Fucus vesiculosus, also called bladderwrack, is one of the most common seaweeds in the Baltic Sea, growing on the rocky bottoms of the Baltic Sea coastal areas and forming dense canopies when detached [22,37,38]. It is reported that in the last decades in some Baltic Sea regions, the amount of these seaweeds have decreased. The disappearance of these seaweeds is explained with the increase of marine pollution, local leakages of toxic components and amount of invasive herbivores using seaweeds as feed [39].

F. vesiculosus is perennial brown seaweed in the Baltic Sea. In Latvia, they grow in depth of 1.5-5.5 m. The length of the *F. vesiculosus* thallus can reach up to even 50 centimetres. Thalli can live for several years, and when they die, new thallus can be developed from rhizoid [40]. Dichotomous forked ribbon thallus with a well-marked central vein is held in a vertical position with the help of the air bladders [41]. *F. vesiculosus* contains 70-85 % water while the dry matter is only 15-30 %. In the coastal areas of the Baltic Sea, the bladderwrack is one of the most ecologically important species, because of its role as an essential nutrient source and habitat for benthic animals, fish and other algae.

The distribution area of *Fucus vesiculosus* spreads from the Atlantic Ocean to Bothnian Gulf, where low salinities decrease successful fertilization processes by reducing the motility of gametes and increasing polyspermy [22,42]. Environmental conditions and survival through the early sporophyte stages are crucial to successful reproduction [43].

Furcellaria lumbricalis is one of few red seaweed members adapted to brackish water and is abundant in the Baltic Sea (Fig. 1.2.).

a b c

Fig. 1.2. a – *F. lumbricalis* on small boulders [44], b – a specimen of *F. lumbricalis* [45], c – washed out *F. lumbricalis* biomass in Latvia [46].

In the attached form, it can be found practically throughout the photic zone on hard substrates at salinities down to 3.6 psu. The floating form has been found in a few habitats near Estonia [47]. Unlike the more common attached form, it can reproduce only vegetative, mainly by thallus fragmentation [32].

Furcellaria lumbricalis has historical economic importance in the Baltic region. Even though it has the most extended harvesting history in this region, it is not cultivated in industrial level yet. In recent years interest in *F. lumbricalis* has increased. In Estonia on seaweed cultivation and reproduction processes as well as industrial production of agar have been researched. Studies have been carried out on vegetative propagation of sporophytes and gametophytes. [9,48,49].

Increase of the global seaweed use raises a question if seaweed biomass available in Latvia could be better, more efficiently and more sustainably exploited. Further research was done focusing on three most abundant seaweed species in Latvia, that represent each of the seaweed types –*Ulva intestinalis* for green seaweeds, *Fucus vesiculosus* for brown seaweeds and *Furcellaria lumbricalis* for red seaweeds.

1.2. Seaweed Biorefinery

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 [50]. 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 [51]. The biomass processing through the biorefinery concept makes production processes economically and environmentally feasible, respecting social and political angles [52].

International Energy Agency (IEA) Bioenergy Task 42 contributes to the development and implementation of sustainable biorefineries – as a part of highly efficient, zero-waste value chains – synergistically producing biobased food and non-food products [53]. Since 2007, when the task was set, European and worldwide view to the task has been changed. The approach has been growing and been developed. Nowadays, global biorefinery concept mainly includes terrestrial biomass with plants and forest on top, and only small part has recently been devoted to aquatic resources like seaweeds [54].

Since mainly first and second-generation biomass is used for biofuels production, there is still a need to find a viable solution for the competition of biomass for food or energy production. In this direction, the concept of biorefinery can be beneficial [55]. Seaweeds have a high potential to partly replace terrestrial biomass. By definition and scientific consensus, third-generation bioresource (i.e. microalgae and macroalgae or seaweeds) avoid competition with food and feed plants, nor is using resources like drinking water and arable land for their growth [7,56]. Valuable substances that can be found in seaweeds can be a way to the promising low-carbon economy [57].

As a biomass source, seaweeds have been appreciated for centuries, but the seaweed natural distribution area is covering all over the world, including Europe and the Baltic Sea [58,59]. Seaweed cultivation industry and mass production of seaweeds are underdeveloped in eastern Europe. Seaweed cultivation could represent a promising opportunity to obtain seaweed biomass, meanwhile reducing eutrophication problems [60]. The opportunities of the seaweed use can thus be vast.

Seaweed biorefinery concept (see Fig. 1.3.) proposes a conceptual model for high value-added product production along with the production of biofuels [61]. 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 [51].

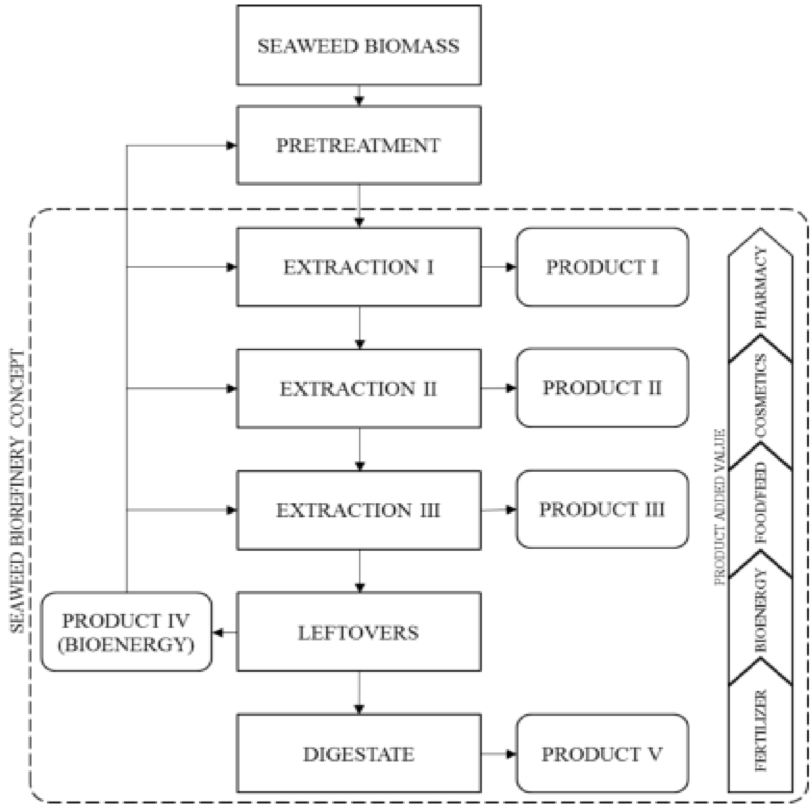

Fig. 1.3. Seaweed biorefinery concept.

Products derived from feedstock can be separated in products which can be already used in the market, or they can be used as feedstock for further manufacturing operations 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 using a cascading approach [62,63].

The heat requirements for the biorefinery may be obtained from the recirculation of heat generated from the cascading process [64]. Different conversion processes (physical, chemical, biological, and thermal) are used either individually or in combination to provide products for economic purposes [65]. 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. The seaweed biorefinery concept is mainly addressed to use seaweed species whose overgrowth, because of eutrophication, is causing ecological damages and affects the sustainability of coastal environments [66]. Instead, seaweeds can be used for remediation of over enrichened environment since together with seaweed biomass a certain amount of nitrogen and phosphorous is removed from the water [67]. Nevertheless, the identification of the thresholds affecting the overall ecosystem service provided by the depletion of natural resources is a sensitive issue. Seaweed biorefinery concept can have a significant impact by showing different aspects for utilizing seaweed to produce a wide range of value-added products, biofuels, and bioenergy within a concept, rather than focusing in a single product [16].

The various uses of products obtained from seaweed biorefineries include transport fuels, therapeutics, food additives and biofertilizers [51]. When developed and implemented in industry, practical impacts of biorefinery concept can be relevant at different levels: within the seaweed farming and industry, as employment increase in the sectors of the bioeconomy, triggering new investments, unlock incentive and favouring the integration of renewable energy within the overall energy systems [68]. 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.

1.3. Chemical Composition of the Seaweeds

Even though seaweeds are often considered as close ancestors to terrestrial plants, substances found in them are different and in more significant quantities than in plants [69]. Known for their high nutritional and pharmaceutical value, they are widely consumed as food and as a herbal remedy to cure health problems like eczema, psoriasis, renal disorders, digestive system problems, heart and cardiovascular diseases, and it is even mentioned as a treatment for cancer [3,70–72]. Seaweed use as feed, food fertilizer, fungicide, the herbicide has developed a demand for seaweed as a valuable resource[3,73,74].

Biochemical composition and general phytochemistry of seaweed have been a popular research object, and is widely investigated worldwide [75]. Nutrients and chemical components provide not only essential substances for human nutrition, but also show bioactive properties that can be used for drugs [3].

Nutritional composition of seaweeds 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.

1.3.1. Proteins

In some Asian countries, seaweeds have been used as a protein source for centuries. Nowadays, seaweeds have become a cheap alternative protein source, mainly due to essential amino acids, especially in developing countries [76].

Amount of proteins in seaweeds varies regarding surrounding environmental factors and species [77]. Highest protein concentrations are reported in winter and early spring months and lowest concentrations regarding nitrogen concentrations have been observed from July to October. 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 [78]. Brackish red seaweed *Furcellaria lumbricalis* sometimes is compared to *Palmaria palmata* for which protein content can represent even up to 35 and 47 % of the dry mass [79]. That is a higher protein amount than 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 [77].

The amino acid composition of seaweeds can be compared to other protein sources such as eggs and soybean. For most seaweeds, glutamic acid and aspartic acids together make a large part of the total amount of amino acids. Some seaweed amino acid profiles show similar concentrations as leguminous plants and ovalbumin [77].

1.3.2. Pigments

As photosynthetic organisms, seaweed contains pigments that determine the colour of brown, green and red seaweeds. 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 (Table 1.1.). In previous years research interest in seaweed pigments has increased due to their antioxidant, anti-obesity and anti-cancer properties.

Table 1.1.

Dominant Pigments Representing the Three Seaweed Groups

Pigment Class	**Green seaweeds**	**Brown seaweeds**	**Red seaweeds**	**Reference**
Chlorophylls	Chlorophyll *a* Chlorophyll *b*, derivatives	Chlorophyll *a* Chlorophyll *b* Chlorophyll *c* derivatives	Chlorophylls *a* Chlorophyll *d* derivatives	[80]
Carotenoids	α, β, γ-carotene, xanthophylls	Fucoxanthin Xanthophylls β-carotene	Xanthophylls α, β-carotene	[80–83]
Phycobiliproteins	-	-	Phycoerythrin Phycocyanin	[80,81]

Green pigment chlorophyll is found in both seaweeds and land plants. It absorbs light and converts it into chemical energy through photosynthesis. Chlorophyll and its by-products such as pheophytin, pyropheophytin and pheophorbide are considered compounds that provide many health benefits, including antioxidant and anti-mutagenic activity which may help to prevent cancer [84]. It is also assumed that chlorophyll-derived compounds can bind certain cancer-causing chemicals, such as heterocyclic amines in the digestive tract, thus reducing their absorption [85].

Carotenoids are pigments that can be found not only in seaweeds but also are present in terrestrial plants, microalgae and photosynthetic bacteria. Animal and human organisms are not able to produce carotenoids on their own, therefore must be uptaken with food. Fucoxanthin and β-carotene are the most well-known carotenoids that can be found in seaweeds. Other carotenoids which can be found in seaweed include astaxanthin, violaxanthin, tocopherol, zeaxanthin and lutein [86]. Carotenoids are potent antioxidants which prevent oxidative damage to cellular components induced by reactive oxygen species [87]. Pigment fucoxanthin is another brown seaweed carotenoid such as *Ascophyllum nodosum* and *Laminaria digitata*. In recent years this pigment has received much attention due to its positive anti-obesity and anti-cancer properties [88].

Phycobiliproteins are water-soluble pigments. They are typically found in red seaweeds, and their types include phycoerythrin, phycocyanin and allophycocyanin. Similarly to other pigments, they also show antioxidant activity and gives health benefiting properties like anti-inflammatory, liver-protecting, anti-viral, anti-tumour, serum lipid reducing and antioxidant activity [72].

1.3.3. Polysaccharides

Seaweed biomass has high polysaccharide amount that is existing in cell wall structures and store polysaccharides, such as laminarin (β-1,3-glucan) in brown seaweeds and floridean starch (amyloprotein-like glucan) in red seaweeds. Polysaccharides are polymers of monosaccharides that are bounded by glycosidic bonds (Table 1.2.). They have various commercial applications in products such as stabilisers, thickeners, emulsifiers, food, feed, beverages, etc. [86,89]. The total amount of polysaccharides can range from 4 % to 76 % of dry weight. The highest contents are found in species such as *Ascophyllum* (42–64%), *Palmaria* (38–74 %) and *Fucus* (66 %); however, green seaweed species such as *Ulva* also have a high content, up to 65 % of dry weight [86].

Table 1.2.

The Major Saccharides and Polysaccharides Found in Seaweeds [83]

Green seaweeds	**Brown seaweeds**	**Red seaweeds**
Cellulose	Cellulose	Cellulose
Starch	Laminarin starch	Floridean starch
Mannan or galactan	Mannitol (monomer)	Agar
Heteroglycan	Alginic acid	Carrageenan
Ulvan	Fucoidans	Xylan
Xylan		Galactan

Seaweed cell walls are formed of cellulose and hemicelluloses (2–10 %) to build thallus and provide physical support for seaweed life in the water. The seaweed cell structure is not so strong as in terrestrial plants. Seaweed polysaccharides are well recognized by the hydrocolloid industry.

Structural features of polysaccharides give them the ability to bind water up to 20 times their weight to give hydrogel, which qualifies them to be referred to as hydrocolloids or phycocolloids [90]. Formation of gel includes non-covalent interactions, like hydrogen bonding, hydrophobic and ionic interactions between the constituents and are composed of cooling of heated solutions of polymers.

Some of the polysaccharides (agars, carrageenans, ulvans and fucoidans), cannot be digested by human intestinal bacteria and therefore can be regarded as dietary fibres [91]. Seaweed polysaccharides show meaningful biological activities (anti-thrombotic, anti-coagulant, anti-cancer, anti-proliferative, anti-viral, and anti-complementary agent, anti-inflammatory). These properties create interest in potential therapeutic applications (Table 1.3.). Xylans and laminarans, they are entirely and rapidly degraded by intestinal bacteria, while alginates are only partly degraded and lead to a production of short-chain fatty acids.

Table 1.3.

Polysaccharides from some Major Seaweed Species, their Bioactivities and Medicinal Uses [92]

Polysaccharides	Major seaweed sources	Bioactive properties
Agar	*Gracillaria spp.* *Gelidium spp.* *Hypnea spp*	Anti-HIV Anti-inflammatory activities Laxative
Alginate	*Macrocystis spp.* *Laminaria spp.* *Ascophyllum spp* *Sargassum spp.*	Antibacterial Hypo-cholesterolemic Anti-hypertensive Anti-ulcer Dietary fiber Constipation treatment Drug delivery Scaffold for reconstructive processes
Carrageenans	*Euchema cottoni* *E. spinosum* *Gigartina stellate* *Chondrus spp*	Anticoagulant Anti-ulcer Immunomodulation Simulates collagen biosynthesis Herpe inhibition Anti-HIV
Fucoidans	*Fucus serratus* *Fucus vesiculosus* *Ascophyllum nodosum* *Laminaria spp.* *Ascophyllum spp.*	Anticoagulant Antitumor Anti-inflammatory Antioxidant Immunostimulant Antiviral Antibacterial Hypoglycemic Ameliorate chronic renal failure
Laminarin	*Laminaria japonica* *Laminaria digitate* *Laminaria hyperborean* *Fucus vesiculosus* *Ascophyllum nodosum*	Antimicrobial Anticoagulant Hypo-cholesteric Antihypertensive Immunostimulant Cytotoxic and wound healing Prebiotic activities Surgical dusting powder
Furcellaran	*Furcellaria lumbricalis* *Furcellaria fastigiate*	Limited biomedical applications
Ulvan	*Ulva conglobate* *Ulva lactuca* *Ulva rigida*	Anticoagulant Heparin-like activity Apoptotic activity Antitumor activity Antiviral Immunomodulating activities
Porphyran	*Palmaria palmata* *Palmaria umbilicalis* *Palmaria umbilicalis*	Apoptotic activity Antitumor activity Use as dietary fibre

1.3.4. Lipids

Seaweeds contain relatively low amounts of lipids as compared to other plant seeds such as soy and sunflower. 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.

The lipid content in seaweed is very variable and have significant differences between species; it also varies by geographically, seasonally, temperature, salinity and light intensity [93]. Recent research reveals that species of tropical areas have significantly lower lipid contents than the cold area species [94]. The brown seaweed sources, location, and extraction methods all affect the chemical properties and structure of fucoidan. Fucoidan from different seaweeds may exhibit different bioactivity. Bioactivities are influenced by the chemical composition and structural properties of fucoidan [3].

Brown seaweeds are the primary producer of fucoidan. Fucoidans are sulphated polysaccharides isolated from brown seaweeds. In recent years, fucoidan has been extracted from various species of brown seaweed and studied for its properties. Although the content of lipids in brown seaweeds is small, it contains biologically active compounds, such as fucoxanthin, omega-3 EPA and SDA and omega-6 arachidonic acid. Among these compounds, fucoxanthin is crucial to understand the essential functions of brown seaweed lipids. The stability of PUFAs is occasionally problematic in the utilization of marine lipids to food and other products. Studies indicate the high oxidative stability of omega-3 PUFAs in brown seaweed lipids. Although oxidative stability of PUFAs in brown seaweed lipids is not precise yet, they can be applied to nutraceuticals and functional foods as a source of oxidative stable omega-3 fatty acid. For the commercial use of brown seaweed lipids, a search seaweed materials with high total lipid content is essential [95]. Bioactivities of fucoidans include antioxidant activity, anticoagulant activity, and anticancer activity.

1.3.5. Minerals

Seaweeds have a high mineral content, that is higher than in terrestrial plants and animal products, and therefore they can be used as food and feed supplements to supply minerals. Seaweeds contain substantial amounts of essential minerals (Na, K, Ca, and Mg) and trace elements (Fe, Zn, Mn, and Cu), which are essential in building human tissues and regulating vital functions [3]. Analyses show that seaweeds contain useful amounts of minerals (K, P, Mg, Ca, Na, Cl, and S), trace metals, and vitamins [3]. Seaweeds accumulate from the water an incomparable amount of mineral elements, macroelements and trace elements [96]. Their mineral content that reaches levels of up to 55 % on a dry weight basis is 10–100 times higher than of traditional vegetables. The mineral content in the form of ash of the seaweeds. Ash content in most land vegetables is usually much lower than in seaweed. e.g. potato 10.4, carrot 7.1, and tomato 7.1. Sweet corn has a lower content (2.6 %), while spinach has an exceptionally high mineral content (20.4 %) for a land plant [97].

The mineral composition varies according to genera as well as various other factors such as seasonal, environmental, geographical and physiological variations and seaweed types such as wild type and cultivated type [3].

The brown seaweeds have commonly been used for treating thyroid goitre due to high contentment of iodine. *Fucus vesiculosus* is registered in the European pharmacopoeia for its high iodine content. As concerns iodine, laminaria is the primary source as it contains 1500 to 8000 ppm dry weight while *Fucus* contain 500 to 1000 ppm dry weight of iodine. Seaweeds are also considered as one of the essential vegetable sources of calcium, which is high as 7 % of the dry weight of the seaweed biomass. The calcium content in seaweeds can reach to 34 % in the chalky coral seaweeds. It is worth to mention that *Enteromorpha spp.* contains 28 times more calcium than spinach, and 30 times more phosphorous than the quantity of any of the common vegetables. Since Ca and P are necessary minerals for the growth, development, maintenance, and function of living forms, *Enteromorpha spp*. is a potentially attractive source of these minerals [36]. Seaweeds also contain high amounts of iron and copper [96]. Seaweed consumption may thus be useful mineral source during pregnancy, four adolescents and elderly that all exposed to a risk of calcium deficiency. Despite high contents, the linkage of certain minerals with anionic polysaccharides (alginate, agar or carrageenan) might limit their absorption. For instance, the strong affinity of divalent cations (particularly calcium) for carboxylic polysaccharides (alginates) probably limits their availability. In contrast, the weakness of the linkages between polysaccharides and iodine allows rapid release of this element [36].

Nevertheless, despite their importance in human health, some of the minerals are necessary for our health, some can be toxic when uptake in higher concentrations [86]. Besides potential health benefits of essential minerals and stimulating compounds in seaweed, other metals, minerals and bioactive may pose a negative effect on health. Depending on the surrounding environment, seaweeds can contain metals in harmful concentrations, heavy metals, and other compounds. For example, lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu), manganese (Mn), zinc (Zn) and the explosive compound trinitrotoluene that may be present in the ambient environment. Marine organisms take up the inorganic arsenic, but usually, the biological system transforms it into an organic and non-toxic form of arsenic. Arsenic concentrations are generally considerably higher in brown seaweed than in red or green seaweeds [86].

This accumulating effect present in seaweed can also be exploited to remediate for undesired compounds in sea and wastewater or the use of seaweed as a bioindicator [3]. However, the number of undesired metals and compounds should be considered and tested if seaweed species are to be used as a feed and food production.

1.3.6. Bioactive Compounds

Seaweeds are also rich with various bioactive compounds which can be characterized by specific biological activities such as anti-inflammatory, antioxidant, cholesterol-lowering, antitumor, prebiotic and antimicrobial capabilities[98]. Presence of these bioactive compounds in seaweeds makes them one of the crucial elements in cosmetic, nutraceutical, biomedical and pharmaceutical industries. While an essential part of bioactive compounds is already described previously as pigments, there are other substances like phenols that make seaweed biomass even more attractive.

Phenolic compounds can be found in both terrestrial and aquatic plants, which include seaweeds. They are a group of secondary metabolites that have a benzene ring with at least one hydrogen replaced with a hydroxyl group. Due to their antioxidant properties, they prevent the formation of many free radicals because of their metal ion chelating capacity [83,99,100]. Phenolic compounds are classified into five broad groups: Flavonoids are the largest group of them and is associated with various bioactivities, including the antioxidant and radical scavenging activity. Other groups are lignans, tannins, tocopherols, and phenolic acids [101]. Phlorotannins are present mostly in brown seaweed, but also in other seaweeds where its concentration is highly variable depending on the geographic area and the species [102]. Phlorotannins are polymers of phloroglucinols (1,3,5-trihydroxybenzene) and can reach up to 15 % of the dry weight of these seaweeds [103]. Seaweed phlorotannin molecules are composed of up to eight rings interconnected with each other, which give much higher antioxidant properties than polyphenols obtained from terrestrial plants, that contain only three or four rings in its structure [101].

Flavonoids that are known as safe and non-toxic antioxidants have an essential function to protect the plant against UV radiation. In plants, the amounts of flavanol in the leaves are directly related to UV-B radiation and reduced levels of oxidative stress [104]. These phenolic compounds provide seaweed capacity to overcome oxidative stress and protect them against grazers, such as marine herbivores [105].

1.4. Seaweed Processing Technologies

Seaweeds are considered a valuable source of chemical compounds with wide application range in the food, feed, pharmaceutical and energy industries [86,106]. The main objective of seaweed processing techniques is to achieve the maximum retrieval of the targeted compounds while preserving the integrity of the molecules of interest and providing maximum purity of the product [107]. The choice of extraction technology depends on the target compound to be obtained, and the field of its application. Application field, in turn, determines the degree of purity of the target compound. In the context of biorefinery, the field of application also determines the technological steps of extraction processes [108,109]. Various species of seaweeds contain a unique proportion of proteins, carbohydrates and lipids. Some are high in lipids, while others are high in protein or carbohydrates. Selection criteria should be based on their nutrient content as well as their specific use requirements [110].

Just before the extraction of the bioactive substances, it is necessary to process the biomass to obtain the maximum yield. Primary pretreatment methods include biomass preparation for extraction. Washing and drying of the biomass remove impurities and excess water [111,112]. Secondary pretreatment methods are divided into mechanical, chemical and enzymatic pretreatment methods [113]. Mechanical pretreatment methods are used to disrupt cell structure mechanically to increase the accessible surface area, and in turn the yield, for the further extraction process. These methods include autoclaving, bead-beating, microwaves, sonication, freeze-drying, mechanical crushing, lyophilization and pulsed electric field technology [114]. Chemical pretreatment methods use chemically active substances like liquid nitrogen, nitric acid, acetic acid. These methods also include hydrolysis by NaOH, HCl, H_2SO_4 or NaCl solution [115–117]. Enzymatic pretreatment methods use enzyme activity to break down macroelements into smaller molecules. Enzymes like cellulase, protease K, driselase, alginate lyase are some of the enzymes that can be applied in this methodology [118,119].

Extraction methods can be divided into conventional and novel methods. Conventional methods are still widely applied by industry and researchers, and they are based on the treatment of biomass under defined conditions with conventional extraction methods to obtain biomolecules. Methods are based on thermomechanical effects and processes of chemical hydrolysis [111,117]. Novel extraction techniques apply technologies that reduce the cost of extraction, reduces the number of steps in the extraction process and increase the yield of obtained biomolecules [3,120,121]. Novel extraction methods are based on the use of physical phenomena (pressure, electric field, ultrasound, microwaves) and biological (enzymes) effects on the biomass [122,123] Conventional, and novel extraction methods can also be combined to get the best extraction yield at the lowest cost and least impact on the environment [98,124].

Conventional Extraction Techniques

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 [113]. 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 [113].

Several types of extraction methods have been applied based on the literature on the extraction of bioactive compounds from various matrices, including those from the seaweeds. Conventional extraction methods applied on seaweed biomass include distillation, Soxhlet extraction, maceration, percolation, infusion, decoction and hot continuous extraction [117].

The effectiveness of these methods depends on various influencing parameters, such as solvent properties (polarity, toxicity, volatility, viscosity, purity), sample size and concentration, particle size, time, the polarity of extractant [125,126]. Main drawbacks of conventional techniques are the long extraction time, the need for very high purity solvents, the energy consumption associated with the evaporation of a large amount of solvent and the relatively low extraction yield [127]. Conventional extraction methods are well described in the scientific literature (lab scale). Environmental policy and resource consumption, scientific research viewpoint has advanced novel extraction methods [122,123,127,128].

Seaweed carbohydrate extraction methods depend on the expected outcome. If the target compound is going to be applied in the food industry, quality standard and purity must meet requirements of food-grade compounds. Seaweed polysaccharides that mostly are used in the food industry are agar, alginate, carrageenan, mannitol, while fucoidan, laminaran and ulvan are used in cosmetology and do not have to meet the same standards [128–130]. Seaweed carbohydrates are obtained using the following methods i) heating in water ii) by heating in water with an alkali compound (e.g., sodium bicarbonate) followed by cooling, separation, and purification. One of the significant drawbacks of the current industrial extraction of seaweed hydrocolloids is the considerable time and energy and water consumption. Extraction of seaweed hydrocolloids usually takes three hours to achieve optimum yield, depending on the types of hydrocolloids involved. Agar, alginate, and carrageenan extraction should take 2 to 4 hours, but with novel methods, it may take up to a few minutes [109,129,130]. Seaweed cellulose also belongs to this product group but is not mentioned because existing land-based biomass is a much more accessible and easily obtainable source of cellulose [131].

Extraction of seaweed proteins, peptides, and amino acids is mainly done on a laboratory scale. The main methods for extracting seaweed protein fractions in the context of conventional methods are solvent extraction, proteolytic hydrolysis (enzymes from microorganisms, plants), hydrolysis by proteolytic microorganisms during fermentation [132–134]. Seaweed proteins are extracted by water, acid and alkali methods followed by several centrifugations, dialysis and recovery steps using methods such as ultrafiltration, precipitation or chromatography. Successful extraction of seaweed proteins can be significantly influenced by the availability of protein molecules, which are significantly inhibited by high viscosity and anion cell wall polysaccharides such as alginates and carrageenans [133].

Seaweeds contain relatively small amounts of lipids. Many seaweeds in nature are not intended for oil extraction with existing technological solutions. Seaweeds are generally considered unsuitable for the production of oil-based products since most species have a low total lipid content <5 % by weight [135,136]. Content of lipids in dry weight can reach 10-20 % in some seaweed like *Dictyotales* [137]. Oils from seaweeds, plant biomass are extracted by a variety of methods, including organic solvents and water [138]. However, the green extraction process is better suited for low oil oxidation and high yield [139]. The most common traditional lipid extraction methods are water vapour extraction or solvent extraction, such as soxhlet [117,124].

Seaweeds contain a large number of minerals, up to 30 % of their dry weight. Minerals include Na, Ca, Mg, K, Cl, S and P and trace elements (Fe, Zn, Mn, and Cu). The mineral content of seaweed is generally high (8-40 %). Minerals and trace elements essential for human consumption are predominantly in brown and red seaweeds [135,136]. Part of the minerals from the seaweed biomass can be extracted by incineration and acid treatment of the resulting material [140]. Seaweeds also contains other groups of minor constituents - pigments, tannins, vitamins, steroids, cellulose, etc. [135,136].

Novel Extraction Techniques

Novel methods can accomplish the extraction of biologically active compounds from seaweeds. They have several advantages over conventional methods, including the reduced amount of solvent used (including its recovery), shorter extraction time, technological performance at lower temperatures. These methods also include improved selectivity for isolation of the desired compounds while avoiding the formation of by-products during the extraction, adverse reactions [141]. Comparing to conventional extraction methods, main advantages of novel extraction methods are higher efficiency, reduced water consumption, application of renewable raw materials, less use of hazardous chemicals, safer co-solvents, energy efficiency, reduced derivatives. [117].

Based on reviewed papers, there are six novel techniques for biomolecule extraction from seaweed [113,117,126,127,141–143]. They are summarized in Table 1.4. and full Table in Annex 1.

Supercritical fluid extraction applies supercritical fluids to separate extractant from the matrix using supercritical CO_2 as a solvent. Microwave-assisted extraction uses microwaves to warm the solvents in contact with the solid matrix to extract the contents from the solution. Ultrasound-assisted extraction utilizes ultrasound to penetrate solvents in contact with the solid matrix to extract the content from the solution. Enzymatic hydrolysis uses exogenous enzymes to digest the material. High-pressure methods use solvents under critical conditions (increased temperature and/or pressure) to speed up the extraction rate of solvents used. Ionic liquid extraction uses specially designed ionic liquids to extract a wide range of compounds. Pulsed electric field extraction utilizes an electric field to disintegrate cell matrix.

Table 1.4.

Overview of Novel Seaweed Compound Extraction Techniques.

Extraction technique	**Extracted bioactive compounds**	**References**
Supercritical CO_2	Fucoxanthin, polyphenols, phlorotannins, carotenoids, pigments, fatty acids, cytokinins, auxins, microelements, macroelements	[139,141,144–147]
Microwave-assisted extraction	Polysaccharides, alkaline, galactans, carrageenans, agar, phlorotannins, phloroglucinol, iodine, bromine, phenols, phytosterols, phytol	[141,148–152]
Ultrasound-assisted extraction	Polyphenols, laminarin, phycobiliproteins, taurine, fucose, uronic acid, antioxidants, prebiotic compounds	[153–157]
High-pressure methods	Polyphenols, phlorotannins, fucoidan, total organic carbon, minerals, monosaccharides, amino acids, polar compounds; fatty acids	[141,158–160]
Enzyme-assisted extraction	Antioxidants, fucoxanthin, fatty acids, polysaccharides	[161,162]
Ionic liquids extraction	Phenolic compounds, polysaccharides, carrageenan, terpenoids, alkaloids	[163–165]
Pulsed electric fields	Phenols, proteins	[126,166]

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 the extracts obtained [141]. Different extraction methods should be used to enhance the scientific understanding of the selectivity of the extraction from different natural sources [127].

1.5. Seaweed Biomass Production

Seaweeds have been collected for centuries, and until recently, seaweed collection from their natural populations was a norm. Recently harvesting from natural habitats have become controversial due to environmental consequences or constraints. It has resulted in strict regulations to define the harvestable amount of the seaweed biomass and determine time intervals between harvests to allow growth and recovery of seaweeds [167,168].

Seaweeds can be harvested using different techniques depending on the location of the biomass and type of seaweed [169]. Biomass can be collected manually by pulling seaweeds with hands or rakes and other tools, that remove seaweeds from the substrate they are attached [170]. Mechanical harvesting is done by machines or large mechanical rakes that collect seaweeds in a way that is not doing damage to the biomass. Harvesting machines are adjusted that they are not degrading the seabed and are leaving enough material for regrowth. A partial collection of material means that only tops of the seaweed plants are cut off by knives or scissors of harvesters [3]. 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 [171]. Overexploitation of natural seaweed resources could have a negative impact on the marine carbon cycle and promote coastal erosion processes [172,173]. To prevent from significant ecological, economic and social consequences at local, regional and even global levels, sustainable management and legislative restrictions are crucial [170]. Legislation regulating seaweed management in terms of harvesting and cultivation have entered in force in countries like Norway, Portugal, Canada, Brazil, Peru and Mexico [171]. These countries are just a few of the 32 countries that rely on the collection of naturally growing seaweeds by collecting around 800 000 t annually [170].

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 a lack of information regarding seaweed distribution and potential use is limited. 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 seaweed biomass without destroying effects on natural seaweed habitats. Development of seaweed cultivation has dramatically increased in the last century, more in Asian countries, but recently also in other continents like America and Europe [8]. Enhancement of natural seaweed meadows was initially achieved by providing additional substrate for seaweeds to attach in the form of tree branches, bamboo shoots and by clearing rocky surfaces [174]. 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 [8].

Seaweed growing does not demand arable land and freshwater. In this way, there is no competition with the agricultural sector for direct or indirect land use

In addition to valuable biomass production, seaweed cultivation provides several essential ecological services. They enrichen water with oxygen and uptake nutrients [171]. Seaweed cultivation not only plays an ecological role but also has socio-economic importance in coastal regions. Many examples of benefits are described all over the world. In Australia there is a kelp forest that covers more than 70 000 km^2 it has both ecological functions – provide biodiversity and economic function – generating an estimated value of US$ 7.7 billion annually in fishing, tourism and cultural services [175]. The European Commission has recognized that 396 microalgae and macroalgae or seaweed species are a promising option for food security that by 2054 seaweed cultivation could reach the production of 56 million metric tonnes of protein, which would constitute 18 % of the global alternative protein market [176]. Sustainable production of seaweeds contributes to society by opening new job positions: at the first level in farms, collection, growing and processing operations; at the second level through industries supplying goods and services to aquacultures such as feed, equipment and advice; and at the third level through the providing of associated jobs, i.e. spending by those employed directly and indirectly in seaweed cultivation [176].

Large-scale seaweed farms can host diverse species and consequently create new habitats and support biodiversity [177]. It can also come with negative impacts like cross-breeding between domesticated and wild strains and the harbouring of parasites, as well as the unintentional introduction of non-indigenous species, including pathogens [3]. The significant problems in the seaweed industry include overexploitation of natural resources leading to a deficiency of raw seaweed material, low-quality biomass, technological barriers to improve quality of the processed product, and a knowledge gaps on new and alternative sources of raw materials. Therefore, efforts are needed to enhance production through improving harvesting techniques, limiting of competing species, creation of artificial habitats and seeding of cleared areas. Despite the development of global seaweed cultivation, there are still many technological challenges to achieve which include thermo-tolerance, disease resistance, high growth rates, high content of desired substances, and development of economically feasible seaweed farm systems [8].

Large-scale seaweed farming is carried out only in Asia, where there is a high demand for seaweed products and growing populations to create market growth. Seaweed cultivation in Asia is a relatively low-technology business in that the whole, attached plants are placed in the sea, and there is a high labour content in operation [178].

The technique applied to seaweed cultivation is dependent on several factors like species, substrate, depth and distance to the coast. Components of the ropes or nets used can be customized in means of size, material and weight, that also have an impact on the price and availability of the tool. There is a range of techniques used for seaweed cultivation on a large scale (Table 1.5.).

Table 1.5.

Large-Scale Cultivation Techniques

Technique	**Principle**	**Located**	**Species**
Line cultivation	Seaweeds are attached to ropes of varying lengths, that are anchored to a substrate	Off-bottom – planting close to the bottom near shore	Small in size
Net cultivation	Seaweed propagules are attached to nets	at the surface or slightly submerged	N/D
Floating raft cultivation	Seaweeds are attached to lines or nets in the form of a float made of bamboo or other material	at the surface	N/D
Integrated multitrophic aquaculture	More than one marine organism is cultivated in a united system.		mollusc and fish cultivation along with seaweeds

Medium-scale seaweed farming is used to design onshore seaweed cultivation farms. They can be large ponds or seaweed photobioreactors, where mixing is controlled to intensify the total micro- and macroalgal yields [176,179]. Pond mixing allows to improve nutrient diffusion and optimize light exposure to capture photons and increase carbon fixation rates [180]. In contrary to offshore seaweed cultivation, environmental conditions in ponds can be controlled. Cultivation experiments were carried out in Denmark using green seaweed *Ulva lactuca*. For five months, seaweeds were cultivated in water ponds, and it allowed to monitor the growth of seaweeds and weekly nutrient uptake [179]. The controllable environment allows doing manipulations with light, mixing and nutrients to monitor seaweed growth in the places where offshore cultivation is not taking place.

Laboratory scale seaweed cultivation. Seaweed cultivation starts in a laboratory. The growth rate and the chemical composition of the seaweeds are affected by various environmental parameters. 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 [175,181,182].

This chapter identified and highlighted the key aspects of seaweed cultivation. Preparation to start seaweed cultivation in any scale includes understanding the seaweed physiological properties and their interaction with surrounding environmental factors: light, temperature, nutrients & CO_2, salinity and water movement, therefore, they are discussed further in the next chapter.

1.6. Factors Affecting Seaweed Growth And Development

Environmental factors affecting seaweed have been vastly described, and it only proves their importance not only in nature but also in seaweed research of cultivations and biotechnologies. It has been vastly described in the literature and research-based findings on growth rates and composition and value of seaweeds (Fig. 1.4.).

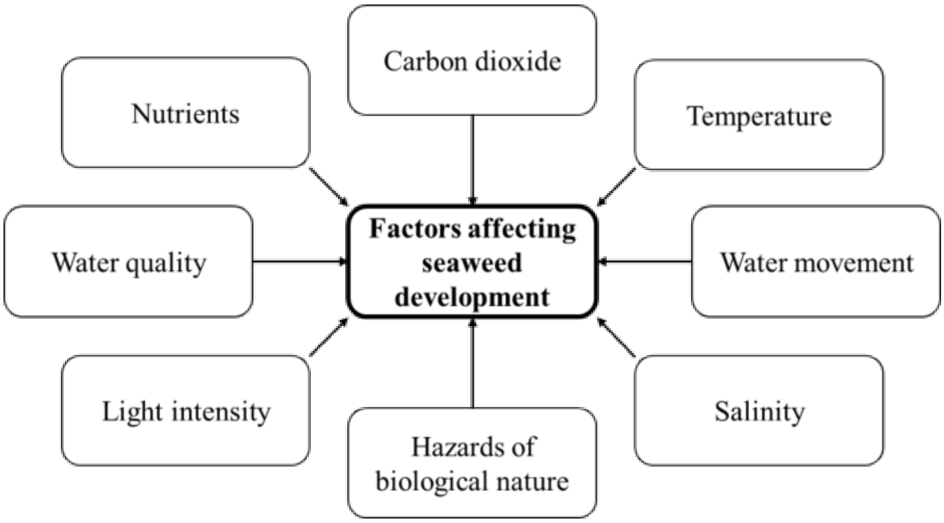

Fig.1.4. Factors affecting seaweed growth and development [183].

Environmental factors have a short or long-term impact on seaweeds at different levels. It can affect the organism in cell level or just limit some functions. Seaweed biological features allow them to adjust to short term external conditions and adapt to permanent changes in environmental conditions. Growth, reproduction, size, and cell compositions can change regarding change of influencing obstacles leading to distinct phenotypical differences even between one species. Every change in the surrounding environment can be a stressor that affects the growth rate, reproduction success and can even cause complete disintegration of the seaweeds. In this way, it can reduce seaweed abundance and biodiversity.

Light

Light is a crucial element to carry out photosynthetic processes with a purpose for chemical energy production. Majority of marine brown and red seaweed species occur from shallow to deep waters, while green species are found in areas where light is more abundant, such as shallow waters and tide pools [184,185]. Despite the depth of the water, seaweeds generally require a high light intensity. However, in cases when required light portion is not provided, for example, when the availability of irradiance is limited because of depth or self-shading, some species have ability to acclimate their photosynthetic characteristics to conditions above or below the optimal light intensity ranges [183]. Seaweeds can increase cellular contents of the photosynthetic pigments (e.g., Chl a, phycoerythrin), and decrease the content of ultra-violet absorbing compounds to acclimate the low solar radiation [183]. Some seaweed species require only 30–70 µmol m^{-2} s^{-1} highlighting and there are also the fastest-growing species with a high intensity of 200–250 µmol m^{-2} s^{-1}. The duration of the light is just as important as light intensity.

Green seaweeds are distinguished from other seaweeds by their association with green terrestrial vascular plants because their photosynthetic processes are the same. Chlorophyll a and chlorophyll b pigments participate in photosynthesis of green seaweeds and vascular plants. These pigments give them a distinctive green colour. Chlorophyll a and b absorb red light rays that are found only in shallow waters. These light rays cannot reach deeper layers of the water, therefore most of the green seaweeds are found in shallow places [186].

Red seaweeds are evolutionarily adapted to live in deeper water in lower light conditions. The seaweeds contain pigment phycoerythrin, which reflects the red-light rays and absorbs blue rays. Therefore, the colour of the seaweeds is dark red or pink. Since blue light rays can penetrate deeper layers of water than light rays with shorter wavelengths, these phycoerythrin pigments allow seaweeds to take photosynthesis and live in deeper waters than seaweeds of other species. Some species of red seaweed contain very little phycoerythrin, so their colour may range from green to bluish, as they are dominated by chlorophyll and other pigments[75,187,188].

Brown seaweeds contain green pigment – i.e. chlorophyll, but they also contain other golden and brown pigments that mask the green colour of the chlorophyll. The dominant pigment in brown seaweeds is fucoxanthin that reflects yellow light rays. Under the influence of all these pigments, brown seaweeds acquire a characteristic colour that varies from light olive-green tone to even dark brown. Most of the brown seaweeds live close to the coast, and most of them are found in cold waters [189]. *Fucus vesiculosus* requires a light intensity of at least 75 µmol m^{-2} $s^{-1,}$ but the maximum growth rate is achieved when light intensity is increased to 175 µmol m^{-2} s^{-1} [190].

Circadian day/night or light/dark regime is an essential requirement to balance light and dark stages of the photosynthesis. This regime has to be complied when seaweeds are cultivated in the laboratory, and artificial lighting is used to provide the required light intensity. The light and dark regimes are needed because excessive lighting limits the growth of seaweeds.

Temperature

Seasonal and geographical changes in temperature determine the distribution of global seaweed populations. It should be underlined that each species is characterized by its geographical habitat. Most species feel comfortable at 20 °C or above temperature, while for some, this temperature is an upper limit above which reproduction decreases or even entirely cease [23,183]. Besides, the temperature may also have an impact on seaweed internal physiological processes, for example, diffusive rates and carrier-mediated nutrient uptake [191]. Altogether, it seems that deviation of the boundaries set for water temperature and lack of light exposure are significant factors limiting the growth of seaweeds and biochemical composition.

Every seaweed species has a specific optimal temperature range that serves the best for growth and surviving. By knowing the necessary temperature and other factors affecting growth, the seaweed cultivation process can give maximum biomass output. The table summarizes the optimal growth temperature for different species of seaweeds (see Table 1.6.).

Table 1.6.

Optimal Seaweed Growth Temperature

	Species	Optimal temperature	Source
Red seaweeds	*Palmaria mollis*	14 – 15 °C	[192]
	Gracilaria lemaneiformis	25 – 30 °C	[193]
	Porphyra dioica	15 °C	[194]
	Chondrus crispus	17 °C	[195]
	Furcellaria lumbricalis	15 °C	[196]
Brown seaweeds	*Fucus vesiculosus*	4 – 10 °C	[197]
	Laminaria digitata	5 – 15 °C	[198]
	Saccharina latissima	5 – 15 °C	[198]
Green seaweeds	*Enteromorpha intestinalis*	10 °C	[199]
	Ulva lactuca	10 °C	[200]

As shown in Table 1.6. some seaweeds are abundant in a warm water environment, and some seaweeds prefer low water temperature. However, it is evident that most seaweeds do not grow in the warm water temperatures and grows more intensively at moderate temperatures not exceeding 15 °C.

Fucus vesiculosus achieves the highest growth rate at a temperature of 20 °C, but with increasing temperature, there is a sharp drop, which leads to the death of the plant. Although the fastest growth of *F. vesiculosus* is at relatively high temperatures, the results of the study confirm that good results can also be achieved at 10 to 15 degrees. However, it should be noted that *F. vesiculosus* is present in several regions and is consistent with the species living in the Irish Sea. Seaweeds occurring in the Baltic Sea are most rapidly growing at temperatures between 4 °C and 10 °C, as there are different weather conditions in the region and the average temperature is lower all year round [197].

The temperature affects seaweed physiological processes, such as the rate of diffusion and the intake of nutrients. For example, with the increase in water temperature, *Gracilaria tikvahiae* is also able to absorb NO_3 [201].

Water movement

Water movement is mentioned as one of the factors affecting seaweed growth and development because of its importance in the acceleration of the diffusion rates of inorganic nutrients and gases in and out seaweed thallus. Absorption of necessary nutrients and other elements occurs throughout the body of seaweed or thallus. Water motion creates favourable environmental conditions for the growth of seaweeds. In addition, Tiwari and Troy (2015) claim that water movement reduces harm by hazards of biological nature [3].

Water streams accelerate the diffusion of nutrients and gases, including CO_2, from water to seaweed thallus and vice versa [201]. In the standing or slowly moving water around the seaweed thallus, a thin boundary layer is formed that limits the supply of CO_2 that is essential for the photosynthesis. A layer reduces the ability of the plant to absorb nutrients from the water and consequently reduces the growth rate of seaweeds. With increasing water movements, the boundary layer decreases and seaweeds, under the influence of the thallus movement, are cleaned. Photosynthesis and nutrient intake are increasing rapidly, and seaweed growth rates are rising [198].

In the seas and ocean, the water is never stagnant. The water enriched with nutrients and minerals is continuously available. Therefore, providing a constant flow of water in a laboratory environment is essential.

In Fig. 1.5. relative daily growth of *Ulva lactuca* in different water flows is depicted. In the natural environment, different factors interact with each other. Therefore, it is not correct to analyse the impact of each of the factors separately. In Fig. 1.5. importance of the light can be seen, and it can be concluded that water flow does not show its importance if the light conditions are insufficient. Limited light conditions impede photosynthetic processes that inhibit the growth of the seaweeds. With optimal light conditions, the positive impact of water flow on seaweed growth is increasing. Sufficient light provides optimal conditions for seaweed growth; therefore, water flow has an impact on the growth rate.

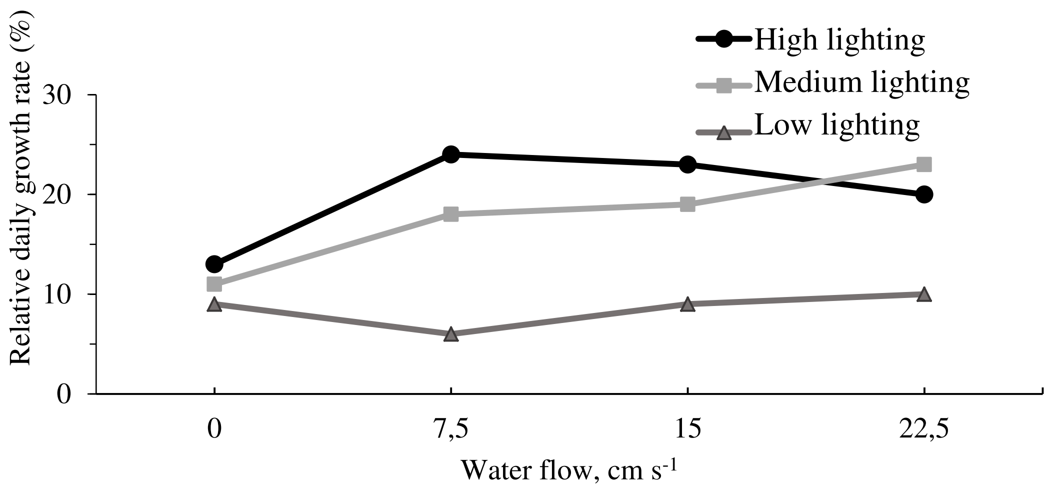

Fig. 1.5. Relative daily growth of *Ulva lactuca* in different water flows and different light conditions [202].

To identify the optimal water flow velocity for every seaweed type, it is necessary to observe their behaviour in their natural environment. When cultivated in artificial conditions, cultivation parameters should simulate the natural environment as close as possible. Accurate data can be obtained only by carrying out targeted experiments, but seaweeds which are naturally living in calm waters will never give their maximum biomass growth if the water flow is too strong. It will be the same with seaweeds that are used to strong waves. If the water flow is not strong enough, they will not grow, or growth will be prolonged because the nutrient uptake will be difficult.

Nutrients and CO_2

The success of seaweed biomass increase depends on multiple factors that interact between them. Amount of available nutrients and carbon dioxide and overall water quality is an essential factor. Similar to terrestrial plants, seaweed use sunlight and consume or adsorb carbon dioxide (CO_2) to provide growing processes. Seaweed growth rates are significantly affected by the number of various chemical components and their concentrations. Importance of optimal nitrogen (N) and phosphorus (P) in seaweed growth has been proven. Increased levels of N and P support optimal growth conditions, while the highest concentration limits are not exceeded. The optimal amount of the nutrients necessary for seaweed growth processes can differ between different seaweed species, their natural growth habitat, because growth conditions can differ in different regions. In optimal nutrient, salinity, light and temperature conditions *U. intestinalis* growth rates exceed, and it can form dense clusters or green tides in the littoral zone [203]. As mentioned before, even though *Ulva sp.* are marine species, they can adjust to a brackish environment by taking up more nitrogen [63].

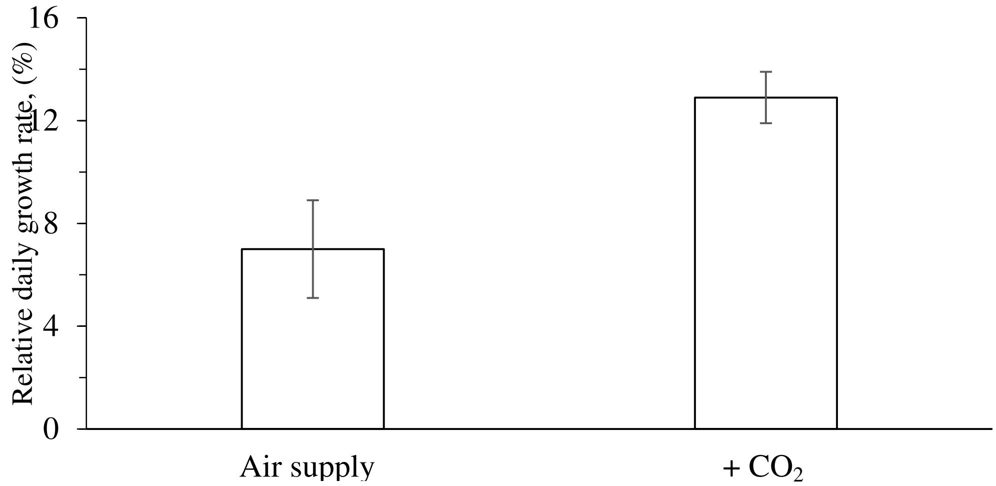

Fig. 1.6. Comparison of the relative daily growth rate of *G. lemaneiformis* with air and air+CO_2 supply [204].

When seaweeds are produced in a closed environment, nutrient concentrations can be controlled, and the desired amount of nitrogen and phosphorous can be added. There has been research showing that higher nutrient concentrations show higher rates of photosynthesis [205]. Regarding the species response to added nutrients, seaweeds can be divided into two parts. Those that grow well with the addition of ammonia or other nitrogenous solution and those that achieve higher growth rates with added PO_4.

Rapidly increasing concentrations of CO_2 in the atmosphere has and will continue to have, an impact on the photosynthesis, growth, metabolism and cell composition of seaweeds. Increasing atmospheric CO_2 stimulates photosynthesis and increases growth rate [206]. Fig. 1.6. shows the growth rate of *G. lemaneiformis* when water in the aquarium is supplied with air or with CO_2-enriched air.

Water motion and turbulence is an important factor affecting the growth of both microalgae and macroalgae. The use of air bubbling in an artificially controlled environment achieves the turbulence, and intense gas and nutrient exchange takes place between the elements in solution and the air bubbles [207]. Air bubbling is one of the most common CO_2 supply methods for cultivating seaweeds, as growth medium can easily be mixed, and carbon, nitrogen and phosphate particles on the cell surface can be restored. Air bubbling is known to help the plant deliver CO_2, even at low concentrations (300-400 ppm) [207].

Salinity

Another important environmental factor affecting seaweed growth is salinity. It has been stated before that mainly seaweeds are abundant in seawater and some can be found in freshwater. Seaweeds can survive in different levels of salinity.

Green seaweed *Enteromorpha intestinalis*, brown seaweed *Fucus vesiculosus*, red seaweed *Furcellaria lumbricalis* mainly inhabits in brackish water or other words in the briny water. Therefore, the species can also be found in the Baltic Sea, the sea which salinity is much lower than ocean water. While habitats of *Fucus*, *Furcellaria* and *Enteromorpha* are not high saline water, the range of the salinity they can be found differs. This factor should be considered when cultivating seaweeds both in a laboratory and natural environment.

When cultivating seaweed in the eastern Baltic Sea region, it should be taken into account that it is not necessary to provide highly saline water. The Baltic Sea has one of the lowest salinity levels, and on the coast of Latvia, its salinity does not exceed 6‰. The Baltic Sea is unique in that its water salinity varies from 0 – 30 ‰ in different places. Coastal areas are rich in river estuaries, which reduce the salinity of the water, requiring seaweeds to adapt to different salinities. Water salinity is a variable factor not only in the Baltic Sea but this trend is also observed elsewhere in the world, so every seaweed species grows most rapidly within the range of its optimal salinity.

Correct determination of water salinity plays a vital role in the cultivation of seaweeds. The wrong choice of water salinity can significantly affect the results. To avoid selecting the wrong salinity, water from the natural habitat of the seaweed growing sit should be used to ensure, that the salinity of the water does not differ from its optimum salinity. Inadequate salinity of water can both reduce seaweed growth rate and cause loss of the seaweed biomass, even though in such cases, the seaweeds are often still able to adapt to the new water salinity, recover and continue to grow. In the case of salt concentrations several times higher than the optimum concentration, seaweed death is inevitable, and cultivation must be restarted.

2. METHODOLOGY

In order to evaluate potential seaweed processing pathways through the biorefinery concept, specific research stages were defined. As the first step, it is necessary to evaluate the availability of seaweed biomass, meaning, species, sources and amount of biomass that could be processed (Paper I and II) [208,209]. The next step is to look for potential products and their extraction methods (Paper III) [210]. Biorefinery concept model was designed, and to evaluate it, SWOT analysis was used (Paper IV) [108]. SWOT analysis gave essential insights, and special attention was paid on seaweed biomass availability. There are more reasons to look for a way to obtain more seaweed resources from artificial sources, meaning cultivation. Therefore, as the first step of the cultivation, research facility – a laboratory stand was needed. Creation of a laboratory stand is described in Paper V [211]. Laboratory was tested by carrying out seaweed cultivation experiments (Paper VI)[212], by artificially changing seaweed growth environment: nutrients and lighting regimes. In this way, it was also possible to identify limitations for seaweed growing (Paper VII) [213].

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. At the same time, the concept must comply with sustainability criteria that are achieved by creating the conceptual design and SWOT analysis.

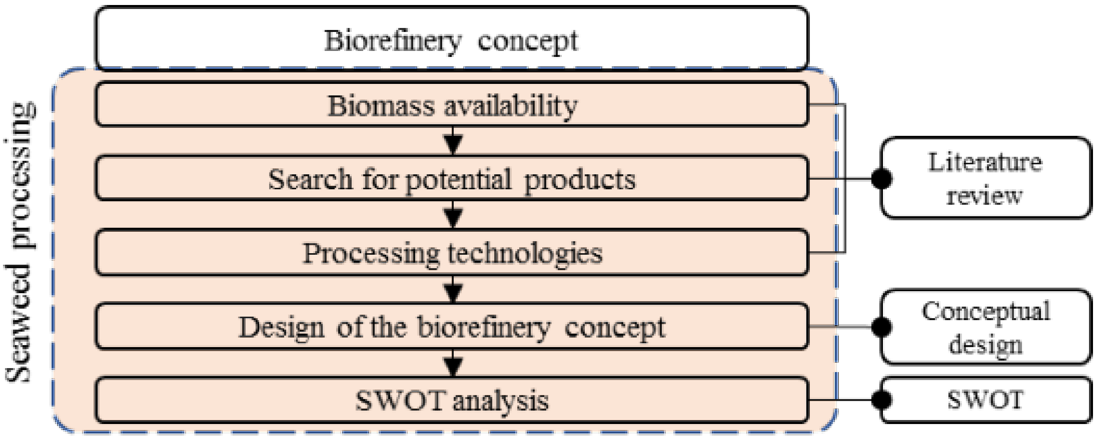

Fig. 2.1. Methodology used to develop the seaweed biorefinery concept.

The core subjects of the biorefinery concept are the supply chain network and the chemical conversion of biomass [214]. 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.

2.1.1. 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 Most abundant seaweed species in Latvia	number species names
Amount of seaweed biomass	Biomass amount in natural growths Washed out seaweed biomass Cultivated seaweed biomass	t/ha m^3 per 100 m 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		

2.1.2. 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 [215].

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.

2.1.3. 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 [216]. 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 [217]. 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 whole biorefinery concept together should be based on bioeconomy principles, but every step and phase of the concept, starting from input material and production and throughout whole life cycle of products should follow the directions given by bioeconomy principles to give the highest social, environmental, and economic benefits.

2.1.4. SWOT Analysis

SWOT analysis is a tool for strategic planning used for different cases, e.g. for analysis of various projects or organizations [218]. SWOT analysis methodology comprises brainstorming for analysis and positioning internal factors, i.e. strengths and weaknesses, and external factors, i.e. opportunities and threats, of the analysed object. By identifying the factors in these four groups, the basis for decision-making and planning strategies is obtained. The main advantage of SWOT analysis is its application simplicity and structuring a particular brainstorming session, whereas the main disadvantage results in no opportunity to rank the significance of one factor versus another [219]. Nevertheless, SWOT analysis is an appropriate tool to address a wide range of business issues and therefore is widely practised

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 alaboratory stand and laboratory experiments (Fig. 2.3.).

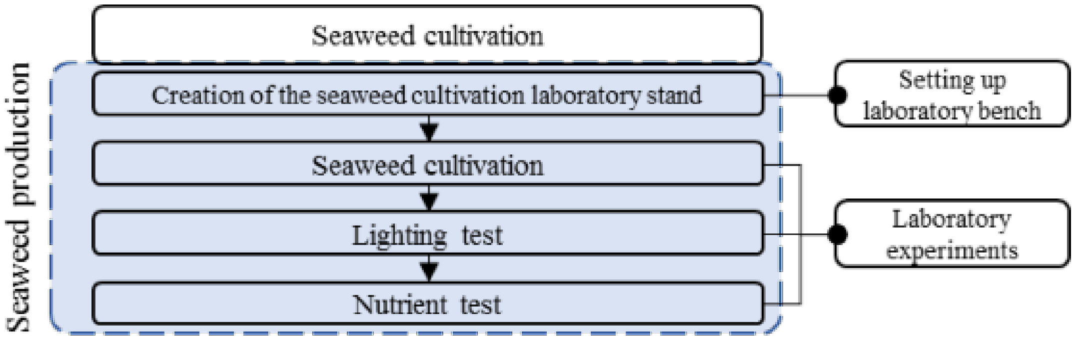

Fig. 2.3. The methodology used to increase biomass availability.

2.2.1. 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). The IESE Biosystems Laboratory was equipped with hot and cold water supplies, electricity and necessary equipment such as a refrigerator, cold store, dishwasher, fume box, drying oven and several tables, chairs and sink.

In order to ensure the conditions necessary for the seaweed cultivation and to perform all necessary measurements and studies, additional equipment were needed (listed below). 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. Therefore, it is necessary to provide the laboratory with all the necessary equipment for different scale seaweed cultivation experiments:

- Different size aquariums,
- Erlenmeyer flasks;
- Petri dishes.

Other equipment is also needed to provide, regulate and measure factors such as light intensity, salinity, a quantity of CO_2 and nutrients:

- a lamp with an LED bulb;
- Plug-in timer;
- Lux Meter;
- Gas cylinder cabinet;
- Multi-Parameter meter;
- Electronic pipette
- Microscope.

A lighting system with LED lamps was set up to provide adequate light exposure for seaweed cultivation. Plug-in timer is used to provide an option to set light/dark regime needed for seaweed growth. The Lux meter is required to precisely determine the light intensity by which seaweeds are screened in different aquariums or flasks. An electrical pipette is handy for the addition of nutrients and water salinity as it can measure the volume more accurately than a manual pipette.

In order to prepare an analysis of seaweed materials and the results as closely as possible, the laboratory also have to contain equipment for additional measurements:

- microscope;
- stereomicroscope;
- analytical weights;
- a tap sink;
- tubing tubes with screw caps;
- tweezers;
- a set of laboratory accessories;
- plastic pipettes.

The laboratory is equipped to carry out experiments with seaweed growing in three scales. At first cultivating seaweeds in large aquariums and experiments in medium and smaller volumes are suggested to avoid unforeseeable results. This means saving time and raw materials, since failure to choose light intensity, temperature or other conditions would result in the disposal of only a few grams of cultivation material. In small volumes, it is also easier to notice any changes in progress and the results are seen more quickly. The small setting with Petri dishes is used to examine morphological changes in small size seaweeds caused by changes in the surrounding environment. For experiments, seaweed fragments of 3 to 4 cm are removed and placed in Petri dishes filled with filtered saltwater. Depending on the conditions of the experiment, the light intensity, temperature and nutrients are regulated. Petri dishes are used to determine the growth rate of small size seaweed by placing them with specimens on them on millimetre paper. Surface area or length and width of the specimen is measured before and after the experiment to detect size changes and to calculate the growth rate. Small size experiments are also usually applied in reproduction tests of the seaweeds.

The medium-scale experiment set up with Erlenmeyer flasks is used to provide a more controlled growth environment for seaweeds. Air bubbling system is installed to provide gas-exchange for seaweed growth. In this case, small size seaweed specimens are the best to use (like *Ulva intestinalis*), and the surface area or length and width is measured to determine the growth rate of seaweeds.

To prepare seaweed material for experiments, aquariums 30-50 L is used for pre cultivation. Aquariums can also be used to maintain alive seaweed material in the long term or for scaling up the experiment, once the most favourable conditions have been identified.

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, but it is not yet possible to create a complete system because some of the necessary equipment and components, such as equipment to control temperature, are missing. By further developing the laboratory, it is suggested to develop a functioning system for the cultivation of seaweeds, which will allow all growth-affecting conditions to be monitored at the same time and will increase the effectiveness of experiments. The complete scheme of seaweed cultivation laboratory stand is shown in Fig. 2.4.

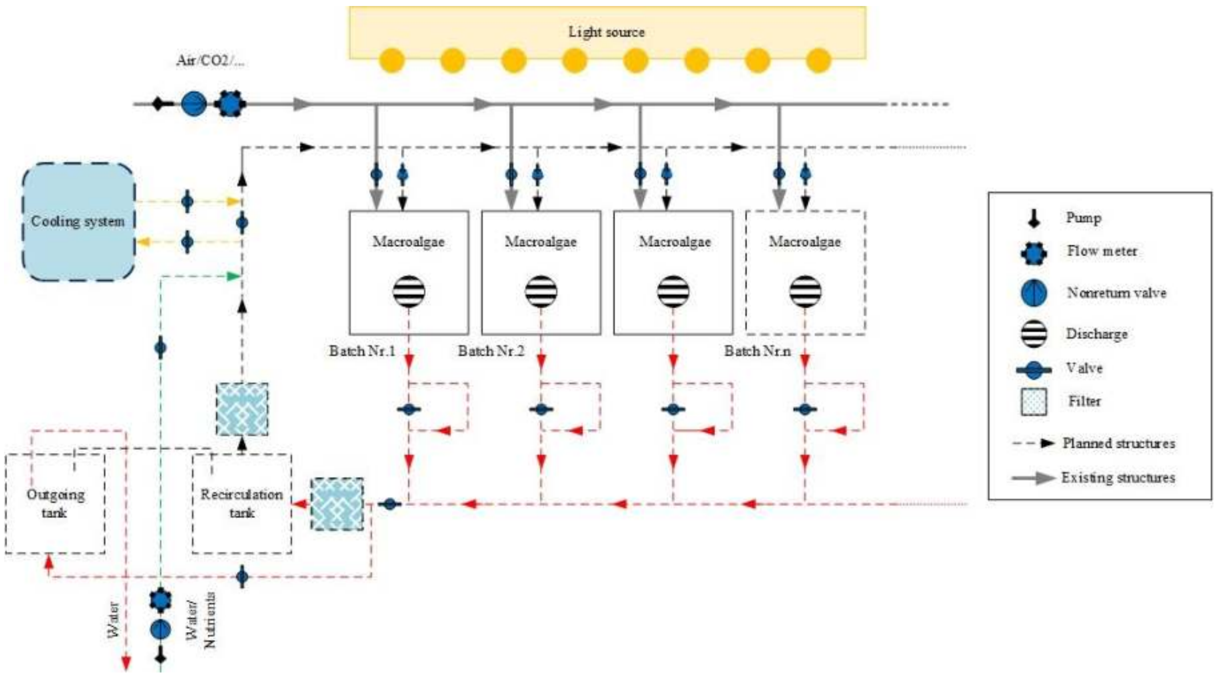

Fig. 2.4. A complete scheme of the seaweed cultivation laboratory stand.

In the scheme grey and black arrows shows the flows entering the aquariums or flasks while red arrows display outgoing flows. The input flows consist of two pumps that are connected to the system to provide air or CO_2 supply and water or nutrient source. The growth medium is cooled or heated to the temperature required for the experiment before injection in aquariums or flasks. Experimental temperature can also be provided by regulation of the temperature of the surrounding room. With outgoing flows, the water is removed from the aquariums, filtered and stored in the recirculation tank, and then returned to the aquariums. If it is needed to replace the water in aquariums, it is drained from the recirculation tank to the outlet tank and then is discharged. After the water discharge, the water level in the system is reduced. Therefore, an additional water supply tube is established to replenish the water in the system. Any of the flows can be stopped and re-opened using valves. A light source is also placed above the flasks or aquariums, which can be set on light/dark mode.

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. 2.5.

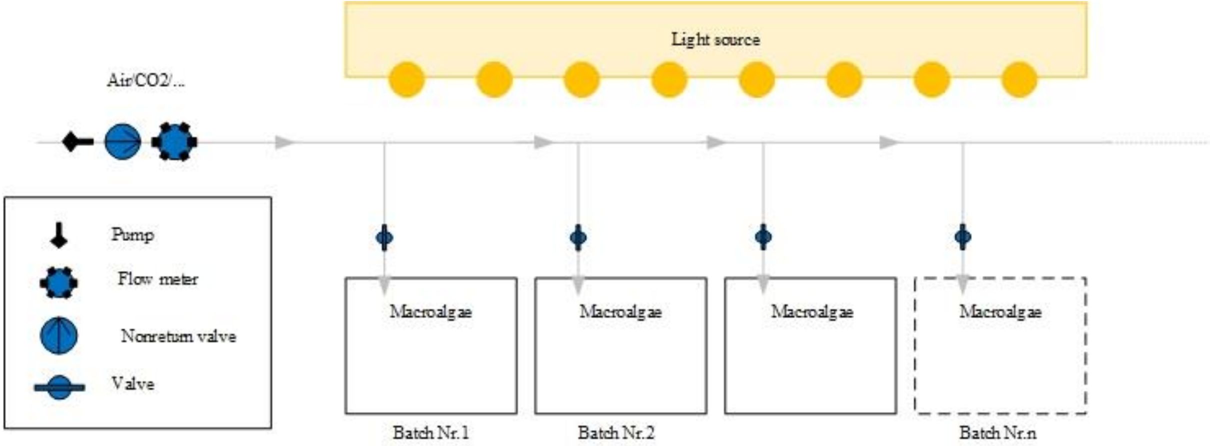

Fig. 2.5. Scheme of implemented seaweed cultivation laboratory stand.

Similarly, to a complete system, presented in Fig. 2.4., 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.

2.2.2. 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. At 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 quickly.

Test organism

Green filamentous seaweed *Ulva intestinalis* was used as a test organism on all experiments. Collection time and location are summarized in Table 2.4. Sites are characterised by shallow depths (0.5–1 m), high nutrient levels and low salinity level (5.5–6 ‰).

Table 2.4.

Test Material Collection Time and Site

Experiment	Collection time	Location	Coordinates
Nutrient test I	April 2016	Tūja beach	57°49′N 24°37′E
Nutrient test II	July 2016	Mērsrags beach	56°59′N 23°51′E
Nutrient test III and IV	June 2017	Tūja beach	57°49′N 24°37′E
Lighting test	June 2017	Tūja beach	57°49′N 24°37′E

Seaweed samples were transported to the laboratory. The first pre-treatment was to wash the samples to remove sediments, small crustaceans and traces of other algae. Seaweed samples were washed to remove impurities.

Seawater to be used in experiments was collected at the same site, using the siphoning method to avoid contaminants from the water surface. Water was collected from the seashore into 20 L plastic buckets and delivered to the laboratory within an hour. The water was filtered through filter paper to remove phytoplankton, zooplankton and debris (Fig. 2.6.).

Fig. 2.6. Seawater filtering process.

Seawater was sterilized in the microwave equipped with rotating base for 10 minutes in 700 W to sterilize the growth media [220]. 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 [174].

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 [221], 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.

Fig. 2.7. Air supply and lighting system.

Flasks were placed under the light source with an irradiance 1000 lux (30 μmol photons $m^{-2} s^{-1}$) (Fig. 2.7.). 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 2.5.).

Table 2.5.

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

For Nutrient test I and Nutrient test II temperature remained constant during the experiment at 20 degrees. For Nutrient test III and Lighting test, batches were held in climate chamber JEIO TECH TH-6 in temperature 13.4 °C, that is the same temperature as it was water temperature on the collection time.


Fig. 2.8. Batches prepared for the experiment.

Air was supplied with rubber tubes to provide constant water motion and gas exchange. Enriched growth media was changed once a week manually by pouring off most of the old media and replacing it with seawater enriched with nutrients. In the case of evaporation, the water level was adjusted by pouring distilled water to maintain previous salinity. Batches prepared for the experiment can be seen in Fig. 2.8.

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" [222]. The growth rate was calculated using Formula (2.1.)

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

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; and t is experiment time in days, d.

3. RESULTS AND DISCUSSION

3.1. Feedstock for Baltic Seaweed biorefinery concept feedstock

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	[33]
Most abundant seaweed species in Latvia	*Ulva intestinalis* *Fucus vesiculosus* *Furcellaria lumbricalis*	species names	[33]
Biomass amount in natural growths	3.5	t/ha	[223]
Washed out seaweed biomass	Summer <16 Autumn <228 Summer <62 Autumn <22	m^3/100 m	[34]

Results summarised in Table 3.1. do not demonstrate all information regarding the indicators set in part 2.1.1. 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 [34].

Regarding the quality of the available seaweed biomass, there is no consistency in the species composition in previously studied seaweed deposits. Composition of deposits varies not only amid to 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.

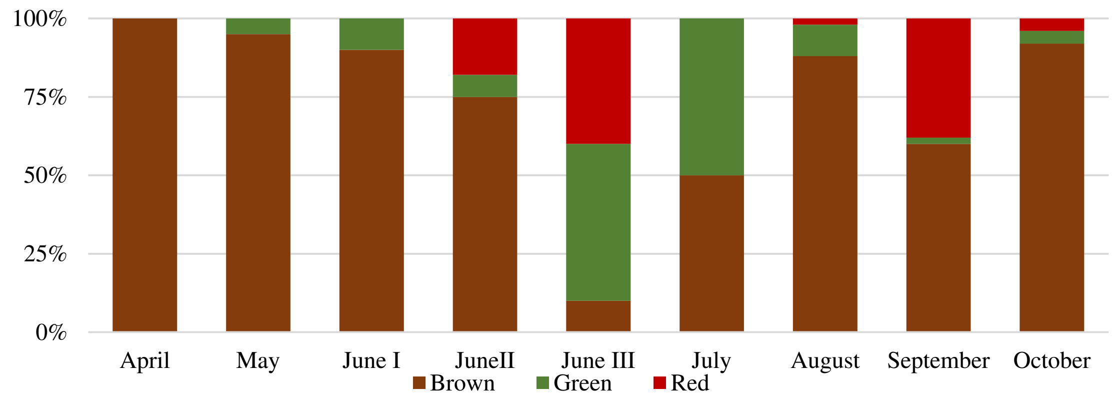

Fig. 3.1. Composition of the washed-out seaweed biomass in Melluži (2018)[34].

Seasonal studies of washed-out seaweed at Melluži beach show significant heterogeneity [34]. Precise species composition could not be determined after literature analysis because it is changing. Just the main trends in terms of dominating species amongst regions could have been determined. Changes of distribution of seaweed species in marine areas in Latvia can be seen in Fig. 3.2.

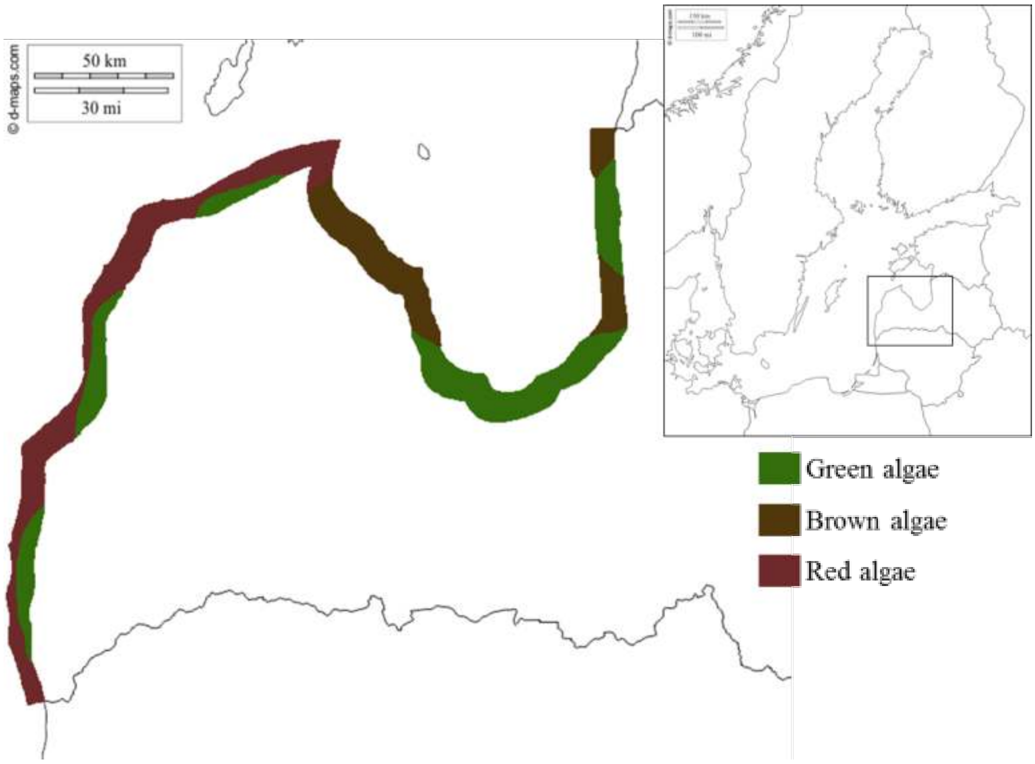

Fig. 3.2. Seaweed species distribution on the coastline of Latvia.

Even though available information on seaweed distribution fluctuates, it can be concluded that the main distribution of red seaweeds *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 [30]. Presence of green seaweeds is observed all along the coastline, where the growing substrate is suitable for seaweed growth [22,32,34]. This distribution pattern is related to salinity that is considered to be the main factor influencing the seaweed distribution in the Baltic Sea [224].

Seaweed mats can float long way and accumulate ashore on the beaches. Although it is a natural coastal process, the problem occurs in its scale and location. Washed out seaweed mats are a disturbing problem during the tourist season when the smelling and decomposing biomass is decreasing the recreational value of beaches. Besides, swimming is not recommended when there is an increased amount of seaweed. Seaweed natural habitats are not located near the beach. Waves and currents are bringing them there, and it mainly depends on wind speed and direction [27]. The appearance of seaweeds on a beach is not a permanent or regular process, so the composition and amount of washed out biomass are not predictable [28].

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 of insufficient quantity for processing that would be cost-efficient and friendly for environment [40]. In order for seaweed processing to become cost-effective, it is necessary to reveal sustainable seaweed cultivation methods for the Baltic region. It is necessary to get acquainted with the most effective techniques in the Latvian climate zone and sea salinity level. However, it is necessary to take into account the fact that seaweed cultivation, like agriculture, requires light and temperature [225]. Many conditions should be met when growing seaweeds: only native species that are already present in the Baltic Sea could be grown, no additional fertilization would be allowed, as they could increase eutrophication in the Baltic Sea. An environmental impact assessment should be carried out before a seaweed cultivation farm is set up.

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 habitats and their slow growth in brackish waters [226]. 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).

Compared to other seas and oceans diversity of species is very low in the Baltic Sea due to low salinity. Salinity near the open coast of the Baltic Sea is 7.2 psu. In the Gulf of Riga where salinity is around 5.2 psu, one of the lowest macro vegetation can be found. For seaweeds as well as for other organisms, it is a challenge to survive and adapt low salinity, especially in combination with the level of eutrophication in the Baltic Sea [25].

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 gained not only of seaweeds from the Baltic Sea but also from the same species of seaweed 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 3.2. 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 seaweed *(Ulva intestinalis)*		Brown seaweed *(Fucus vesiculosus)*		Red seaweed *(Furcellaria lumbricalis)*	
Carbohydrates ,% DW	31.34–92	[227–231]	65.7	[232]	55.4	[233]
Polysacchrides	4.9–59	[227,230,234–236]	2.31–22	[237]		
Alginate	2–59	[230]				
Fucoidan			7.54–11.1	[238]		
Furcellaran					40–50	[239]
Cellulose					3.4–5.7	[9] [240]
Proteins, % DW	9.49-20.60	[227,229–231,235,236,241,242]	1.4–11.3	[243,244]	13.1–28	[9] [245] [246] [20]
Pigments, % of total pigments						
Chl a	0.394	[247]	0.157–5	[106,247]	0.228	[247]
Chl b					0.078	
Chl c			0.035	[247]		
B carotenoids			0.2	[106]	13.3–28.6	[9] [247]
Fucoxanthin			1			
R-phycoerythrin					0.1	[9]
Xantophyll (mg/kg)					32.8	[246]
Phenolic compounds, % water extracts			18.4	[83,106,248]	2.25–4.6	[9,246]
Lipids, % DW	1.16–22.0	[231,242,244,249–251]	3.95–4.8	[243,252]	1	[245,246]
Fatty acids (FA)		[244,249,250,253]		[243,244,254]		[20,246,254]
<u>SFA, % of total FA</u>	25.0–60.6		24.3		38	
C10:0			2.8–18.8			
C12:0	0.1					
C14:0	1.8–5.38		7.5–13.9		5.07	
C16:0	17.9–23.2		9.6–12.1		29.36	
<u>MUFA, % of total FA</u>	21.81–24.8		47.1		28.80	
C16:1, n7	1.8–6.56		31.9–46.9		8.54	
C18:1, n7	7.6–15.2				4.80	
C18:1. n9	1.5–5.4		46.0		10.22	
<u>PUFA, % of total FA</u>	14.8–37.1		25.8		14.45	
C16:4. n3	4.8–10.0					
C18:2. n6	4.6–5.8		7.5–10		2.48	
C18:3. n3	8.55–24.1		2.7–3.4		2.05	
C18:4. n3	4.39–14.4		2.2		0.92	
C20:4. n6	1.4–1.5		7.4		1.63	
C20:5. n3	0.8–5.43		3.7–6.7		3.26	
Minerals, mg/100g		[242,254]		[97,244,248,254]		[254]
Mg	11		6.7		8.9	
K	12		25		42	
Ca	29		30		3.7	
Na	8.5		18		10	
P	1.7		1		1.2	
Cu	5.7		3.7		6.2	
Fe	580		290		130	
I	130		260		84	
Mn	180		37		7.5	
Se	0.76		0.08		0.1	
Zn	21		28		23	
<u>Total ASH, % DW</u>	5.42–29.4	[230,236,241,242,255]	18.74–30.30	[97,232,244,248]	9–41	[20,240,246]

Findings that are summarised in the Table 3.2. shows that carbohydrates can create even more than half of the total seaweed biomass. They are stored in seaweed cell structures in the form of polysaccharides. 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).

The yield of furcellaran depends mostly on the conditions of extraction and the raw material used. There is a considerable difference in extracting yield between the attached and unattached form of *F. lumbricalis*. While the unattached form of this seaweed species yields only 19 % of furcellaran during a 4-h extraction in pure water, the attached one affords 32 % under the same conditions. The lower yields in the case of the unattached form are caused by the thallus morphology, i.e., by the higher amount of the cortex due to thinner thallus filaments resulting in a lower total content of galactan. The concentration of fucoidan differs from the applied extraction method [238]. Literature analysis results show that cellulose fraction in *F. lumbricalis* can reach 5.7 % DW. The quantity of the cellulose for washed-out seaweed deposits can create up to 30 % DW that could be explained with the plant material in washed-out biomass.

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 seaweeds. 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 [133].

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 %).

Similarly, Matanjun et al. (2009) [256] and Polat and Ozogul (2008) [257] reported that palmitic acid and oleic acid were the major fatty acids found in the seaweeds that they examined. Of the three Baltic seaweed species investigated, U. intestinalis (25.0-60.6 %) and *F. lumbricalis* (38 %) had the highest relative concentrations of SFA in their total lipid content of which at least 20 % consisted of palmitic acid. The brown seaweed species F. vesiculosus (47.1 %) and red seaweed F. lumbricalis (28.8 %) had the highest relative concentrations of MUFA, consisting mainly of oleic acid (C18:1 n9) and palmitoleic acid

(C16:1 n7). The green seaweed *U. intestinalis* had the highest content of PUFA at 37.1 % of total fatty acids consisting mainly of α-linolenic acid, known as one of the omega-3 fatty acids.

Obtained results of fatty acid composition in these seaweeds are in line with the findings of Rohani [253] who obtained similar trend in fatty acid composition. Amount of fatty acids in seaweeds is dependent on the water temperature. It has been reported that species in colder regions contain higher levels of fatty acid composition comparing to tropical species [253].

Regarding mineral composition, it is strictly dependant on the surrounding environment and mineral amount in the water. All of the studied seaweed species are rich with iron but have low concentrations of selenium and phosphorous. The iodine content in *F. vesiculosus* (260 mg/100 g) is significantly higher compared to green (130 mg/100 g) and red seaweeds (84 mg/100 g). For this reason, *Fucus vesiculosus* is still registered in the European pharmacopoeia. Green seaweed *U. intestinalis* contains a considerable amount of iron and manganese, red seaweeds and brown seaweeds show high levels of iron and iodine. Mineral content in seaweeds is reported to be higher than in edible plants. As an example it is worth to mention leafy vegetable spinach, which is suggested to be consumed in case of iron deficiency, contain 26.69 mg/ 100g DW while in *U. intestinalis* it can reach even up to 580 mg/100 g DW [258]. Extraction of minerals in seaweed to use them as a food supplement is not a viable solution, but seaweed can be consumed as a food supplement.

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 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 can be used for nutritional and pharmaceutical purposes. Values of minerals and phenolic compounds in *Fucus vesiculosus* shows that those could be potential pathways of use of these seaweeds.

It has to be taken into account that the amount of the substances detected in the biomass depends mainly on the used extraction technologies. Most of the data summarized in the table are gathered from research carried out in small scale for analytical purposes. When produced in upscaled facilities, obtained quantities might be different, and it can affect purity and form of the compounds.

Seaweed processing technologies are vastly described in Chapter 1.4. Furthermore, there are no significant differences in the application of extraction technologies on the seaweeds in the Baltic region. The overview of novel seaweed compound extraction techniques is summarized in Table 1.4. (full table in Annex). When applying these technologies in the biorefinery concept, it is necessary to define the application of the end-product.

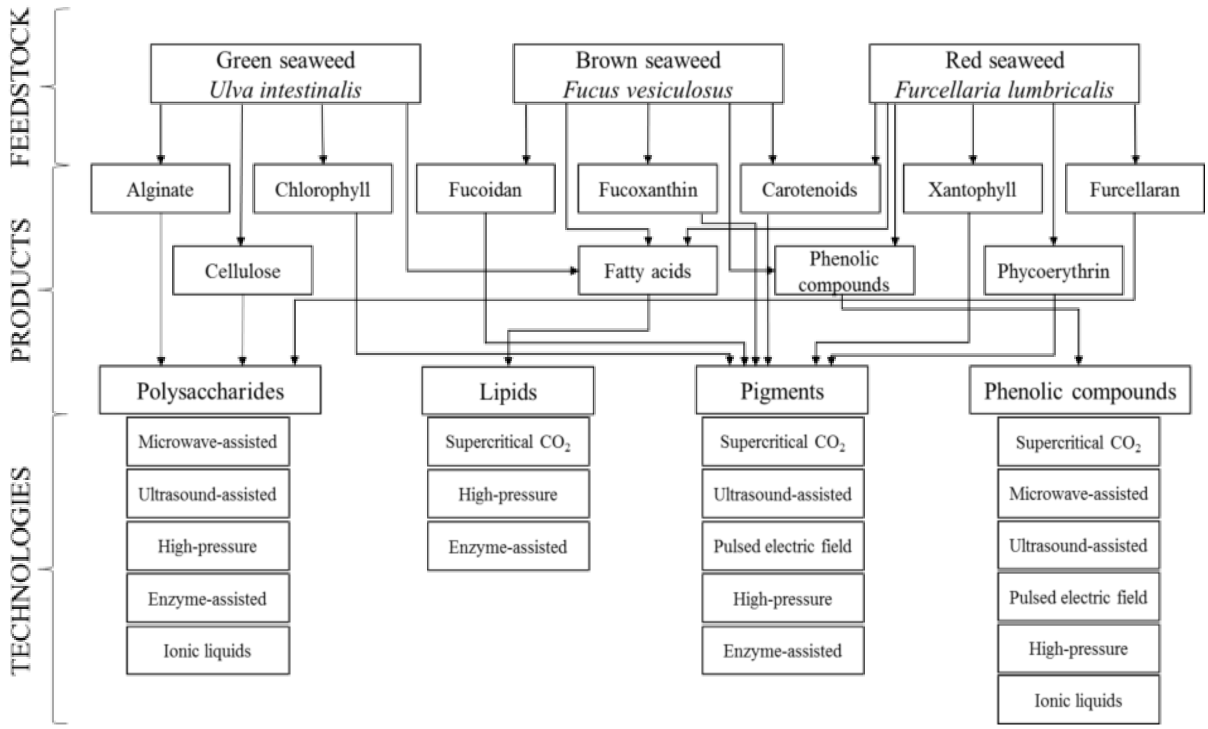

Fig. 3.3. Baltic seaweed biomass transformation routes.

Fig. 3.3. summarize valuable compounds obtainable from Baltic seaweed biomass and offers transformation routes using novel extraction technologies. Scheme of transformation routes can be applied in multiple ways, by choosing available feedstock type, available technology or desired product.

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 [232,259]. The seaweed mineral content is higher than the mineral level in terrestrial plants and animal products [97,244]. High mineral content and low-fat content represents seaweeds as 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 to the food industry but also for the pharmaceutical industry. Fucoidans found in brown seaweeds exhibit various biological activities with potential health benefits [244,260]. 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 [51].

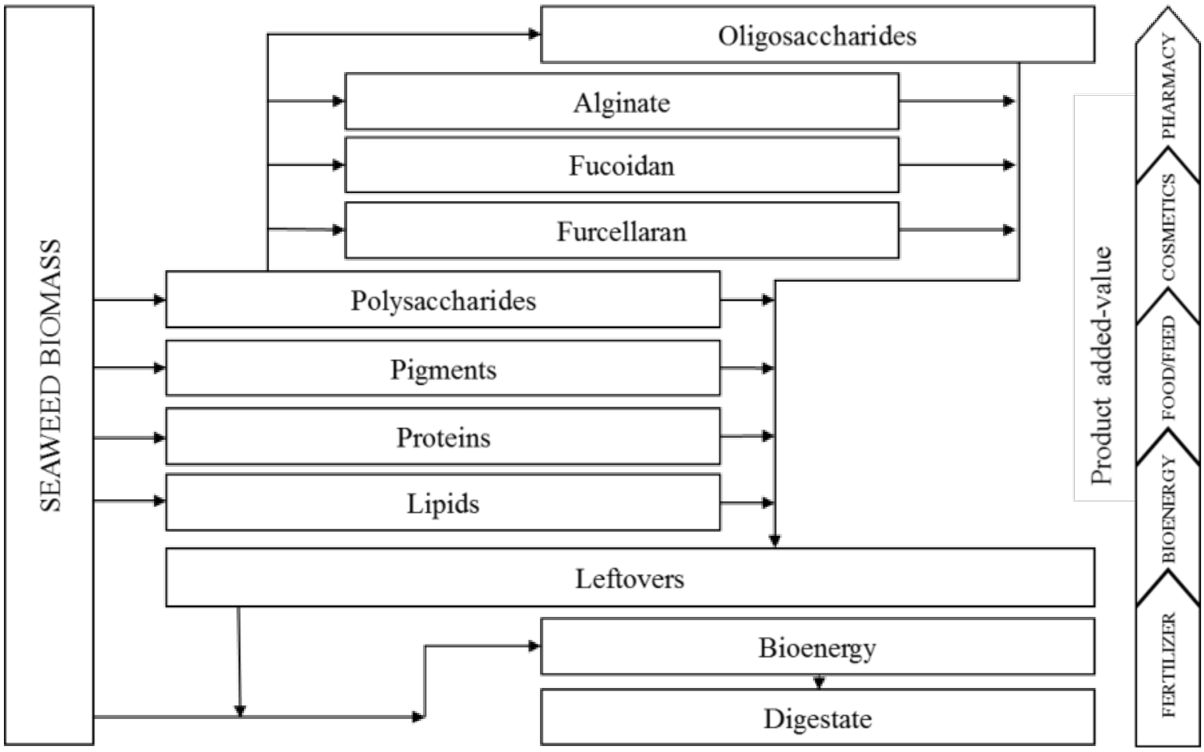

Fig. 3.4. The Baltic seaweed biorefinery concept..

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 [51]. 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 seaweed biorefinery concept is mainly addressed to use particular seaweed species whose overgrowth, as a result of eutrophication, is causing ecological damages and affects the sustainability of coastal environments. Cultivated seaweed biomass can be used as an additional feedstock to provide smooth production throughout the year and strengthen seasonal independence. Seaweed biorefinery concept can have a significant impact by showing different aspects for utilizing seaweed to produce a wide range of value-added products, biofuels, and bioenergy within a concept, rather than focusing on a single product [20]. The various uses of products obtained from seaweed biorefineries include transport fuels, therapeutics, food additives and biofertilizers [51].

When developed and implemented in industry, practical impacts of biorefinery concept can be relevant at different levels: within the seaweed farming and industry, as employment increase in the sectors of the bioeconomy, triggering new investments, unlock incentive and favouring the integration of renewable energy within the overall energy systems [68]. 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 [55].

Analysing the concept under the bioeconomy principles looks toward the identification of prerequisites for better exploitation of locally available (and valuable) biorefinery feedstocks within bioeconomy overarching perspective. This is essential in the direction of reaching the set targets of low-carbon economy, higher sustainability and optimal bioresource use efficiency.

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 [216]. 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 [217]. The Baltic seaweed biorefinery concept is following these principles, and it has a significant role in strengthening the bioeconomy.

Food first

Food safety is set as a first principle, and it is focusing on food quality and availability. The seaweed biorefinery concept is matching the aim of this principle. Nutritional value and health benefits of seaweeds are already well known for years. Seaweeds can provide a human organism with necessary vitamins, minerals and antioxidants and is a valuable calorie source [261,262]. Moreover, seaweeds are not competing for land with plants, and land can still be used for agriculture or forestry. Grown in the marine environment, they do not require fresh water and additional nutrients, like terrestrial plants. It is predicted that in 2050, the global population will reach 9 billion people and with current food production it is possible to provide food only for half of them [263], so it is crucial to find additional food resources or increase food quality. High nutritional value of seaweeds matches with this demand for this additional food base.

Sustainable yields

Availability of sustainable seaweed feedstock currently is the main challenge, since seaweed cultivation in Latvia is non-existent, natural seaweeds are protected by law, and washed-out seaweed biomass is inconsistent in terms of availability and composition. Seaweed cultivation is the way to provide a sustainable yield of biomass in terms of availability. Seaweed cultivation is a relatively recent form of aquaculture. World production has exponentially increased during the last 50 years, and it tripled between 1997 and 2012, from 7 million tons to 24 million tons, and it is still increasing [264]. Seaweed yield sustainability is dependent on the region. Seaweed farming is rapidly increasing in some countries, while in others, it is slowly gaining acceptance [3]. There are still more than 150 countries with the coastal area which are not yet considering seaweed cultivation. Reasons are different: seasonality, lack of infrastructure and missing demand from society. These are the main causes that hamper the development of the area and slow down the seaweed cultivation around the globe and also in the Baltic region.

Cascading approach

The cascading approach is an essential detail of the seaweed biorefinery concept to ensure sustainable use of the biomass. Cascading approach promotes that product with the highest value is made first, and leftover biomass is used to produce the next product until leftover biomass cannot be used anymore and becomes a feedstock for bioenergy production. Furthermore obtained bioenergy can be used to provide the energy for biorefinery processes. This approach increases resource efficiency and adds an even higher value to used biomass, which is a part of the circular economy. Increased resource efficiency also is saving the raw material supply because biomass can be used repeatedly. With this principle usually, the problem is that leftover biomass has to be transported from one location to another to continue with the next step of production, but biorefinery concept solves this problem since the concept is meant to be implemented in one location.

Circularity

Since cascading approach does not address the waste problem by itself, the circularity principle has to be followed. The circular economy is built on three basic rules: (1) waste does not exist, as products are designed for a cycle of disassembly and reuse; (2) consumables should be returned to the biosphere without harm after a cascading sequence of uses, contributing to its restoration, while durables are designed to maximise their reuse or upgrade; and (3) renewable energy should be used to fuel the process. The same rules are used in the seaweed biorefinery concept, and the amount of waste is reduced to the minimum, and waste energy is used to supply energy to production processes [265].

Diversity

The seaweed biorefinery concept is made to be diverse, and it covers many sectors. Production systems have to be various and produce different outputs using different techniques. Production chains can be transformed to meet the market demand. Washed-out seaweed biomass deposits can be used to diversify the feedstock for bioenergy production on the last step of the production chain. Biorefinery concept can be adapted and be used for other types of feedstock, just in-depth analysis of feedstock availability, potential products and processing technologies are needed before. Diversity is a key to resilience, and bioeconomy requires to develop more innovations to create diversity rather than limit it.

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

This subchapter presents a SWOT analysis that was made to indicate the role biorefinery concept can play to support the development of sustainable bioeconomy. The perspective of the constant development of biorefineries leads to the use of new feedstocks, higher conversion efficiencies, new technologies and co-products. Opportunities will inevitably arise in all areas of the present economy. Research and development will feed in rural economic development, new industrial areas and an opening in existing and newly created markets. Developing technologies that are based on high yielding, low input feedstocks or wastes offer the hope to be even more sustainable. The biorefinery is a technology that is dependent upon a growing innovation, presenting opportunities to all sectors [57]. The building of a bioeconomy has the capacity to not only move the world through existing challenges on biobased resources scarcity but will be also beneficial for industries in terms of reducing environmental footprint. Specifically, in this section, the strengths, weaknesses, opportunities and threats of seaweed biorefinery concept are addressed, and the role 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. Applying SWOT analysis to the state-of-art Baltic seaweed biorefinery concept aims to recognize the strategies to use opportunities and acknowledge weaknesses, that with the implementation of additional research could be shifted to strengths. Opportunities and threats of the concept are considered in general, without a comprehensive approach. This SWOT analysis does not include pros and cons of the seaweed biorefinery concept in comparison to fossil-based refineries, such as water depletion, greenhouse gas emissions, etc.

Strengths and weaknesses of the biorefinery concept are summarized in Table 3.3.

Table 3.3.

Strengths and Weaknesses of the Baltic Seaweed Biorefinery Concept

Strengths	**Weaknesses**
• Adding value to the seaweed biomass • Development of novel biotechnologies • Environmentally friendly resource since it does not require drinking water and nutrients and it reduces eutrophication • Maximizing biomass conversion efficiency – minimizing raw material requirements • Production of biobased products (food, feed, materials, chemicals) and bioenergy (fuels, power and/or heat) feeding the full bioeconomy • Circular approach with no waste principle	• Involvement of stakeholders of different market sectors (agro, energy, chemical, ...) over full biomass value chain necessary • Most promising biorefinery processes/concepts not clear • Scientific and technological challenges in not mature technologies • Studying and concept development instead of real market implementation • Variability of quality and energy density of 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 the competing fossil-based refineries, etc., hampering developments of the biorefinery. Opportunities and threats of the Baltic seaweed biorefinery concept are summarised in Table 3.4.

Table 3.4.

Opportunities and Threats of the Baltic Seaweed Biorefinery Concept

Opportunities	Threats
• Strengthening of the economic position of numerous bioeconomy sectors (e.g. agriculture, fishery, chemical and energy) • Seaweed biorefinery can give significant input in sustainable bioeconomy • The popularization of sustainable use of seaweed for the production of energy is setting new goals for policy • An international agreement that resource should be used as efficiently as possible develops spreading of biorefinery concept in other sectors • International development of technological aspects of biorefinery concepts.	• Economic change and fluctuation of fossil fuel prices • Competition of other renewable energy technologies satisfying the market needs • Higher quality standards are applied to bioresource and bioenergy than to traditional products • Availability and quality of raw materials (e.g. climate change, policies, logistics) • Difficulties in finding investment capital for pilot and demo refineries, and adjusting existing refineries • Changing governmental policies • No market demand for products • Increase of environmental pollution can limit the range of products in the biorefinery concept

Application of the seaweed biorefinery concept can strengthen the economic position of various bioeconomy sectors, improve regional development and sustainability of the bioeconomy. In the international level, this approach would support the technological development of the biorefinery and increase the application of bioeconomy principles in resource processing. Where processes for unique materials, chemicals or biofuels have been developed, markets have not yet been established. Although the most likely approach to integrated biorefineries is to add further feed streams and product streams onto an existing biorefinery, rather than building an integrated biorefinery from scratch, large investments are required.

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 [5]. Thereby the approach could allow accessing to different markets. In particular, each possible use of seaweed biomass has different timescale to commercialization, if the proposed use is not at its mature phase. Most commonly, the low-value products have the shortest timescale to commercialization [266].

3.5. Seaweed Cultivation Laboratory

Seaweed cultivation laboratory stand is set up as a part of the Biosystems Laboratory of the Riga Technical University Institute of Energy Systems and Environment (IESE). Laboratory setting allows to carry out cultivation processes in artificially lit aquaria/ water tanks using natural or artificial seawater as a growth medium. In addition, the effect of light and nutrient type and concentrations were tested experimentally [175].

Preparation of the laboratory

Necessary equipment and instruments to provide essential environmental parameters – light, temperature, growth medium, bubbling, that has crucial importance for successful cultivation. Different needs for separate seaweed species make the preparation process even harder. Available literature and cultivation manuals were analysed individually for each species. Previous experience has to be taken into account to determine the most favourable growth conditions and their interactions for specific species. Inadequate culture conditions limit seaweed growth and can cause their death [174].

Biological pollution may cause problems in the seaweed cultivation process. Particular attention was paid on sterile instruments and growth medium because the cultivation environment can also be the right place for the development of microbiological organisms, that can consume nutrients and overtake seaweeds. Microorganisms can also create shadowing and release harmful substances, in this way limiting access to the light or toxic substances causes seaweed death [174].

Isolation and purification of seaweed cultures, together with long-term seaweed culture maintenance, are also carried out in the laboratory. Seaweed cultivation in lab conditions allows to provide desirable growth conditions and manipulate with seaweed physiology in this way, selecting the best growing environment to achieve a specific objective. Growing of *Ulva intestinalis* in pilot-scale appears to achieve comparatively high biomass growth rates in *in vitro* culturing, relative to other seaweed – showing in that sense a high potential for such culturing [267]. Different kind of variables can be controlled to optimize biomass production. The seawater must be of optimal temperature and salinity; water movement must be sufficient to provide seaweeds with nutrients and carbon dioxide; enough light must be available to allow photosynthesis, and UV radiation should not be at damaging levels [268]. For optimal growth, all conditions must be within a specific, usually narrow, range. Tolerance to these conditions varies between species with some able to grow well under conditions where another is excluded. An understanding of these tolerances is therefore essential to allow optimal site selection and so maximise the cultivation potential. Suboptimal conditions such as deficient nutrients or very high light can lead to physiological stress, reductions in growth rate, increase in tissue degradation or even death. Seaweeds are considered to have very high phenotypic plasticity allowing them to adapt to a wide range of fixed and changeable environmental conditions [269–271].

Several considerations should be included when compare field studies with laboratory studies. Firstly, environmental conditions in studies carried out in the laboratory are much more controlled and, in some way, more limited. High nutrient conditions in laboratory studies might not have the same response as in natural conditions. Nutrients can compensate for lack of water movement and exchange, but it is unlikely that this substitution will give the same results. In the laboratory, species are isolated and are not subject to competition and grazing. Laboratory cultures are uniform, but in nature, there are often unpredictable environmental fluctuations. In culture flasks, some of the cells can shade another, and without enough mixing, they can create nutrient-depleted zones around them, creating a mosaic of nutrient concentrations. In nature, seaweed morphology may differ even between single specie demonstrating different reactions on environmental conditions [75]. For some species, environmental parameters differ from one seaweed growth to another, and parameters like specific growth rate versus nutrient supply vary among populations [272].

In eastern Baltic conditions seasonality might be an issue – the average annual temperature is 5.9 °C. Starting from late autumn (November) until early spring (March) there may be difficulties to prepare cultivation material and carry out any cultivation because the sea is covered by ice and seaweeds are prepared for winter. During this period average temperature fluctuates around -7.5 °C, but it can drop to even - 30 °C. These temperatures are too low for seaweed to grow, so they are preserving and stop their metabolic processes. Lot of seaweeds decay during the winter season to start growing again on the spring [273].

Culture collection and preparation

Culture collection is a significant step in seaweed cultivation. It is necessary to gather biological material from its natural environment and transfer it to the laboratory without negative impact on seaweed and their cells. Collection principles may differ for different types of seaweed according to the climate where seaweeds are living because their ability to adapt to stress conditions can be different. Seaweed can be collected both from the coast and from the open sea. Red seaweed species like *Palmaria palmata* and *Gracilaria caudata* can be collected when washed on the coast because they do not lose their ability to reproduce when left in dry conditions for a more extended time [274,275]. These species, as well as many coastal species, have adapted to environmental pressure, like rapid temperature or water level change and drying. On the opposite, seaweed species which inhabit in greater depths (under 5 – 10 m), are more tolerant to changes of environment like an increase or decrease of the temperature or impact of direct sunlight. For this reason, sampling and transporting of these species are more complicated. Sensitive deep-water seaweed species right after collecting should be placed in containers filled with seawater in the same temperature. Containers must be sealed to avoid the negative impact of the light regime, temperature and air exposure [276]. After sampling, they should be moved to relevant size plastic bags, ice boxes, bottles or containers filled with the water from the site [197,276]. It is suggested to keep water 5 – 10 °C colder than it is in nature, respectively it should be 5 – 10 °C degrees for cold-water species like the Baltic Sea and 20 – 25 °C degrees for seaweeds collected in warmer areas, to maintain liveability and increase survival [276].

To sustain the viability of seaweeds and in order for planned experiments to be successful, right after delivering to the laboratory, it is necessary to transfer material to the appropriate environment – filtered and sterilized seawater. If the seaweed growth medium is not prepared yet, seaweed can be kept in the same water that was used for transport, but not for a long time, because it is not sterile and can be a source of unfavourable microorganisms.

Seaweed cultivation medium

The seaweed cultivation medium is a solution based on water, and it is enriched with vitamins and minerals, essential for the growth of seaweeds. Sterilization of the cultivation medium is an essential precondition to prevent the reproduction of various undesirable organisms that could interfere with the growth of seaweeds. Sterilization is a process used to eliminate microorganisms, resulting in sterile conditions [174]. That is a particularly important step in the cultivation of different organisms, as it is usually necessary to observe only one particular species, not several organisms together, but innumerable microorganisms are present in the environment and on different objects. Environmental sterilization and the use of sterile equipment and accessories significantly reduce the presence of unwanted organisms, which leads to more accurate and more adequate experimental results, but also many conditions for handling these sterile materials must be taken into account in order not to contaminate the micro-organisms from the hands and objects repeatedly. As soon as the sterilized material encounters the air, it quickly overlaps with dust, spores and microorganisms, but their volume is much smaller.

There are several sterilization methods, and they are divided into four major categories: thermal sterilization, sterilization of electromagnetic waves, sterilization using filtration and chemical sterilization. Thermal sterilization is the most used method and usually requires high temperatures above 100 °C, so sterilization requires materials that can withstand high temperatures such as glass, metal and aluminium foil. The liquids are sterilized by the filtration method if they contain the components required for the experiment, which are not resistant to high temperatures and which die during thermal sterilization. Chemical sterilization involves the use of various chemical compounds such as ethanol and formaldehyde, but after sterilization, these compounds tend to stay on the surface of the sterilized object or in the fluid and thus damage the survival of seaweeds. For this reason, chemical sterilization in laboratories is no longer used [174,277].

Seaweed cultivation laboratory stand was set up based on knowledge on seaweed biology, physiology and experiences gained from other research. To examine if the laboratory stand is appropriate to cultivate seaweeds, experiments to test growing conditions were carried out. Structure of this created seaweed cultivation laboratory stand allows to carry out experiments not only with seaweeds, but it also can be adjusted for microalgae cultivation.

3.6. Seaweed Growing Conditions

Tests on growing conditions were carried out with seaweeds collected in different time periods, and for every test laboratory condition were different. Therefore, the results of each test are presented separately and compared in summary at the end of the section.

Nutrient Test I

To carry out the Nutrient test I, the nutrient stock solution was prepared using NPK fertilizer with microelements in chelation structure “Vito Universal” [278]. It contains 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. Concentrations for the stock solution added to the seaweed growth medium in this experiment was 2, 5, 10, 30 mL L^{-1}. The main goal of the experiment was to determine optimal nutrient concentration for seaweed growing. Even though the suggested time to carry out the test like this is at least 28 days, the test was stopped after 14 days of experimenting, because growing material started to decay. Seaweed samples were measured using millimetre paper and length and width of the seaweed specimen were documented.

Measurements after the first week of the experiment show growth in all concentrations, except the highest concentration of 30 mL L^{-1}. This concentration caused seaweed deterioration already after the first week of the experiments. Lower concentrations have shown a better effect on the seaweed growth. Several seaweed pieces in concentration 2 mL L^-1 increased their size from 66 mm to 116mm that is 48 mm a week or 10.58 % a day. The second week of the experiment was not showing the same trend anymore. It is considered to be caused by the bubbling system that caused unpredictable evaporation and increased salinity in batches. Hypersalinity in the growth environment caused irretrievable damage for seaweed material, and further seaweed growth was not possible. It could be explained by plasmolysis of seaweed cells due to the high concentration of growth medium. It is possible that for seaweed species, that are naturally growing in more saline water, water reduction would not cause as much stress, but for The Baltic seaweed *U. intestinalis,* it was lethal [279]. Seaweed sample on the first day and the seventh day of the experiment can be seen in Fig. 3.5.

Fig. 3.5. Seaweed sample on day 1 (left) and day 7 (right), 5 mL L^{-}1 concentration.

The seaweed growth rate was calculated using the data from the first seven days of the experiment. In applied nutrient stock solution concentrations, *Ulva intestinalis* showed the specific growth pattern (Fig. 3.6.).

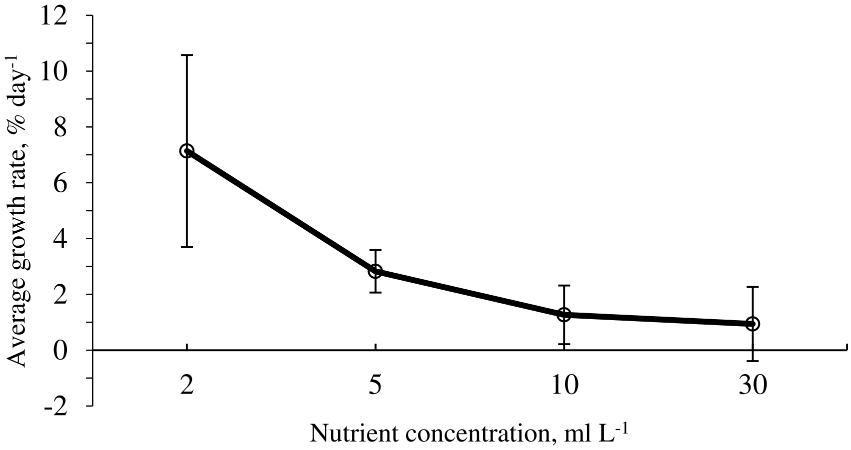

Fig. 3.6. Growth of *U. intestinalis* under different nutrient concentrations (7 days).

Fig. 3.6. shows the effect of different nutrient concentrations on seaweed growth. Data shows that nutrient concentrations significantly affected the growth rate of *U. intestinalis*. Increasing concentration above 2 ml L^{-1} significantly slows down the growth rate. The maximum growth rate was achieved with 2 ml L^{-1} high nutrient concentration, and with such conditions growth rate reached an average 7.13 ± 3.44 % per day. Increasing concentration to 10 ml L^{-1}, *U. intestinalis* growth rate decreased and showed only 1.27 ± 1.05 % growth per day, which is less than the average growth rate for this species. Previous research with *U. intestinalis* showed that average growth rate for this type of alga is 0.15 – 0.25 cm per day which is 3.39 – 5.32 % per day [280]. These results were obtained with naturally occurring alga in the Black sea, not with cultivated alga in the laboratory, and that could be the cause of better growth [281,282].

The experimental laboratory provided sufficient conditions for seaweed cultivation. Main issues for seaweed cultivation lab would be the adjustment of the bubbling system to provide smooth bubbling. The current situation was that changing of water level, changed the strength of bubbling in many batches and evaporation happened at a high rate. This situation could be solved using valves, which regulate airflow before every batch. Also, the temperature would be an essential factor that should be provided. Optimal water temperature for seaweed cultivation would be 10 °C – 15 °C [282]. This experiment was carried out in room temperature, which in this case was 20 °C. The necessary temperature should be provided using an incubator or cooling system.

Nutrient test II

In Nutrient test II, three different concentrations of nutrient were tested, in addition to a sample with no nutrients. The nutrient stock solution was used the same as in Nutrient test I. The experiment was screened for 21 days, and the area of the seaweed was measured manually using a millimetre paper. The concentrations of nutrient were equal to 0 ml L^{-1} (A), 2 ml L^{-1} (B), 10 ml L^{-1} (C) and 30 ml L^{-1} (D). While the average data showed the seaweed growth peak of 3.78 % on the 3rd day, comparing to the beginning of the experiment, for 2 mL/L fertilizer samples, the peaks were even smaller for A and C samples (Fig. 3.7.). All samples showed the growth, except samples in 30 ml L^{-1} concentration. This concentration caused perishing of the seaweed fragments. Therefore concentration 30 ml L^{-1} can be described as an inhibitive concentration for the seaweed growth. No growth can also be partially attributed to the drowsiness (and consequently lack of light) which occurred due to the accumulation of salt and nutrients and crystal formation, which was over the time the case for all other samples, where fertilizers were used.

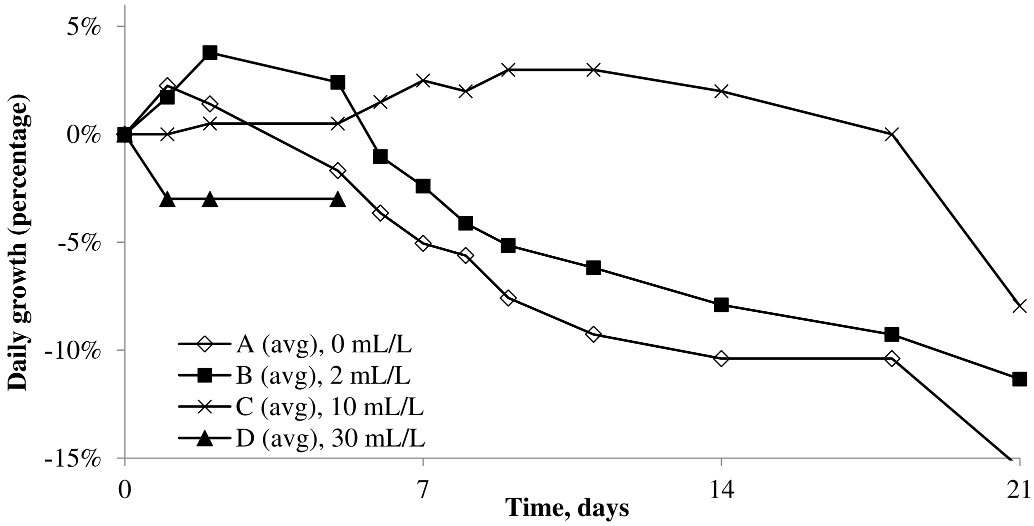

Fig. 3.7. Daily change of *U. intestinalis* growth in different nutrient concentrations – average values

While most of the screened samples had an increase in biomass, in the beginning, days six to eight were crucial for samples in concentrations 0 and 2 ml L^{-1}, as these were the days when the biomass decrease is being observed. This could be attributed to generally undesirable conditions for *U. intestinalis* growth, as the water temperature was 21 °C and the salinity level fluctuating significantly during the night, as a result of evaporation. The optimal temperature and salinity values for *Ulva* are, however, documented to be 15 °C and 24 ‰, respectively, while 20 °C is deemed critical [283].

Nutrient test III and Nutrient test IV

Previously done experiments identified weaknesses in water bubbling and temperature of the environment. In previous experiments, seaweeds showed a loss of viability after a week in experimental conditions. *U. intestinalis* growth material was acclimatised in natural seawater prior the test, to exclude change of the surrounding environment as a cause for seaweed death. Only healthy green specimens were used in the experiment. The growth response on *Ulva intestinalis* was tested using two types of nutrients at nutrient concentrations: 0 mL·L^{-1}, 2.5 mL·L^{-1}, 5 mL·L^{-1}, 10 mL·L^{-1}, 15 mL·L^{-1} and 20 mL·L^{-1}.

Each of these nutrient sources showed a different impact on growth rate (Fig. 3.8.).

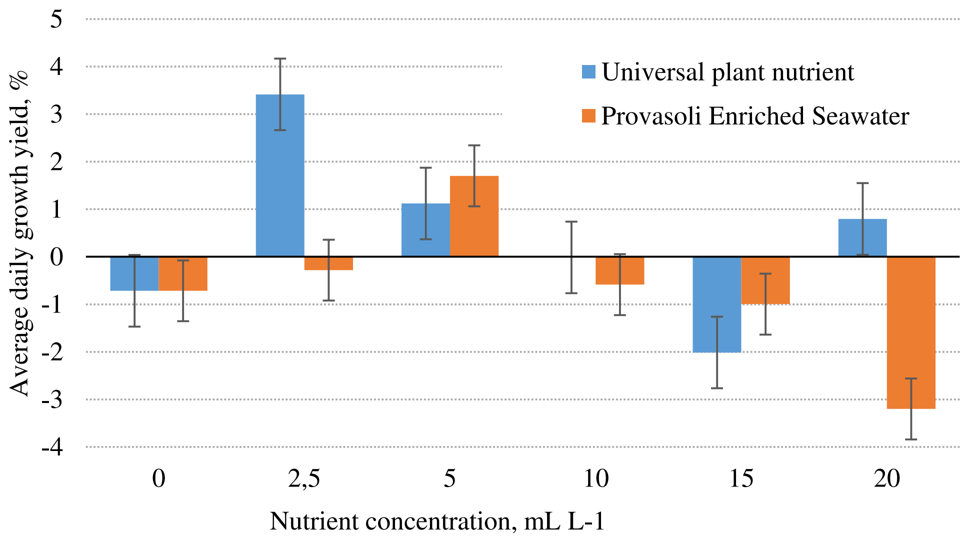

Fig. 3.8. *Ulva intestinalis* average daily growth yield in two types of nutrients in six different concentrations.

Using universal plant fertilizer in concentrations that are relevant to the Provasoli growth medium gives higher growth results. Best daily average growth yield was achieved on the concentration of 2.5 mL·L^{-1} where average daily growth was more than 3 %. An increase of the nutrient concentration to 5 mL·L^{-1} reduced seaweed growth to 1 % per day, but concentrations 10 and 15 mL L^{-1} not only stopped seaweed growing, but also caused degradation of *U. intestinalis* tissue. That could be caused by proliferation by microscopic algae which consumed nutrients necessary for the growth of *Ulva* (Fig. 3.9.).

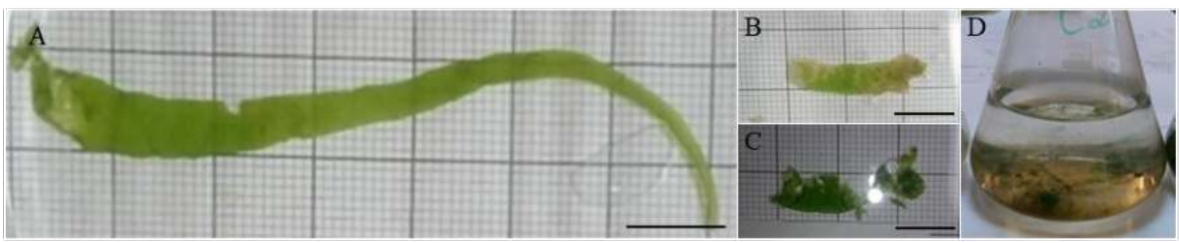

Fig. 3.9. *U. intestinalis* development in 21 days in plant fertilizer concentration 15 mL L^{-1}
A 0 days B 7 days C 14 days D 21 days

It was also observed that in these concentrations seaweed material started to fragment.

The highest concentration used in this experiment was 20 mL·L^{-1} and it did have a positive impact on seaweed growth, and fragmentation did not occur comparing to lower concentrations (Fig. 3.10.). It was also noticed that seaweed thalli were dark green and healthy.

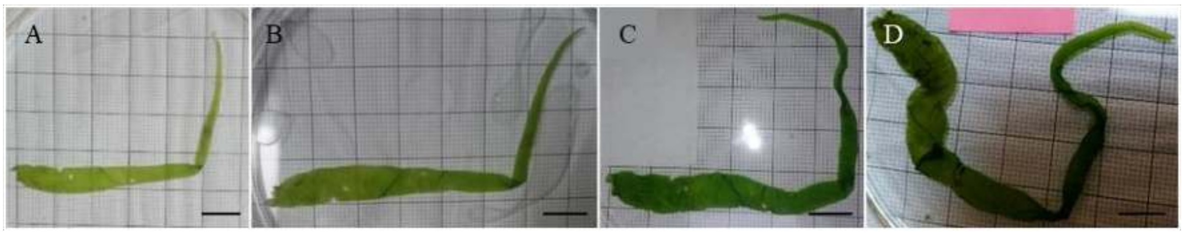

Fig. 3.10. *Ulva intestinalis* development in 21 days in plant fertilizer concentration 20 mL·L^{-1}
A. 0 days B. 7 days C. 14 days D. 21 day.

The concentration 20 mL·L^{-1} of plant fertilizer solution, provided better growth conditions and promoted the preservation of seaweed material. It also has to be mentioned that there was no microalgal pollution observed.

Results with using Provasoli Enriched seawater stock solution show that the highest growth was achieved in concentration 5 mL·L^{-1} and other concentrations were not giving positive results. These results show that *Ulva* growing in brackish Baltic Sea environment prefer lower medium concentration than suggested in the literature [174].

For both nutrient sources, lower concentrations showed better growth results. Highest growth rate can be achieved when universal plant fertilizer was used as a nutrient source. Highest biomass yield was achieved with concentration with 2.5 mL plant fertilizer stock solution to a litre filtered seawater. More than 3.4% daily growth was registered in this concentration. This trend is in line with previous findings in which plant fertiliser was used as a nutrient source. Concentration of 2 mL L^{-1} allowed to reach concentration 7 % per day [211]. Special growth media often contain a lot of metals, vitamins and stabilizers, that should provide metabolic processes and support seaweeds with necessary diet. Results achieved in this research shows that it is possible to grow seaweeds using plant fertiliser, and it allows to achieve a higher growth rate.

From a morphological point of view, there were no significant differences observed between types of nutrients. Between concentrations proliferation of microscopic algae was observed, and some unidentified thalli forming seaweeds was noticed. Further microscopic analysis should be made to get more precise results.

Lighting test

Results of the experiment with different lighting colours can be seen in Fig. 3.11.

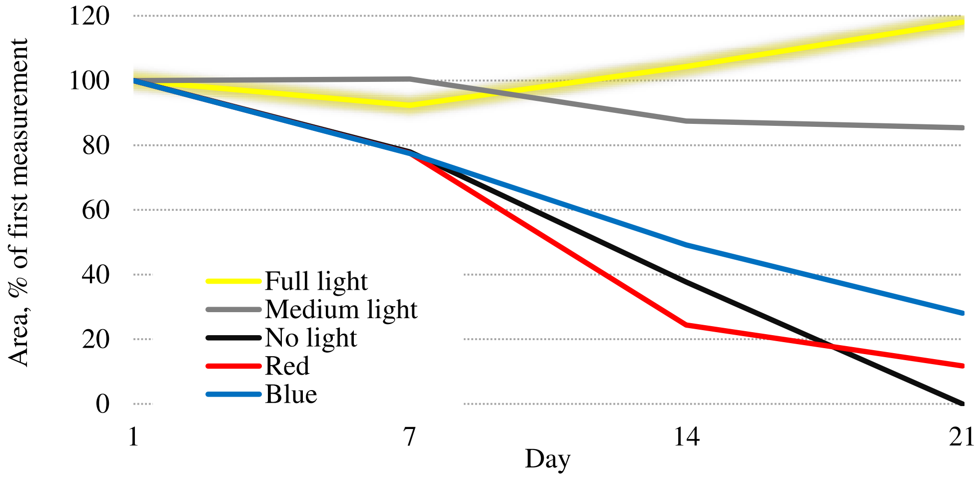

Fig. 3.11. Effects of different lighting conditions on *Ulva intestinalis* size.

The most significant growth was reached in full light conditions without any filters. Even though the degradation of seaweed material was observed after the first week of the experiment, it might be caused by the stress of changing growth environment. During the further experiment, seaweed growing in full light conditions was observed, and total growth during the experiment was achieved 18 % (Fig. 3.12.).

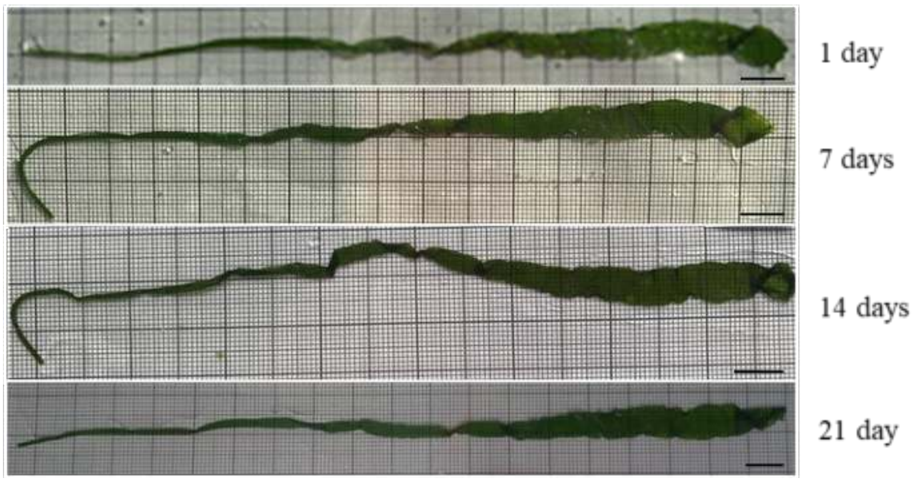

Fig. 3.12. Growing of *U. intestinalis* in full light conditions in 21 day.

Fig. 3.13. Growing of *U. intestinalis* in partial light conditions in 21 day.

Reduced light conditions did not have any significant changes in size on the first week, but on next week size of the seaweed reduced and at the end of the experiment it was decreased to 85% of the initial size. Testing material was fresh and green, giving us insight that reduced light conditions did not affect viability but only growing (Fig. 3.13.).

When coloured lights were used, the reduction of initial seaweed material was observed. Red light initiated 12 % and blue light 28 % reduction in comparison to the initial sample size. These shades caused poor light conditions for seaweed life and growth (Fig. 3.14.). The specific filters did not provide enough light to continue photosynthetic processes.

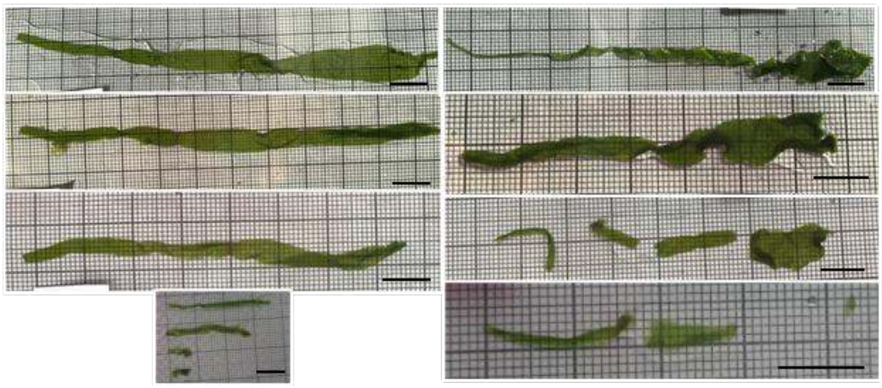

Fig. 3.14. Growing of *U. intestinalis* in red and blue light conditions in 21 day.

Seaweeds kept in the dark showed no growth, and after 14 days 38 % reduction was observed, and in 21 days all *U. intestinalis* material was pale coloured and decayed with no signs of life (Fig. 3.15.).

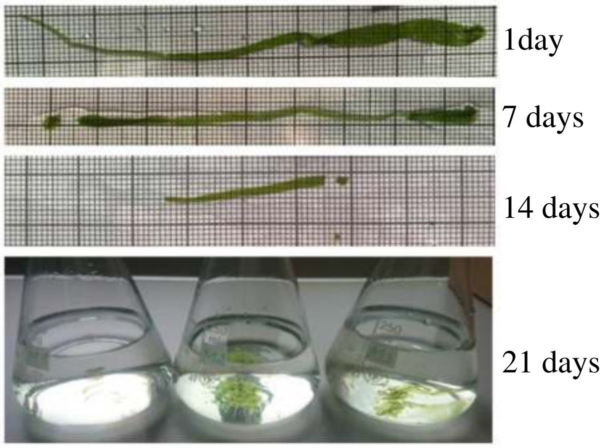

Fig. 3.15. *U. intestinalis* in no-light conditions in 21 day.

Photosynthesis is a vital process to support metabolism in seaweeds. Lack of light inhibits photosynthesis and cause seaweed death, and decay. Blue and red coloured lights caused seaweed material fragmentation, but the material still retained its colouring and firm condition. It could be that these colours stopped some photosynthetic processes but did not cause the complete death of seaweeds. These colours should be researched more to see the full impact on photosynthesis of seaweed. Literature suggests that red light (540nm-630nm) activates the photosynthesis even though blue light is absorbed at the highest.

Application of laboratory tests

Environmental conditions for seaweed cultivation were provided in seaweed cultivation laboratory stand. 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 [75]. Enriched Natural Seawater (Provasoli) Media is widely used as a nutrient source for seaweed cultivation [221]. The suggested amount of stock solution added to filtered natural seawater is 20 ml L^{-1}. 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^{-1} 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 [183]. 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 [199]. 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 [198,201]. 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 [284].

Another issue for seaweed growing in laboratory conditions was recognized. Biological pollution in the form of microscopic algae was abundant in Nutrient test III, IV and Lighting test (Fig. 3.16.).

Fig. 3.16. Microalgae pollution in Nutrient test IV.

Even though all preparation and sterilization processes were done as described, it was not possible to avoid microalgae abundance. Biological hazards can likely be brought in with test organism. Even though seaweed specimens were washed before using in the experiment, seaweed fragments are fragile. Regards the cleaning of the test organism, it also has to be noted that microorganisms like bacteria play an essential role in cell differentiation in seaweeds [182].

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 stand 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*. 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.

REFERENCES

[1] Wei N., Quarterman J., Jin Y.-S. S. Marine macroalgae: An untapped resource for producing fuels and chemicals. *Trends in Biotechnology* 2013:**31**(2):70–77. doi:10.1016/j.tibtech.2012.10.009.

[2] Buschmann A. H., Camus C., Infante J., et al. Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology* 2017:**52**(4):391–406. doi:10.1080/09670262.2017.1365175.

[3] Tiwari B. K., Troy D. J. *Seaweed Sustainability: Food and Non-Food Applications*. Seaweed Sustainability: Food and Non-Food Applications. Elsevier; 2015.

[4] Harmsen P., Blaauw R., Haveren J. Van. Seaweed Biorefinery Wageningen UR Food & Biobased Research. 2013:25–26.

[5] Bixler H. J., Porse H. A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology* 2011:**23**(3):321–335. doi:10.1007/s10811-010-9529-3.

[6] Milledge J. J., Smith B., Dyer P. W., Harvey P. Macroalgae-derived biofuel: A review of methods of energy extraction from seaweed biomass. *Energies* 2014:**7**(11):7194–7222. doi:10.3390/en7117194.

[7] Adenle A. A., Haslam G. E., Lee L. Global assessment of research and development for algae biofuel production and its potential role for sustainable development in developing countries. *Energy Policy* 2013:**61**:182–195. doi:10.1016/j.enpol.2013.05.088.

[8] Kim J. K., Yarish C., Hwang E. K., Park M., Kim Y. Seaweed aquaculture: Cultivation technologies, challenges and its ecosystem services. *Algae* 2017; **32**(1):1–13.

[9] Kersen P., Paalme T., Pajusalu L., Martin G. Biotechnological applications of the red alga Furcellaria lumbricalis and its cultivation potential in the Baltic Sea. *Botanica Marina* 2017:**60**(2):207–218. doi:10.1515/bot-2016-0062.

[10] European Commission. *The EU Blue Economy Report. 2020*. The EU Blue Economy Report. 2020.; 2020.

[11] Kerrison P. D., Stanley M. S., Edwards M. D., Black K. D., Hughes A. D. The cultivation of European kelp for bioenergy: Site and species selection. *Biomass and Bioenergy* 2015:**80**:229–242. doi:10.1016/j.biombioe.2015.04.035.

[12] Boonstra A. The Macroalgae-Based Biorefinery. 2014:

[13] Ferdouse F., Holdt S. L., Smith R., Murúa P., Yang Z. The global status of seaweed production, trade and utilization. *FAO Globefish Research Programme* 2018:**124**:120.

[14] Torres M. D., Kraan S., Domínguez H. *Seaweed biorefinery*. Seaweed biorefinery 18.; 2019.

[15] Van Hal J. W., Huijgen W. J. J. J. J., López-Contreras A. M. M. Opportunities and challenges for seaweed in the biobased economy. *Trends in Biotechnology* 2014:**32**(5):231–233. doi:10.1016/j.tibtech.2014.02.007.

[16] Álvarez-Viñas M., Flórez-Fernández N., Torres M. D., Domínguez H. Successful approaches for a red seaweed biorefinery. *Marine Drugs* 2019:**17**(11). doi:10.3390/md17110620.

[17] van Hal J., Huijgen W., Bjerre A.-B., López Contreras A., Stanley M., Hreggvidsson G. Carbohydrate analysis of seaweed in the biorefinery to chemicals and fuel context. *22nd International Seaweed Symposium 2016 : Academia meets Industry, Copenhagen, Denmark.* 2016:(October):54.

[18] Beacham T. A., Cole I. S., DeDross L. S., et al. Analysis of seaweeds from South West England as a biorefinery feedstock. *Applied Sciences (Switzerland)* 2019:**9**(20). doi:10.3390/app9204456.

[19] Ramirez V., Carlos H., Ramirez V. Biogas production from seaweed biomass : a biorefinery approach. 2016:197.

[20] Naseri A., Holdt S. L., Jacobsen C. Biochemical and Nutritional Composition of Industrial Red Seaweed Used in Carrageenan Production. *Journal of Aquatic Food Product Technology* 2019:**28**(9):967–973. doi:10.1080/10498850.2019.1664693.

[21] Seghetta M., Marchi M., Thomsen M., Bjerre A. B., Bastianoni S. Modelling biogenic carbon flow in a macroalgal biorefinery system. *Algal Research* 2016:**18**. doi:10.1016/j.algal.2016.05.030.

[22] Torn K., Krause-Jensen D., Martin G. Present and past depth distribution of bladderwrack (Fucus vesiculosus) in the Baltic Sea. *Aquatic Botany* 2006:**84**(1):53–62. doi:10.1016/j.aquabot.2005.07.011.

[23] Bird N. L., Chen L. C.-M. C.-M., McLachlan J., McLachla J. Effects of Temperature, Light and Salinity on Growth in Culture of Chondrus crispus. Furcellaria lumbricalis, Gracilaria tikvahiae (Gigartinales, Rhodophyta), and Fucus serratus (Fucales, Phaeophyta). *Botanica Marina* 1978:**XXII**:521–527.

[24] Bäck S., Collins J. C., Russell G. Effects of salinity on growth of Baltic and Atlantic Fucus vesiculosus. *British Phycological Journal* 1992:**27**(1):39–47. doi:10.1080/00071619200650061.

[25] Suursaar Ü., Torn K., Martin G., Herkül K., Kullas T. Formation and species composition of stormcast beach wrack in the Gulf of Riga, Baltic Sea. *Oceanologia* 2014:**56**(4):673–695. doi:10.5697/oc.56-

4.673.
[26] Znotiņa V. Jūras biotopi. 2014.
[27] Burkholder J. M., Tomasko D. A., Touchette B. W. Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology* 2007:**350**(1–2):46–72. doi:10.1016/j.jembe.2007.06.024.
[28] Filipkowska A., Lubecki L., Szymczak-Zyła M., Łotocka M., Kowalewska G. Factors affecting the occurrence of algae on the Sopot beach (Baltic Sea). *Oceanologia* 2009:**51**(2):233–262.
[29] EU. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Official Journal of the European Union* 2006:**L 064**(1882):37–51.
[30] Strunga A. Mikro un makro aļģu audzēšanas un pārstrādes iespējas Baltijas jūras reģionā. In: *SUBMARINER*. Liepāja; 2013:7.
[31] Dumay J., Morançais M., Nguyen H. P. T., Fleurence J. Extraction and purification of r-phycoerythrin from marine red algae. *Methods in Molecular Biology* 2015:**1308**:109–17. doi:10.1007/978-1-4939-2684-8_5.
[32] Kersen P. Red seaweeds Furcellaria lumbricalis and Coccotylus truncatus: community structure, dynamics and growth in the Northern Baltic sea. 2013; (January).
[33] Riekstiņš N. *Latvijas zivsaimniecības gadagrāmata2014*. Latvijas zivsaimniecības gadagrāmata2014. The Latvian Rural Advisory and Training Centre; 2014.
[34] Biedrība "Baltijas krasti." Jūras aļģu sanesumu izvērtēšanas un apsaimniekošanas plāns Latvijas piekrastē. 2018:
[35] Alström-Rapaport C., Leskinen E., Pamilo P. Seasonal variation in the mode of reproduction of Ulva intestinalis in a brackish water environment. *Aquatic Botany* 2010:**93**(4):244–249. doi:10.1016/j.aquabot.2010.08.003.
[36] Aguilera-Morales M., Casas-Valdez M., Carrillo-Domínguez S., González-Acosta B., Pérez-Gil F. Chemical composition and microbiological assays of marine algae Enteromorpha spp. as a potential food source. *Journal of Food Composition and Analysis* 2005:**18**(1):79–88. doi:10.1016/j.jfca.2003.12.012.
[37] Lago-Lestón A., Mota C., Kautsky L., Pearson G. A. Functional divergence in heat shock response following rapid speciation of Fucus spp. in the Baltic Sea. *Marine Biology* 2010:**157**(3):683–688.
[38] Piazzi L., Balata D., Cinelli F. Species composition and morphological groups of macroalgal assemblages around Gorgona Island (north-western Mediterranean Sea). *Cryptogamie Algologie* 2004:**25**(1):19–38.
[39] Wikström S. A., Kautsky L. Structure and diversity of invertebrate communities in the presence and absence of canopy-forming Fucus vesiculosus in the Baltic Sea. *Estuarine, Coastal and Shelf Science* 2007:**72**(1–2):168–176. doi:10.1016/j.ecss.2006.10.009.
[40] Kautsky L., Svensson S. Life in the Baltic Sea. *Environmental Science: Understanding, Protecting and Managing the Environment in the Baltic Sea Region* 2003:148–181.
[41] Piterāns A., Vimba E. *Zemāko augu sistemātikas praktikums*. Zemāko augu sistemātikas praktikums. Rīga, ZVAIGZNE; 1987.
[42] Serrão E. a. E. A. E. a., Brawley S. H. S. H., Hedman J., et al. Reproductive success of Fucus vesiculosus (Phaeophyceae) in the Baltic Sea. *Journal of Phycology* 1999:**35**(2):254–269. doi:10.1046/j.1529-8817.1999.3520254.x.
[43] Steen H., Rueness J. Comparison of survival and growth in germlings of six fucoid species (Fucales, Phaeophyceae) at two different temperature and nutrient levels. *Sarsia* 2004:**89**(3):175–183. doi:10.1080/00364820410005818.
[44] Bučas M., Daunys D., Olenin S. Overgrowth patterns of the red algae Furcellaria lumbricalis at an exposed Baltic Sea coast: The results of a remote underwater video data analysis. *Estuarine, Coastal and Shelf Science* 2007:**75**(3):308–316. doi:10.1016/J.ECSS.2007.04.038.
[45] Guiry M. J. AlgaeBase. World-wide electronic publication 2014.
[46] Pludmalē traucē izskalotās aļģes | liepajniekiem.lv. https://www.liepajniekiem.lv/zinas/sabiedriba/pludmale-trauce-izskalotas-alges-205881#foto-322057. Accessed December 11, 2018.
[47] Malm T., Isæus M. Distribution of macroalgal communities in the central Baltic Sea. 2005:(August):257–266.
[48] Pajusalu L., Albert G., Fachon E., et al. Ocean acidification may threaten a unique seaweed community and associated industry in the Baltic Sea. *Journal of Applied Phycology* 2019:(Ipcc 2014). doi:10.1007/s10811-019-01935-1.
[49] Kersen P., Paalme T., Treier R. Variation in epibiotic load on the macroalgae Furcellaria lumbricalis and Fucus vesiculosus in the Baltic Sea. 2016:(November 2013).
[50] Bechtold T., Mussak R. *Biorefinery Co-Products*. Biorefinery Co-Products.; 2012.
[51] Laurens L. M. L., McMillan J. D., Baxter D., et al. *State of Technology Review – Algae Bioenergy*. State

of Technology Review – Algae Bioenergy.; 2017.
[52] Stuart P. R. *Integrated Biorefineries*. Integrated Biorefineries.; 2012.
[53] IEA. IEA bioenergy Task 42 on biorefineries: co-production of fuels, chemicals, power and materials from biomass. *Minutes of the third Task meeting* 2007.
[54] Ree R. van, Zeeland A. van. IEA Bioenergy: Task42 Biorefining. 2014:1–66.http://www.ieabioenergy.com/wp-content/uploads/2014/09/IEA-Bioenergy-Task42-Biorefining-Brochure-SEP2014_LR.pdfAccessed.
[55] Xuan T. D., Sakanishi K., Nakagoshi N., et al. Biorefinery : Concepts , Current Status , and Development Trends. 2012:(January 2015).
[56] Hou X., From N., Angelidaki I., Huijgen W. J. J., Bjerre A. B. Butanol fermentation of the brown seaweed Laminaria digitata by Clostridium beijerinckii DSM-6422. *Bioresource Technology* 2017:**238**(May):16–21. doi:10.1016/j.biortech.2017.04.035.
[57] Jung K. A., Lim S.-R. R., Kim Y., Park J. M. Potentials of macroalgae as feedstocks for biorefinery. *Bioresource technology* 2013:**135**:182–90. doi:10.1016/j.biortech.2012.10.025.
[58] FAO (Food and Agriculture Organization of the United Nations). Fishery and Aquaculture Statistics. ftp://ftp.fao.org/FI/CDrom/ CD_yearbook_2010/index.htm 2012.
[59] NetAlgae. Seaweed Industry in Europe. 2012:11.www.netalgae.euAccessed.
[60] Bruhn A., Tørring D., Thomsen M., et al. Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of Saccharina latissima. *Aquaculture Environment Interactions* 2016:**8**:619–636. doi:10.3354/aei00200.
[61] Rajak R. C., Jacob S., Kim B. S. A holistic zero waste biorefinery approach for macroalgal biomass utilization: A review. *Science of the Total Environment* 2020:**716**:137067. doi:10.1016/j.scitotenv.2020.137067.
[62] Julio R., Albet J., Vialle C., Vaca-Garcia C., Sablayrolles C. Sustainable design of biorefinery processes: existing practices and new methodology. *Biofuels, Bioproducts and Biorefining* 2017:**11**(2):373–395. doi:10.1002/bbb.1749.
[63] Groenendijk F., Bikker P., Blaauw R., et al. North-Sea-Weed-Chain: Sustainable seaweed from the North Sea; an exploration of the value chain. 2016:(July).
[64] Andersson V., Broberg S., Hackl R. *Integrated Algae Cultivation for Biofuels Production in Industrial Clusters*. Integrated Algae Cultivation for Biofuels Production in Industrial Clusters.; 2011.
[65] Aristizábal-Marulanda V., Cardona Alzate C. A. Methods for designing and assessing biorefineries: Review. *Biofuels, Bioproducts and Biorefining* 2019:**13**(3):789–808. doi:10.1002/bbb.1961.
[66] Lakshmi D. S., Sankaranarayanan S., Gajaria T. K., et al. A short review on the valorization of green seaweeds and ulvan: Feedstock for chemicals and biomaterials. *Biomolecules* 2020:**10**(7):1–20. doi:10.3390/biom10070991.
[67] Sanderson J. C., Dring M. J., Davidson K., Kelly M. S. Culture, yield and bioremediation potential of Palmaria palmata (Linnaeus) Weber & Mohr and Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders adjacent to fish farm cages in northwest Scotland. *Aquaculture* 2012:**354–355**:128–135. doi:10.1016/J.AQUACULTURE.2012.03.019.
[68] Blumberga D., Muizniece I., Blumberga A., Baranenko D. Biotechonomy Framework for Bioenergy Use. In: *Energy Procedia*, vol 95; 2016:76–80.
[69] Uju, Wijayanta A. T., Goto M., Kamiya N. Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid–ionic liquid pretreatment. *Biomass and Bioenergy* 2015:**81**:63–69. doi:10.1016/j.biombioe.2015.05.023.
[70] Taylor S. *Marine Medicinal Foods: Implications and Applications. Macro and Microalgae*. Marine Medicinal Foods: Implications and Applications. Macro and Microalgae. Elsevier Inc.; 2011.
[71] Senthilkumar N., Kurinjimalar C., Thangam R., et al. Further studies and biological activities of macromolecular protein R-Phycoerythrin from Portieria hornemannii. *International Journal of Biological Macromolecules* 2013:**62**:107–116. doi:10.1016/j.ijbiomac.2013.08.004.
[72] Dumay J., Morançais M., Munier M., Le Guillard C., Fleurence J. Phycoerythrins: Valuable proteinic pigments in red seaweeds. *Advances in Botanical Research* 2014:**71**:321–343. doi:10.1016/B978-0-12-408062-1.00011-1.
[73] Rajauria G. Seaweeds: A sustainable feed source for livestock and aquaculture. In: *Seaweed Sustainability: Food and Non-Food Applications*; 2015.
[74] Bikker P., van Krimpen M. M., van Wikselaar P., et al. Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology* 2016:**28**(6):1–15. doi:10.1007/s10811-016-0842-3.
[75] Hurd C. L., Harrison P. J., Bischof K., Lobban C. S. *Seaweed Ecology and Physiology*. Seaweed Ecology and Physiology.; 2014.
[76] Bruton T. (BioXL), Lyons H., Lerat Y., Stanley M., Rasmussen M. B. A Review of the Potential of

Marine Algae as a Source of Biofuel in Ireland. *Sustainable Energy Ireland* 2009:92.
[77] Fleurence J., Morançais M., Dumay J. *Seaweed proteins*. Seaweed proteins 10 Second Edi. Elsevier Ltd.; 2018.
[78] Burtin P. Nutritional Value of Seaweeds. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 2003:**2**(4):1579–4377.
[79] Fleurence J. R-Phycoerythrin from red macroalgae: Strategies for extraction and potential application in Biotechnology. *Applied Biotechnology, Food Science and Policy* 2003:**1**(1):63–68.
[80] Werlinger C., Alveal K., Romo H. *Biología marina y oceanografía: conceptos y procesos*. Biología marina y oceanografía: conceptos y procesos.; 2009.
[81] Bonanno G., Orlando-Bonaca M. Chemical elements in Mediterranean macroalgae. A review. *Ecotoxicology and Environmental Safety* 2018:**148**(July 2017):44–71. doi:10.1016/j.ecoenv.2017.10.013.
[82] Leliaert F., Smith D. R., Moreau H., et al. Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* 2012:**31**(1):1–46. doi:10.1080/07352689.2011.615705.
[83] Spurr H. I. Extraction, separation and purification of polyphenols, polysaccharides and pigments from British seaweed for high-value applications. 2014:102–108.
[84] Nunes N., Ferraz S., Valente S., Barreto M. C., Pinheiro de Carvalho M. A. A. Biochemical composition, nutritional value, and antioxidant properties of seven seaweed species from the Madeira Archipelago. *Journal of Applied Phycology* 2017:**29**(5):2427–2437. doi:10.1007/s10811-017-1074-x.
[85] Breinholt V., Bailey G., Schimerlik M., Dashwood R. Mechanisms of Chlorophyllin Anticarcinogenesis against Aflatoxin B 1 : Complex Formation with the Carcinogen. *Chemical Research in Toxicology* 1995: doi:10.1021/tx00046a004.
[86] Holdt S. L., Kraan S. Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology* 2011; **23**(3):543–597.
[87] Sanjeewa K. K. A., Kim E. A., Son K. T., Jeon Y. J. Bioactive properties and potentials cosmeceutical applications of phlorotannins isolated from brown seaweeds: A review. *Journal of Photochemistry and Photobiology B: Biology* 2016:**162**:100–105. doi:10.1016/j.jphotobiol.2016.06.027.
[88] Milledge J. J., Nielsen B. V., Bailey D. High-value products from macroalgae: the potential uses of the invasive brown seaweed, Sargassum muticum. *Reviews in Environmental Science and Biotechnology* 2016; **15**(1).
[89] Venkatesan J, S A. Sk., Venkatesan J., Anil S., Kim S. K. *Seaweed Polysaccharides Isolation, Biological and Biomedical Applications*. Seaweed Polysaccharides Isolation, Biological and Biomedical Applications 1.; 2017.
[90] Qazi T. H., Mooney D. J., Pumberger M., Geißler S., Duda G. N. Biomaterials based strategies for skeletal muscle tissue engineering: Existing technologies and future trends. *Biomaterials* 2015:**53**:502–521. doi:10.1016/j.biomaterials.2015.02.110.
[91] Lahaye M., Robic A. Structure and function properties of Ulvan, a polysaccharide from green seaweeds. *Biomacromolecules* 2007; **8**(6):1765–1774.
[92] Venugopal V. Cronicon EC NUTRITION Review Article Sulfated and Non-Sulfated Polysaccharides from Seaweeds and their Uses: An Overview. 2019:**2**:126–141.
[93] Sánchez-Machado D. I., López-Cervantes J., López-Hernández J., Paseiro-Losada P. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry* 2004: doi:10.1016/j.foodchem.2003.08.001.
[94] Bhaskar N., Kazuo M., Masashi H. Comparative Evaluation of Fatty Acid Composition of Different Sargassum (Fucales , Phaeophyta) Species Harvested from Temperate and Tropical Waters. *Journal of Aquatic Food Product Technology* 2004: doi:10.1300/J030v13n04.
[95] Ahmed N., Ahmed K. Chemical and different nutritional characteristics of brown seaweed lipids. *Advances in Science, Technology and Engineering Systems* 2016:**1**(1):23–25. doi:10.25046/aj010104.
[96] Venkatesan J, S A. Sk. *Seaweed Polysaccharides Isolation, Biological and Biomedical Applications*. Seaweed Polysaccharides Isolation, Biological and Biomedical Applications 1.; 2017.
[97] Ruperez P. Mineral content of edible marine seaweeds. *Food Chemistry* 2002:**79**(1):23–26. doi:10.1016/S0308-8146(02)00171-1.
[98] Misra N. N., Rai D. K., Hossain M. Chapter 10 – Analytical techniques for bioactives from seaweed. In: *Seaweed Sustainability*; 2015:271–287.
[99] Yanik J., Stahl R., Troeger N., Sinag A. Pyrolysis of algal biomass. *Journal of Analytical and Applied Pyrolysis* 2013:**103**(September):134–141. doi:10.1016/j.jaap.2012.08.016.
[100] Duan X. J., Zhang W. W., Li X. M., Wang B. G. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata. *Food Chemistry* 2006: doi:10.1016/j.foodchem.2004.12.015.
[101] Gomez-Zavaglia A., Prieto Lage M. A., Jimenez-Lopez C., Mejuto J. C., Simal-Gandara J. The potential

of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. *Antioxidants* 2019:**8**(9). doi:10.3390/antiox8090406.
[102] Pádua D., Rocha E., Gargiulo D., Ramos A. A. Bioactive compounds from brown seaweeds: Phloroglucinol, fucoxanthin and fucoidan as promising therapeutic agents against breast cancer. *Phytochemistry Letters* 2015:**14**:91–98. doi:10.1016/j.phytol.2015.09.007.
[103] Wang T., Jónsdóttir R., Liu H., et al. Antioxidant capacities of phlorotannins extracted from the brown algae Fucus vesiculosus. *Journal of Agricultural and Food Chemistry* 2012:**60**(23):5874–5883. doi:10.1021/jf3003653.
[104] Bergeron C., Carrier D. J., Ramaswamy S. *Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing*. Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing.; 2012.
[105] Van Altena I. A., Steinberg P. D. Are differences in the responses between North American and Australasian marine herbivores to phlorotannins due to differences in phlorotannin structure? *Biochemical Systematics and Ecology* 1992: doi:10.1016/0305-1978(92)90003-V.
[106] Hermund D. B. Extraction, characterization and application of antioxidants from the Nordic brown alga Fucus vesiculosus. 2016:(February):312.
[107] Garcia-Vaquero M., Rajauria G., Tiwari B., Sweeney T., O'Doherty J. Extraction and yield optimisation of fucose, glucans and associated antioxidant activities from laminaria digitata by applying response surface methodology to high intensity ultrasound-assisted extraction. *Marine Drugs* 2018: doi:10.3390/md16080257.
[108] Balina K., Romagnoli F., Blumberga D. Seaweed biorefinery concept for sustainable use of marine resources. In: *Energy Procedia*, vol 128. Elsevier B.V.; 2017:504–511.
[109] Zollmann M., Robin A., Prabhu M., et al. Green technology in green macroalgal biorefineries. *Phycologia* 2019:**58**(5):516–534. doi:10.1080/00318884.2019.1640516.
[110] Rajauria G., Cornish L., Ometto F., Msuya F. E., Villa R. *Identification and selection of algae for food, feed, and fuel applications*. Identification and selection of algae for food, feed, and fuel applications. Elsevier Inc.; 2015.
[111] Chemat F., Boutekedjiret C. Extraction // Steam Distillation☆. In: *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*; 2015.
[112] Allen E., Browne J., Hynes S., Murphy J. D. The potential of algae blooms to produce renewable gaseous fuel. *Waste management (New York, N.Y.)* 2013:**33**(11):2425–33. doi:10.1016/j.wasman.2013.06.017.
[113] Michalak I., Chojnacka K. Algal extracts: Technology and advances. *Engineering in Life Sciences* 2014:**14**(6):581–591. doi:10.1002/elsc.201400139.
[114] Kadam S. U., Álvarez C., Tiwari B. K., O'Donnell C. P. *Processing of seaweeds*. Processing of seaweeds. Elsevier Inc.; 2015.
[115] Jard G., Dumas C., Delgenes J. P., et al. Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalga Palmaria palmata. *Biochemical Engineering Journal* 2013:**79**. doi:10.1016/j.bej.2013.08.011.
[116] Tyagi V. K., Lo S. L. Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: An up to date review. *Reviews in Environmental Science and Biotechnology* 2011:**10**(3):215–242. doi:10.1007/s11157-011-9244-9.
[117] Sosa-Hernández J. E., Escobedo-Avellaneda Z., Iqbal H. M. N., Welti-Chanes J. State-of-the-art extraction methodologies for bioactive compounds from algal biome to meet bio-economy challenges and opportunities. *Molecules* 2018:**23**(11). doi:10.3390/molecules23112953.
[118] Saluri M., Kaldmäe M., Tuvikene R. Extraction and quantification of phycobiliproteins from the red alga Furcellaria lumbricalis. *Algal Research* 2019:**37**(December 2018):115–123. doi:10.1016/j.algal.2018.11.013.
[119] Mensi F., Ksouri J., Seale E., Romdhane M. S., Fleurence J. A statistical approach for optimization of R-phycoerythrin extraction from the red algae Gracilaria verrucosa by enzymatic hydrolysis using central composite design and desirability function. *Journal of Applied Phycology* 2012:**24**(4):915–926. doi:10.1007/s10811-011-9712-1.
[120] Hayes M. *Marine bioactive compounds: Sources, characterization and applications*. Marine bioactive compounds: Sources, characterization and applications 9781461412.; 2012.
[121] Garcia-Vaquero M., Lopez-Alonso M., Hayes M. Assessment of the functional properties of protein extracted from the brown seaweed Himanthalia elongata (Linnaeus) S. F. Gray. *Food Research International* 2016: doi:10.1016/j.foodres.2016.06.023.
[122] Ledesma-Escobar C. A., de Castro M. D. L. Coverage Exploitation of By-Products from the Agrofood Industry. *Green Extraction of Natural Products: Theory and Practice* 2014:265–306. doi:10.1002/9783527676828.ch8.

[123] Cravotto G., Binello A., Orio L. Green extraction techniques. *AgroFOOD industry hi-tech* 2011:**22**(6):57–59.
[124] Baiano A. Recovery of biomolecules from food wastes - A review. *Molecules* 2014:**19**(9):14821–14842. doi:10.3390/molecules190914821.
[125] Razi Parjikolaei B., Errico M., Bahij El-Houri R., Mantell C., Fretté X. C., Christensen K. V. Process design and economic evaluation of green extraction methods for recovery of astaxanthin from shrimp waste. *Chemical Engineering Research and Design* 2017:**117**:73–82. doi:10.1016/j.cherd.2016.10.015.
[126] Grosso C., Valentão P., Ferreres F., Andrade P. B. Alternative and efficient extraction methods for marine-derived compounds. *Marine Drugs* 2015:**13**(5):3182–3230. doi:10.3390/md13053182.
[127] Azmir J., Zaidul I. S. M., Rahman M. M., et al. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering* 2013:**117**(4):426–436. doi:10.1016/j.jfoodeng.2013.01.014.
[128] Praveen M. A., Parvathy K. R. K., Balasubramanian P., Jayabalan R. An overview of extraction and purification techniques of seaweed dietary fibers for immunomodulation on gut microbiota. *Trends in Food Science and Technology* 2019:**92**(August):46–64. doi:10.1016/j.tifs.2019.08.011.
[129] Rioux L., Turgeon S. L. *Chapter 7 - Seaweed carbohydrates*. Chapter 7 - Seaweed carbohydrates. Elsevier Inc.; 2015.
[130] Abdul Khalil H. P. S., Lai T. K., Tye Y. Y., et al. A review of extractions of seaweed hydrocolloids: Properties and applications. *Express Polymer Letters* 2018:**12**(4):296–317. doi:10.3144/expresspolymlett.2018.27.
[131] Ge L., Wang P., Mou H. Study on saccharification techniques of seaweed wastes for the transformation of ethanol. *Renewable Energy* 2011:**36**(1):84–89. doi:10.1016/j.renene.2010.06.001.
[132] Kazir M., Abuhassira Y., Robin A., et al. Extraction of proteins from two marine macroalgae, Ulva sp. and Gracilaria sp., for food application, and evaluating digestibility, amino acid composition and antioxidant properties of the protein concentrates. *Food Hydrocolloids* 2019:**87**(February 2018):194–203. doi:10.1016/j.foodhyd.2018.07.047.
[133] Bleakley S., Hayes M. Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods* 2017:**6**(5):33. doi:10.3390/foods6050033.
[134] Pangestuti R., Kim S. *Seaweed proteins, peptides, and amino acids*. Seaweed proteins, peptides, and amino acids. Elsevier Inc.; 2015.
[135] Hamid N., Ma Q., Boulom S., et al. *Seaweed minor constituents*. Seaweed minor constituents. Elsevier Inc.; 2015.
[136] Peng Y., Hu J., Yang B., et al. *Chemical composition of seaweeds*. Chemical composition of seaweeds. Elsevier Inc.; 2015.
[137] Prato E., Biandolino F. Total lipid content and fatty acid composition of commercially important fish species from the Mediterranean, Mar Grande Sea. *Food Chemistry* 2012:**131**(4):1233–1239. doi:10.1016/j.foodchem.2011.09.110.
[138] Chemat F., Cravotto G. *Enhancing Extraction Processes in the Food Industry*. Enhancing Extraction Processes in the Food Industry.; 2011.
[139] Ivanovs K., Blumberga D. Extraction of fish oil using green extraction methods: A short review. In: *Energy Procedia*, vol 128. Elsevier Ltd; 2017:477–483.
[140] Gribovskaya I. V., Gladchenko I. A., Zinenko G. K. Extraction of mineral elements from inedible wastes of biological components of a life-support system and their utilization for plant nutrition. *Advances in Space Research* 1996:**18**(4–5):93–97. doi:10.1016/0273-1177(95)00865-C.
[141] Ciko A. M., Jokić S., Šubarić D., Jerković I. Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. *Marine Drugs* 2018:**16**(10). doi:10.3390/md16100348.
[142] Kadam S. U., Álvarez C., Tiwari B. K., O'Donnell C. P. *Extraction of biomolecules from seaweeds*. Extraction of biomolecules from seaweeds. Elsevier Inc.; 2015.
[143] Sabeena S. F., Alagarsamy S., Sattari Z., et al. Enzyme-assisted extraction of bioactive compounds from brown seaweeds and characterization. *Journal of Applied Phycology* 2019: doi:10.1007/s10811-019-01906-6.
[144] Quitain A. T., Kai T., Sasaki M., Goto M. Supercritical carbon dioxide extraction of fucoxanthin from undaria pinnatifida. *Journal of Agricultural and Food Chemistry* 2013:**61**(24):5792–5797. doi:10.1021/jf400740p.
[145] Heffernan N., Smyth T. J., FitzGerald R. J., et al. Comparison of extraction methods for selected carotenoids from macroalgae and the assessment of their seasonal/spatial variation. *Innovative Food Science and Emerging Technologies* 2016:**37**:221–228. doi:10.1016/j.ifset.2016.06.004.
[146] Sivagnanam S. P., Yin S., Choi J. H., Park Y. B., Woo H. C., Chun B. S. Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO2 extraction. *Marine*

Drugs 2015:**13**(6):3422–3442. doi:10.3390/md13063422.
[147] Michalak I., Górka B., Wieczorek P. P., et al. Supercritical fluid extraction of algae enhances levels of biologically active compounds promoting plant growth. *European Journal of Phycology* 2016:**51**(3):243–252. doi:10.1080/09670262.2015.1134813.
[148] Zhang R., Yuen A. K. L., Magnusson M., et al. A comparative assessment of the activity and structure of phlorotannins from the brown seaweed Carpophyllum flexuosum. *Algal Research* 2018:**29**(November 2017):130–141. doi:10.1016/j.algal.2017.11.027.
[149] Magnusson M., Yuen A. K. L., Zhang R., et al. A comparative assessment of microwave assisted (MAE) and conventional solid-liquid (SLE) techniques for the extraction of phloroglucinol from brown seaweed. *Algal Research* 2017:**23**:28–36. doi:10.1016/j.algal.2017.01.002.
[150] Quitain A. T., Kai T., Sasaki M., Goto M. Microwave-hydrothermal extraction and degradation of fucoidan from supercritical carbon dioxide deoiled Undaria pinnatifida. *Industrial and Engineering Chemistry Research* 2013:**52**(23):7940–7946. doi:10.1021/ie400527b.
[151] Yuan Y., Xu X., Jing C., Zou P., Zhang C., Li Y. Microwave assisted hydrothermal extraction of polysaccharides from Ulva prolifera: Functional properties and bioactivities. *Carbohydrate Polymers* 2018:**181**(November):902–910. doi:10.1016/j.carbpol.2017.11.061.
[152] Romarís-Hortas V., Moreda-Piñeiro A., Bermejo-Barrera P. Microwave assisted extraction of iodine and bromine from edible seaweed for inductively coupled plasma-mass spectrometry determination. *Talanta* 2009:**79**(3):947–952. doi:10.1016/j.talanta.2009.05.036.
[153] Wang F., Guo X. Y., Zhang D. N., Wu Y., Wu T., Chen Z. G. Ultrasound-assisted extraction and purification of taurine from the red algae Porphyra yezoensis. *Ultrasonics Sonochemistry* 2015:**24**:36–42. doi:10.1016/j.ultsonch.2014.12.009.
[154] Mittal R., Tavanandi H. A., Mantri V. A., Raghavarao K. S. M. S. Ultrasound assisted methods for enhanced extraction of phycobiliproteins from marine macro-algae, Gelidium pusillum (Rhodophyta). *Ultrasonics Sonochemistry* 2017:**38**:92–103. doi:10.1016/j.ultsonch.2017.02.030.
[155] Lee S. H., Kang M. C., Moon S. H., Jeon B. T., Jeon Y. J. Potential use of ultrasound in antioxidant extraction from Ecklonia cava. *Algae* 2013:**28**(4):371–378. doi:10.4490/algae.2013.28.4.371.
[156] Kadam S. U., Donnell C. P. O., Rai D. K., et al. Laminarin from Irish Brown Seaweeds Ascophyllum nodosum and Laminaria hyperborea. *Obesity, Fitness & Wellness Week* 2015:(December):1247–1247. doi:10.3390/md1307427.
[157] Dang T. T., Van Vuong Q., Schreider M. J., Bowyer M. C., Van Altena I. A., Scarlett C. J. Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant activities of the alga Hormosira banksii using response surface methodology. *Journal of Applied Phycology* 2017:**29**(6):3161–3173. doi:10.1007/s10811-017-1162-y.
[158] Saravana P. S., Choi J. H., Park Y. B., Woo H. C., Chun B. S. Evaluation of the chemical composition of brown seaweed (Saccharina japonica) hydrolysate by pressurized hot water extraction. *Algal Research* 2016:**13**:246–254. doi:10.1016/j.algal.2015.12.004.
[159] Plaza M., Amigo-Benavent M., del Castillo M. D., Ibáñez E., Herrero M. Facts about the formation of new antioxidants in natural samples after subcritical water extraction. *Food Research International* 2010:**43**(10):2341–2348. doi:10.1016/j.foodres.2010.07.036.
[160] Anaëlle T., Serrano Leon E., Laurent V., et al. Green improved processes to extract bioactive phenolic compounds from brown macroalgae using Sargassum muticum as model. *Talanta* 2013:**104**:44–52. doi:10.1016/j.talanta.2012.10.088.
[161] Sánchez-Camargo A. D. P., Montero L., Stiger-Pouvreau V., et al. Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae. *Food Chemistry* 2016:**192**:67–74. doi:10.1016/j.foodchem.2015.06.098.
[162] Hammed A. M., Jaswir I., Simsek S., Alam Z., Amid A. Enzyme aided extraction of sulfated polysaccharides from Turbinaria turbinata brown seaweed. *International Food Research Journal* 2017:**24**(4):1660–1666.
[163] Vo Dinh T., Saravana P. S., Woo H. C., Chun B. S. Ionic liquid-assisted subcritical water enhances the extraction of phenolics from brown seaweed and its antioxidant activity. *Separation and Purification Technology* 2018:**196**:287–299. doi:10.1016/j.seppur.2017.06.009.
[164] Yi J., Dong B., Jin J., Dai X. Effect of increasing total solids contents on anaerobic digestion of food waste under mesophilic conditions: Performance and microbial characteristics analysis. *PLoS ONE* 2014:**9**(7). doi:10.1371/journal.pone.0102548.
[165] Gereniu C. R. N., Saravana P. S., Chun B. S. Recovery of carrageenan from Solomon Islands red seaweed using ionic liquid-assisted subcritical water extraction. *Separation and Purification Technology* 2018:**196**:309–317. doi:10.1016/j.seppur.2017.06.055.
[166] Bozinou E., Karageorgou I., Batra G., G. Dourtoglou V., I. Lalas S. Pulsed Electric Field Extraction and Antioxidant Activity Determination of Moringa oleifera Dry Leaves: A Comparative Study with Other

Extraction Techniques. *Beverages* 2019:**5**(1):8. doi:10.3390/beverages5010008.
[167] Roesijadi G., Jones S. B., Zhu Y. Macroalgae as a Biomass Feedstock : A Preliminary Analysis. *Analysis* 2010:(September):1–50.http://www.pnl.gov/main/publications/external/technical_reports/PNNL-19944.pdfAccessed.
[168] Environment & Heritage Service. *Environmentally Sustainable Seaweed Harvesting in Northern Ireland*. Environmentally Sustainable Seaweed Harvesting in Northern Ireland.; 2007.
[169] Stévant P., Rebours C., Chapman A. Seaweed aquaculture in Norway: recent industrial developments and future perspectives. *Aquaculture International* 2017; **25**(4).
[170] Mac Monagail M., Cornish L., Morrison L., Araújo R., Critchley A. T. Sustainable harvesting of wild seaweed resources. *European Journal of Phycology* 2017:**52**(4):371–390. doi:10.1080/09670262.2017.1365273.
[171] Rebours C., Marinho-Soriano E., Zertuche-González J. A., et al. Seaweeds: An opportunity for wealth and sustainable livelihood for coastal communities. *Journal of Applied Phycology* 2014:**26**(5):1939–1951. doi:10.1007/s10811-014-0304-8.
[172] Gutow L., Beermann J., Buschbaum C., M. Rivadeneira M., Thiel M. Castaways can't be choosers — Homogenization of rafting assemblages on floating seaweeds. *Journal of Sea Research* 2015:**95**:161–171. doi:10.1016/j.seares.2014.07.005.
[173] Ugarte R. A., Sharp G. A new approach to seaweed management in Eastern Canada: The case of Ascophyllum nodosum. *Cahiers de Biologie Marine* 2001:
[174] Andersen R. A. *Algal Culturing Techniques*. Algal Culturing Techniques. Elsevier; 2005.
[175] Winberg P., Skropeta D., Ullrich A. *Seaweed Cultivation Pilot Trials - Towards culture systems and marketable products*. Seaweed Cultivation Pilot Trials - Towards culture systems and marketable products.; 2011.
[176] Hafting J. T., Craigie J. S., Stengel D. B., et al. Prospects and challenges for industrial production of seaweed bioactives. *Journal of Phycology* 2015; **51**(5):821–837.
[177] Gröndahl F. *Seafarm - Cultivation of macroalgae*. Seafarm - Cultivation of macroalgae.; 2014.
[178] Khan S. I., Satam S. B. Seaweed Mariculture : Scope And Potential In Seaweed Mariculture : Scope And Potential In India. 2015:(JANUARY 2003).
[179] Bruhn A., Dahl J., Nielsen H. B., et al. Bioenergy potential of Ulva lactuca: biomass yield, methane production and combustion. *Bioresource technology* 2011:**102**(3):2595–604. doi:10.1016/j.biortech.2010.10.010.
[180] Golberg A., Liberzon A. Modeling of smart mixing regimes to improve marine biorefinery productivity and energy efficiency. *Algal Research* 2015:**11**:28–32. doi:10.1016/j.algal.2015.05.021.
[181] Kerrison P. D., Stanley M. S., Black K. D., Hughes A. D. Assessing the suitability of twelve polymer substrates for the cultivation of macroalgae Laminaria digitata and Saccharina latissima (Laminariales). *Algal Research* 2017:**22**. doi:10.1016/j.algal.2016.10.001.
[182] Wichard T. Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). *Frontiers in Plant Science* 2015:**6**(March). doi:10.3389/fpls.2015.00086.
[183] Yang Y., Chai Z., Wang Q., Chen W., He Z., Jiang S. Cultivation of seaweed Gracilaria in Chinese coastal waters and its contribution to environmental improvements. *Algal Research* 2015:**9**:236–244. doi:10.1016/j.algal.2015.03.017.
[184] Van Den Hoek C., Mann D. G., Jahns H. M. Algae: an introduction to phycology. *Search* 1995:627.
[185] Makkar H. P. S., Tran G., Heuzé V., et al. Seaweeds for livestock diets: A review. *Animal Feed Science and Technology* 2016; **212**.
[186] Par gaismas ietekmi uz zaļaļģu augšanu. http://oceanlink.island.net/biodiversity/seaweeds/Chlorophyta.html. Accessed February 14, 2016.
[187] Par gaismas ietekmi uz sārtaļģu augšanu. http://www.ucmp.berkeley.edu/protista/rhodophyta.html. Accessed December 10, 2015.
[188] Reece J. B., Meyers N., Urry L. A., et al. *Campbell Biology Australian and New Zealand version*. Campbell Biology Australian and New Zealand version. Pearson Higher Education AU; 2014.
[189] Par gaismas ietekmi uz brūnaļģu augšanu. http://oceanlink.island.net/biodiversity/seaweeds/Phaeophyta.html.
[190] Rohde S., Hiebenthal C., Wahl M., Karez R., Bischof K. Decreased depth distribution of Fucus vesiculosus (Phaeophyceae) in the Western Baltic: effects of light deficiency and epibionts on growth and photosynthesis. *European Journal of Phycology* 2008:**43**(2):143–150.http://www.tandfonline.com/doi/abs/10.1080/09670260801901018AccessedApril 22, 2016.
[191] Yang Z., Cheng J., Li K., Zhou J., Cen K. Optimizing gas transfer to improve growth rate of Haematococcus pluvialis in a raceway pond with chute and oscillating baffles. *Bioresource Technology* 2016:**214**:276–283. doi:10.1016/j.biortech.2016.04.107.
[192] Demetropoulos C. L., Langdon C. J. Enhanced production of Pacific dulse (Palmaria mollis) for co-

culture with abalone in a land-based system: nitrogen, phosphorus, and trace metal nutrition. *Aquaculture* 2004:**235**(1–4):433–455.http://www.sciencedirect.com/science/article/pii/S0044848603006148AccessedNovember 21, 2015.
[193] Raikar S. V., Iima M., Fujita Y. Effect of temperature, salinity and light intensity on the growth of Gracilaria spp. (Gracilariales, Rhodophyta) from Japan, Malaysia and India. *Indian Journal of Geo-Marine Sciences* 2001:**30**(2):98–104.https://www.researchgate.net/publication/285783434_Effect_of_temperature_salinity_and_light_intensity_on_the_growth_of_Gracilaria_spp_Gracilariales_Rhodophyta_from_Japan_Malaysia_and_India AccessedMay 3, 2016.
[194] Pereira R., Yarish C., Sousa-Pinto I. The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of Porphyra dioica (Bangiales, Rhodophyta). *Aquaculture* 2006:**252**(1):66–78.http://www.sciencedirect.com/science/article/pii/S0044848605007544AccessedNovember 21, 2015.
[195] Corey P., Kim J. K., Garbary D. J., Prithiviraj B., Duston J. Bioremediation potential of Chondrus crispus (Basin Head) and Palmaria palmata: effect of temperature and high nitrate on nutrient removal. *Journal of Applied Phycology* 2011:**24**(3):441–448.http://link.springer.com/10.1007/s10811-011-9734-8AccessedMay 3, 2016.
[196] Bird N. L., Chen L. C.-M., McLachlan J. Effects of Temperature, Light and Salinity on Growth in Culture of Chondrus crispus. Furcellaria lumbricalis, Gracilaria tikvahiae (Gigartinales, Rhodophyta), and Fucus serratus (Fucales, Phaeophyta). *Botanica Marina* 1979:**22**(8).http://www.degruyter.com/view/j/botm.1979.22.issue-8/botm.1979.22.8.521/botm.1979.22.8.521.xmlAccessedMay 3, 2016.
[197] Graiff A., Liesner D., Karsten U., Bartsch I. Temperature tolerance of western Baltic Sea Fucus vesiculosus – growth, photosynthesis and survival. *Journal of Experimental Marine Biology and Ecology* 2015:**471**:8–16. doi:10.1016/j.jembe.2015.05.009.
[198] Kerrison P. D., Stanley M. S., Edwards M. D., Black K. D., Hughes A. D. The cultivation of European kelp for bioenergy: Site and species selection. *Biomass and Bioenergy* 2015:**80**:229–242.http://www.sciencedirect.com/science/article/pii/S0961953415001725AccessedOctober 21, 2015.
[199] Kim K. Y., Lee I. K. The germling growth of Enteromorpha intestinalis (Chlorophyta) in laboratory culture under different combinations of irradiance and salinity and temperature and salinity. *Phycologia* 1996:**35**(4):327–331.https://www.researchgate.net/publication/240794250_The_germling_growth_of_Enteromorpha_intestinalis_Chlorophyta_in_laboratory_culture_under_different_combinations_of_irradiance_and_salinity_and_temperature_and_salinityAccessedMay 3, 2016.
[200] Kalita T. L., Titlyanov E. A. Influence of temperature on the infradian growth rhythm in Ulva lactuca (Chlorophyta). *European journal of phycology* 2013:http://agris.fao.org/agris-search/search.do?recordID=US201500061799AccessedMay 3, 2016.
[201] Yang Y., Chai Z., Wang Q., Chen W., He Z., Jiang S. Cultivation of seaweed Gracilaria in Chinese coastal waters and its contribution to environmental improvements. *Algal Research* 2015:**9**:236–244. doi:10.1016/j.algal.2015.03.017.
[202] Koch E. W. The effect of water flow on photosynthetic processes of the alga Ulva lactuca L. *Hydrobiologia* 1993:**260**–**261**(1):457–462.http://link.springer.com/10.1007/BF00049056AccessedMay 29, 2016.
[203] Martins I., Oliveira J. M., Flindt M. R., Marques J. C. No Title. *Acta Oecologica* 1999:**20**(4):259–265.
[204] Zou D., Gao K. Effects of elevated CO 2 on the red seaweed Gracilaria lemaneiformis (Gigartinales, Rhodophyta) grown at different irradiance levels. *Phycologia* 2009:**48**(6):510–517. doi:10.2216/08-99.1.
[205] Nygård C. A., Dring M. J. Influence of salinity, temperature, dissolved inorganic carbon and nutrient concentration on the photosynthesis and growth of Fucus vesiculosus from the Baltic and Irish Seas. *European Journal of Phycology* 2008:**43**(3):253–262.http://www.tandfonline.com/doi/abs/10.1080/09670260802172627#.VyjurvmyOkoAccessedMarch 11, 2016.
[206] Kubler J. E., Johnston A. M., Raven J. A. The effects of reduced and elevated CO2 and O2 on the seaweed Lomentaria articulata. *Plant, Cell and Environment* 1999:**22**(10):1303–1310. doi:10.1046/j.1365-3040.1999.00492.x.
[207] Rodríguez-Maroto J. M., Jiménez C., Aguilera J., Niell F. X. Air bubbling results in carbon loss during microalgal cultivation in bicarbonate-enriched media: experimental data and process modeling. *Aquacultural Engineering* 2005:**32**(3–4):493–508.http://www.sciencedirect.com/science/article/pii/S0144860904001001AccessedNovember 21, 2015.
[208] Balina 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**:403–

410. doi:10.1016/j.egypro.2017.04.022.
[209] Balina K., Romagnoli F., Blumberga D. Chemical Composition and Potential Use of Fucus Vesiculosus from Gulf of Riga. *Energy Procedia* 2016:**95**:43–49. doi:10.1016/j.egypro.2016.09.010.
[210] 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):178–195.
[211] Balina K., Lika A., Romagnoli F., Blumberga D. Seaweed Cultivation Laboratory Testing: Effects of Nutrients on Growth Rate of Ulva intestinalis. *Energy Procedia* 2017:**113**:454–459. doi:10.1016/j.egypro.2017.04.038.
[212] Sabunas A., Romagnoli F., Pastare L., Balina K. Laboratory Algae Cultivation and BMP Tests with Ulva intestinalis from the Gulf of Riga. *Energy Procedia* 2017:**113**:277–284. doi:10.1016/j.egypro.2017.04.066.
[213] Balina K., Piscika A., Gruduls A., Romagnoli F., Blumberga D. Lab scale cultivation of Baltic Ulva intestinalis in different light and nutrient conditions: effects on growth on morphology. In: *26th European Biomass Conference and Exhibition, 14-17 May 2018, Copenhagen, Denmark*, vol 40. Copengagen; 2018:14–17.
[214] Özdenkçi K., De Blasio C., Muddassar H. R., et al. A novel biorefinery integration concept for lignocellulosic biomass. *Energy Conversion and Management* 2017: doi:10.1016/j.enconman.2017.04.034.
[215] Paré G., Trudel M. C., Jaana M., Kitsiou S. Synthesizing information systems knowledge: A typology of literature reviews. *Information and Management* 2015: doi:10.1016/j.im.2014.08.008.
[216] Muizniece I., Timma L., Blumberga A., Blumberga D. The Methodology for Assessment of Bioeconomy Efficiency. In: *Energy Procedia*, vol 95; 2016:482–486.
[217] Mathijs E., Brunori G., Carus M., Griffon M., Last L. *Sustainable Agriculture, Forestry and Fisheries in the Bioeconomy -A Challenge for Europe*. Sustainable Agriculture, Forestry and Fisheries in the Bioeconomy -A Challenge for Europe.; 2015.
[218] Araujo R. M., Assis J., Aguillar R., et al. Status, trends and drivers of kelp forests in Europe: an expert assessment. *Biodiversity and Conservation* 2016:**25**(7):1319–1348. doi:10.1007/s10531-016-1141-7.
[219] GÜREL E. SWOT ANALYSIS: A THEORETICAL REVIEW. *Journal of International Social Research* 2017: doi:10.17719/jisr.2017.1832.
[220] Stengel D. B., Connan S., Popper Z. a. Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. *Biotechnology advances* 2011:**29**(5):483–501. doi:10.1016/j.biotechadv.2011.05.016.
[221] Anderson R. A., Berges R. A., Harrison P. J., Watanabe M. M. Recipes for Freshwater and Seawater Media; Enriched Natural Seawater Media. In: *Algal Culturing Techniques*; 2005:596.
[222] ImageJ. https://imagej.nih.gov/ij/. Accessed May 9, 2018.
[223] Alberte M. Makrobentosa sugu izplatību un daudzveidību regulējoši faktori Baltijas jūras austrumu daļas akmeņainajā piekrastē. 2012.
[224] Schramm W. The Baltic Sea and Its Transition Zones. In: 1996.
[225] Medne R., Purviņa S. *Inovatīvas pieejas un tehnoloģiju izmantošana ilgtspējīgas akvakultūras attīstībai Baltijas jūras reģionā*. Inovatīvas pieejas un tehnoloģiju izmantošana ilgtspējīgas akvakultūras attīstībai Baltijas jūras reģionā.; 2012.
[226] SUBMARINER. *Compendium An Assessment of Innovative and Sustainable Uses of Baltic Marine Resources*. Compendium An Assessment of Innovative and Sustainable Uses of Baltic Marine Resources.; 2012.
[227] Li X., Xiong F., Liu Y., Liu F., Hao Z., Chen H. Total fractionation and characterization of the water-soluble polysaccharides isolated from Enteromorpha intestinalis. *International Journal of Biological Macromolecules* 2018:**111**:319–325. doi:10.1016/j.ijbiomac.2018.01.018.
[228] Wright R. T., Boorse D. F. *Environmental Science: Toward a Sustainable Future, 12th Edition*. Environmental Science: Toward a Sustainable Future, 12th Edition. Upper Saddle River: Pearson Education Inc.; 2014.
[229] Peasura N., Laohakunjit N., Kerdchoechuen O., Wanlapa S. Characteristics and antioxidant of Ulva intestinalis sulphated polysaccharides extracted with different solvents. *International Journal of Biological Macromolecules* 2015:**81**:912–919. doi:10.1016/j.ijbiomac.2015.09.030.
[230] Kidgell J. T., Magnusson M., de Nys R., Glasson C. R. K. Ulvan: A systematic review of extraction, composition and function. *Algal Research* 2019:**39**(January):101422. doi:10.1016/j.algal.2019.101422.
[231] Szaniawska A., Normant M. Szaniawska, Normant - 2000 - The biochemical composition of Enteromorpha spp . from the Gulf of Gdańsk coast on the southern Balt.pdf. 2000:
[232] Rioux L.-E., Turgeon S. L., Beaulieu M. Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers* 2007:**69**(3):530–537. doi:10.1016/j.carbpol.2007.01.009.

[233] Stiger-Pouvreau V., Bourgougnon N., Deslandes E. *Carbohydrates from Seaweeds*. Carbohydrates from Seaweeds. Elsevier Inc.; 2016.
[234] de Reviers B., Leproux A. Characterization of polysaccharides from Enteromorpha intestinalis (L.) link, chlorophyta. *Carbohydrate Polymers* 1993:**22**(4):253–259. doi:10.1016/0144-8617(93)90128-Q.
[235] Tabarsa M., Han J. H., Kim C. Y., You S. G. Molecular characteristics and immunomodulatory activities of water-soluble sulfated polysaccharides from ulva pertusa. *Journal of Medicinal Food* 2012:**15**(2):135–144. doi:10.1089/jmf.2011.1716.
[236] Tabarsa M., You S. G., Dabaghian E. H., Surayot U. Water-soluble polysaccharides from Ulva intestinalis: Molecular properties, structural elucidation and immunomodulatory activities. *Journal of Food and Drug Analysis* 2018:**26**(2):599–608. doi:10.1016/j.jfda.2017.07.016.
[237] Graiff A., Liesner D., Karsten U., Bartsch I. Temperature tolerance of western Baltic Sea Fucus vesiculosus – growth, photosynthesis and survival. *Journal of Experimental Marine Biology and Ecology* 2015:**471**:8–16. doi:10.1016/j.jembe.2015.05.009.
[238] Ptak S. H., Christensen K. V., Meichßner R., Fretté X. Improving fucoidan yield from fucus brown algae by microwave extraction. *Chemical Engineering Transactions* 2019:**74**(August 2018):109–114. doi:10.3303/CET1974019.
[239] Yu G., Yang B., Ren W., Zhao X., Zhang J., Barrow C. A comparative analysis of four kinds of polysaccharides purified from Furcellaria lumbricalis. *Journal of Ocean University of China* 2007: doi:10.1007/s11802-007-0016-7.
[240] Bird C. J., Saunders G. W., McLachlan J. Biology of Furcellaria lumbricalis (Hudson) Lamouroux (Rhodophyta: Gigartinales), a commercial carrageenophyte. *Journal of Applied Phycology* 1991:**3**(1):61–82.
[241] Rahimi F., Tabarsa M., Rezaei M. Ulvan from green algae Ulva intestinalis: optimization of ultrasound-assisted extraction and antioxidant activity. *Journal of Applied Phycology* 2016:**28**(5):2979–2990. doi:10.1007/s10811-016-0824-5.
[242] Benjama O., Masniyom P. Nutritional composition and physicochemical properties of two green seaweeds (Ulva pertusa and U. intestinalis) from the Pattani Bay in Southern Thailand. *Songklanakarin Journal of Science and Technology* 2011:**33**(5):575–583.
[243] Peinado I., Girón J., Koutsidis G., Ames J. M. Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds. *Food Research International* 2014:**66**:36–44. doi:10.1016/j.foodres.2014.08.035.
[244] Maehre H. K., Malde M. K., Eilertsen K.-E., Elvevoll E. O. Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *Journal of the Science of Food and Agriculture* 2014:**94**(15):3281–3290. doi:10.1002/jsfa.6681.
[245] Cherry P., O'hara C., Magee P. J., Mcsorley E. M., Allsopp P. J. Risks and benefits of consuming edible seaweeds. *Nutrition Reviews* 2019:**77**(5):307–329. doi:10.1093/nutrit/nuy066.
[246] Parjikolaei B. R., Bruhn A., Eybye K. L., et al. Valuable Biomolecules from Nine North Atlantic Red Macroalgae: Amino Acids, Fatty Acids, Carotenoids, Minerals and Metals. *Natural Resources* 2016:**07**(04):157–183. doi:10.4236/nr.2016.74016.
[247] Dumay J., Morançais M. *Proteins and Pigments*. Proteins and Pigments. Elsevier Inc.; 2016.
[248] Truus K., Vaher M., Koel M., Mähar A., Taure I. Analysis of bioactive ingredients in the brown alga Fucus vesiculosus by capillary electrophoresis and neutron activation analysis. *Analytical and Bioanalytical Chemistry* 2004:**379**(5–6):849–852. doi:10.1007/s00216-004-2666-2.
[249] Rozentsvet O. A., Nesterov V. N. Lipids and fatty acids from ulva intestinalis from estuaries of the caspian basin (elton region). *Chemistry of Natural Compounds* 2012:**48**(4):544–547. doi:10.1007/s10600-012-0305-2.
[250] Ragonese C., Tedone L., Beccaria M., et al. Characterisation of lipid fraction of marine macroalgae by means of chromatography techniques coupled to mass spectrometry. *Food Chemistry* 2014:**145**:932–940. doi:10.1016/j.foodchem.2013.08.130.
[251] Jeong G. T., Park D. H. Optimization of lipid extraction from marine green macro-algae as biofuel resources. *Korean Journal of Chemical Engineering* 2015:**32**(12):2463–2467. doi:10.1007/s11814-015-0083-1.
[252] Alam M., Chakravarti A., Ikawa M. Lipid composition of the brown alga Fucus vesiculosus. *Journal of Phycology* 1971:**7**(3):267–268. doi:10.1111/j.1529-8817.1971.tb01515.x.
[253] Rohani-Ghadikolaei K., Abdulalian E., Ng W. K. Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of Food Science and Technology* 2012:**49**(6):774–780. doi:10.1007/s13197-010-0220-0.
[254] Biancarosa I., Belghit I., Bruckner C. G., et al. Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and

feed. *Journal of the Science of Food and Agriculture* 2018:**98**(5):2035–2042. doi:10.1002/jsfa.8798.
[255] Yin-Hu W., Yin Y., Xin L., Hong-Ying H., Zhen-Feng S. Biomass production of a Scenedesmus sp. under phosphorous-starvation cultivation condition. *Bioresource technology* 2012:**112**:193–8. doi:10.1016/j.biortech.2012.02.037.
[256] Matanjun P., Mohamed S., Mustapha N. M., Muhammad K. Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. *Journal of Applied Phycology* 2009: doi:10.1007/s10811-008-9326-4.
[257] Polat S., Ozogul Y. Biochemical composition of some red and brown macro algae from the Northeastern Mediterranean Sea. *International Journal of Food Sciences and Nutrition* 2008:**59**(7–8):566–572. doi:10.1080/09637480701446524.
[258] El-Sayed S. M. Use of spinach powder as functional ingredient in the manufacture of UF-Soft cheese. *Heliyon* 2020:**6**(1):e03278. doi:10.1016/j.heliyon.2020.e03278.
[259] Li Y., Cui J., Zhang G., et al. Optimization study on the hydrogen peroxide pretreatment and production of bioethanol from seaweed Ulva prolifera biomass. *Bioresource Technology* 2016:**214**:144–149. doi:10.1016/j.biortech.2016.04.090.
[260] Rioux L. E., Turgeon S. L., Beaulieu M. Structural characterization of laminaran and galactofucan extracted from the brown seaweed Saccharina longicruris. *Phytochemistry* 2010:**71**(13):1586–1595. doi:10.1016/j.phytochem.2010.05.021.
[261] Kumar S., Sahoo D., Levine I. Assessment of nutritional value in a brown seaweed Sargassum wightii and their seasonal variations. *Algal Research* 2015:**9**:117–125. doi:10.1016/j.algal.2015.02.024.
[262] Mendis E. Chapter 1 – Present and Future Prospects of Seaweeds in Developing Functional Foods. In: *Advances in Food and Nutrition Research*, vol 64; 2011:1–15.
[263] FAO. How to Feed the World in 2050. *Insights from an expert meeting at FAO* 2009:**2050**(1):1–35. doi:10.1111/j.1728-4457.2009.00312.x.
[264] FAO Fisheries and Aquaculture Department. FAO yearbook of fishery and aquaculture statistics summary tables. 2016:**0**:26–28. doi:10.5860/CHOICE.50-5350.
[265] Liguori R., Faraco V. Biological processes for advancing lignocellulosic waste biorefinery by advocating circular economy. *Bioresource Technology* 2016; **215**:13–20.
[266] Capuzzo E., McKie T. Seaweed in the UK and abroad – status, products, limitations, gaps and Cefas role. 2016:(April):66.
[267] Ruangchuay R., Dahamat S., Chirapat A., Notoya M. Effects of culture conditions on the growth and reproduction of Gut Weed, Ulva intestinalis Linnaeus (Ulvales, Chlorophyta). *Songklanakarin Journal of Science and Technology* 2012:**34**(5):501–507.
[268] Dean T. A., Jacobsen F. R. Growth of juvenile Macrocystis pyrifera (Laminariales) in relation to environmental factors. *Marine Biology* 1984: doi:10.1007/BF00397463.
[269] Fowler-Walker M. J., Wernberg T., Connell S. D. Differences in kelp morphology between wave sheltered and exposed localities: Morphologically plastic or fixed traits? *Marine Biology* 2006: doi:10.1007/s00227-005-0125-z.
[270] Brown M. T., Nyman M. A., Keogh J. A., Chin N. K. M. Seasonal growth of the giant kelp macrocystis pyrifera in New Zealand. *Marine Biology* 1997: doi:10.1007/s002270050182.
[271] Spurkland T., Iken K. Seasonal growth patterns of Saccharina latissima (Phaeophyceae, Ochrophyta) in a glacially-influenced subarctic estuary. *Phycological Research* 2012: doi:10.1111/j.1440-1835.2012.00657.x.
[272] Kopczak C. D., Zimmerman R. C., Kremer J. N. Variation in nitrogen physiology and growth among geographically isolated populations of the giant kelp, Macrocystis pyrifera (Phaeophyta). *Journal of Phycology* 1991: doi:10.1111/j.0022-3646.1991.00149.x.
[273] Par Latvijas klimatu. http://meteo.lv/lapas/laika-apstakli/klimatiska-informacija/latvijas-klimats/latvijas-klimats?id=1199&nid=562. Accessed March 4, 2016.
[274] Edwards M. D., Dring M. J. Open-sea cultivation trial of the red alga, Palmaria palmata from seeded tetraspores in Strangford Lough, Northern Ireland. *Aquaculture* 2011: doi:10.1016/j.aquaculture.2011.04.007.
[275] Marinho-Soriano E., Panucci R. A., Carneiro M. A. A., Pereira D. C. Evaluation of Gracilaria caudata J. Agardh for bioremediation of nutrients from shrimp farming wastewater. *Bioresource Technology* 2009: doi:10.1016/j.biortech.2009.06.102.
[276] Anderson R. A. *Algal culturing techniques*. Algal culturing techniques 53.; 2013.
[277] Hamilton R. D. *Handbook O Phycological Methods: Phusiological and Biochemical Methods*. Handbook O Phycological Methods: Phusiological and Biochemical Methods. Cambridge University Press; 1978.
[278] (No Title). http://spodriba.lv/musu-produkti/darzam/vito-universal-augu-bariba/. Accessed October 13, 2020.

[279] Fong P., Boyer K. E., Desmond J. S., Zedler J. B. Salinity stress, nitrogen competition, and facilitation: What controls seasonal succession of two opportunistic green macroalgae? *Journal of Experimental Marine Biology and Ecology* 1996:**206**(1–2):203–221. doi:10.1016/S0022-0981(96)02630-5.

[280] Zbikowski R., Szefer P., Latała A. Comparison of green algae Cladophora sp. and Enteromorpha sp. as potential biomonitors of chemical elements in the southern Baltic. *The Science of the total environment* 2007:**387**(1–3):320–32. doi:10.1016/j.scitotenv.2007.07.017.

[281] Lignell A., Ekman P., Pedersén M. Cultivation Technique for Marine Seaweeds Allowing Controlled and Optimized Conditions in the Laboratory and on a Pilotscale. *Botanica Marina* 1987:**30**(5):417–424. doi:10.1515/botm.1987.30.5.417.

[282] Parchevskij V., Rabinovich M. Growth rate and harvest of the green alga Enteromorpha intestinalis on artificial substrata. *Biologiya Morya* 1991:**2**:56–60.

[283] Kang E. J., Kim J. H., Kim K., Kim K. Y. Adaptations of a green tide forming Ulva linza (Ulvophyceae, Chlorophyta) to selected salinity and nutrients conditions mimicking representative environments in the Yellow Sea. *Phycologia* 2016: doi:10.2216/15-67.1.

[284] Eriksson B. K., Bergström L. Local distribution patterns of macroalgae in relation to environmental variables in the northern Baltic Proper. *Estuarine, Coastal and Shelf Science* 2005:**62**(1–2):109–117. doi:10.1016/j.ecss.2004.08.009.

APPENDIX

Appendix 1

Overview of novel methods for seaweed bioactive compound extraction

Extraction technique Conditions (C) and influencing parameters (IP)	Seaweed species under investigation	Extracted bioactive compounds	Application outlook	References
Supercritical CO_2 (SC-CO_2) (C) Pressure 9.1 – 40 MPa, Temp. 25 – 75 C, Time 50 – 360 min, > 2 mL CO2/min Co-solvents: EtOH 0,5 – 15 % Sunflower, soybean, canola oil 0,5 – 2 %. (IP) Water %, T°C, pressure. Flow of CO_2; Extraction type: continuous, co-solvent, soaking.	*Cladophora glomerata, Chara fragilis, Chondrus crispus, Dictyopteris membranacea, Fucus serratus,* *Gracilaria mammillaris, Hypnea charoides,* *Hypnea spinella, Halopytis incurvus,* *Porphyra sp., Laminaria digitata,* *Sargassum muticum, Sargassum vulgare,* *Ulva clathrata* *Undaria pinnatifida, Polysiphoniucoides, Saccharina japonica, Sargassum horneri,* *Undaria pinnatifida, Ulva flexuosa,*	Fucoxanthin, polyphenols, phlorotannins, carotenoids, pigments, fatty acids, cytokinins, auxins, microelements, macroelements	High investment cost; Operates in elevated pressure (safety); High power consumption.	[139,141,144 –147]
Microwave-assisted extraction (MAE) (C) Power 300 – 1000 W; Frequancy – 2450 Mhz; Temperature – 10 – 185 °C; Solvents – EtOH, H_2O, acetone, propanol, ethyl acetate, 0,1 M HCl, petroleum ether, ethyl acetate; Time – 2 – 30 min. (IP) Particle size, solvent used, time, capacity, and frequency of microwave	*Ascophyllum nodosum, Carpophyllum flexuosum, Carpophyllum plumosum, Caulerpa racemose, Carpophyllum flexuosum, Ecklonia radiata, Enteromorpha prolifera, Fucus vesiculosus, Padina pavonica, Sargassum thunbergii, Monostroma latissimum, Ulva meridionalis, Ulva ohnoi, Ulva prolifera, Undaria pinnatifida,*	Poly-saccharides, alkaline, galactans, carrageenans, agar, phlorotannins, phloroglucinol, iodine, bromine, phenols, phytosterols, phytol	Hard to scale up; Generation of heat leads to degradation of thermolabile compounds; Low efficiency when using volatile solvents.	[141,148–152]
Ultrasound-assisted extraction (UAE) (C) Ultrasound Equipment – Ultrasonic bath, Ultrasound probe; Frequency – 20 – 60 kHz; Power – 100 – 750 W; Temperature – 20 – 60 °C; Time – 2 – 720 min; Solvents: ethanol, 0,03 M HCl, methanol, water; Small sample – 1 – 10 g. (IP) Ultrasonic frequency, power, time and medium.	*Hormosira banksia, Ascophyllum nodosum, Ascophyllum nodosum, Laminaria hyperborean, Ecklonia cava, Gelidium pusillum, Sargassum muticum, Osmundea pinnatifida, Codium tomentosum, Laurencia obtuse, Porphyra yezoensis*	Polyphenols, laminarin, phycobili-proteins, taurine, fucose, uronic acid, antioxidants, prebiotic compounds	High power consumption and difficult to scale up.	[153–157]

High pressure methods "Subcritical Water Extraction (SWE)" "Pressurized liquid extraction (PLE)" "Accelerated solvent extraction (ASE)" (C) Water extraction: Pressure – 1,3 – 52 MPa; Temperature – 50 – 420 °C; Time – 5 – 25 min; Solvent Extraction: 50–200 °C; 3.5–20 MPa (IP) Temperature (C), solvent concentration (%), static time (min), pressure (psi), weight of sample (g), and flush volume (%).	*Ascophyllum nodosum, Fucus spiralis, Codium fragile, Cystoseira abies-marina, Sargassum muticum, Padina pavonica, Fucus serratus, Laminaria digitata, Gracilaria gracilis, Porphyra spp., Sargassum vulgare, Undaria pinnatifida, Halopitys incurvus, Himanthalia elongate, Pelvetia canaliculata, Ulva intestinalis. Saccharina japonica, Ulva lactuca, Fucus vesiculosus, Dictyota dichotoma, Cystoseira baccata, Himanthalia elongate*	Polyphenols, phlorotannins, fucoidan, total organic carbon, minerals, monosaccharides, amino acids, polar compounds; fatty acids	Not suitable for thermolabile compounds; Less selective than SFE.	[141,158–160]
Enzyme-assisted extraction (EAE) (C) Time 1−4 h Temperature 40−60 °C The ratio of enzyme to substrate ~ 0.5–5 % (IP) Type, activity and amount of enzyme used, pH. Absence of endogenous enzymes.	*Sargassum horneri,brown seaweeds, Undaria pinnatifida, Sargassum coreanum*	Antioxidants, fucoxanthin, fatty acids, polysaccharides	Costs of enzymes are very high; Selectivity of enzymes.	[161,162]
Ionic liquids extraction (ILE) (C) Chemicals: For phenolic extraction 0.5 M [C4C1im][BF4], 1:32 w/v mixing ratio; time 24 h, stirring at 500 rpm; Optional extraction vessel and pressure. Extraction conditions (ionic liquids used) strongly depends on target compound. (IP) Chemicals, vessel, pressure used.	*Kappaphycus alvarezii,* *S. japonica*	Phenolic compounds, polysaccharides, carrageenan, terpenoids, alkaloids	Some ILEs require purification process	[163–165]
Pulsed electric fields (PEFs) (C)field strength of 0.5–1.0 kV/cm treatment time 100–10,000 µs or 1–10 kV/cm and shorter time (5–100 µs) (IP) Field strength, time, conductivity of intact and disintegrated cells	-	Phenols, proteins	Optimization of process by using different parameters is needed. These include pulse duration, pulse interval, electric field strength, or other electrical pulse shapes.	[126,166]

PUBLICATIONS ARISING FROM THESIS

Paper I: 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)

Paper II: 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)

Paper III: 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 (accepted)

Paper IV: 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)

Paper V: 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)

Paper VI: Sabūnas, A., Romagnoli, F., Pastare, L., Bāliņa, K. Laboratory Algae Cultivation and BMP Tests with Ulva intestinalis from the Gulf of Riga. *Energy Procedia*, 2017, 113, pp. 277-284 (Indexed in Scopus)

Paper VII: 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)

Paper I

Use of Macroalgae for Bioenergy Production in Latvia: Review on Potential Availability of Marine Coastline Species

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Use of macroalgae for bioenergy production in Latvia: review on potential availability of marine coastline species

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Abstract

Macroalgae have recently attracted attention as a possible feedstock for energy. Macroalgae have high productivity, which is high as or higher than terrestrial plants, and macroalgae do not compete with crops for arable land. Macroalgal biomass provides environmentally and economically feasible alternatives to fossil fuels.

The Baltic Sea is under great environmental stress and suffers from environmental problems such as eutrophication. Macroalgae have a great potential eutrophication effect through nutrient removal processes, even if an excess of algae growing could create a lack of oxygen within the biota with an impact on the biodiversity dimension. Thus a macroalgae-based industrial system would be beneficial for the overall nutrient level in the Baltic Sea and become a favourable and sustainable feedstock for energy purposes.

Intensive cultivation of macroalgae is likely to increase with the development of an algal biofuels industry and algal bioremediation. However, target macroalgae species suitable for cultivation on the Latvian coastline have not yet been identified.

The review focuses on macroalgae species abundant on the coastline of Latvia. Biomass potential of the three Baltic Sea species representative of genera: *Fucus vesiculosus, Furcellaria lumbricalis, Ulva intestinalis* were compared and their suitability for energy production was investigated. Productivity, growth and biochemical composition was evaluated to estimate potential for biomass applications.

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Keywords: seaweeds; chemical composition; biomass; Baltic Sea; third generation

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1. Introduction and background

Seaweed or marine macroalgae have received attention as third generation biofuels feedstock, which present advantages over first and second generation biofuels [1]. Marine macroalgae have been considered as a valuable resource for energy production [2, 3]. Utilization of marine macroalgal biomass to produce bioenergy is re-emerging due to several reasons. Firstly, macroalgae are consuming CO_2 and thus lowering CO_2 emissions and maintaining closed carbon cycle [4]. Secondly, the use of macroalgae is beneficial for the food industry by avoiding direct and indirect competition on terrestrial food crops utilization for biofuel production, and preventing high food prices [5, 6]. Thirdly, compared to terrestrial crops, macroalgae have a much higher growth rate and it has been proven that macroalgae can reach 2–20 times the production potential of conventional terrestrial energy crops [7, 8]. The fourth reason is related to the environmental benefit preserving the use of forest-based biomass and preventing the conversion to biofuel from crop-based cultivation [9].

It is estimated that the energy potential of marine biomass can be even five times higher than land-based biomass [10]. Seaweed also has a higher carbon capturing potential than terrestrial crop. Macroalgal primary productivity rates are approximately 1600 g $Cm^{-2}y^{-1}$ compared to the primary productivity of land crops which have 470 g $Cm^{-2}y^{-1}$ [11]. Carbon can create approximately half of the dry weight of algal biomass but composition of macroalgae strongly depends on the season and growth conditions [12].

In Latvia most of the produced and harvested seaweed biomass is an unused resource. Some seaweed is incorporated into compost and used as a soil supplement, however mainly seaweed is left to decompose on the shore creating waste problems [13]. Seaweed can successfully be used as a bioenergy feedstock [6, 14].

2. Baltic Sea situation

The Baltic Sea is a marginal brackish sea that is poor in natural species poor and where the most species live close to their physical limits [15]. The Baltic Sea is a shallow sea with an average depth of only 60 m. Main freshwater sources include Gulf of Bothnia, Gulf of Finland and Gulf of Riga which supply equal amounts of freshwater equal as to that flowing in from the North Sea [16]. The regions of the Baltic Sea belonging to Latvia are the Gulf of Riga and the northern part of the eastern Baltic Sea. The Baltic Sea border along the coast is approximately 490 km long. The Baltic Sea near Latvia is reaching a level of salinity around 4–6 psu, while the world oceans salinity level reaches up to 30 psu [17]. Compared to other seas and oceans, the diversity of species is very low in the Baltic Sea due to its low salinity. In the Gulf of Riga where salinity is around 5.2 psu, one of the lowest macro vegetation can be found. For macroalgae as well as for other organisms it is a challenge to survive and adapt under low salinity, especially in combination with the level of eutrophication in the Baltic Sea [18].

Eutrophication is one of the most severe environmental problems of the Baltic Sea. The large amount of nutrients, especially in the last five decades, has increased nitrogen and phosphorus concentrations [19]. Eutrophication and pollution in the Baltic Sea are also endangering the growth of seaweed [20]. Eutrophication processes have raised a concentration of organic material in the water column and increased the amount of sediment. It is changing ecological and biological processes not only in open seas, but also in coastal areas [21]. In the study of Berger et al. [22] the authors have reported that, as result of eutrophication, perennial macroalgae stocks have decreased, but it is not clear which stage of algal development it is affecting. Opposite processes are occurring in microalgae and annual macroalgae [23, 24]. A large blooming of macroalgae biomass and changes in the composition of species is a sign of eutrophication [25].

Macroalgae mats are able to float far distances and accumulate ashore on the beaches. Although it is a natural coastal process, problems occurs in its scale and location. Washed out algal mats are an important problem during the tourist season when the smell and decomposing algae decreases the recreational value of beaches. Macroalgal natural habitats are not located near the beach, waves and currents are bringing them there and it mainly depends on wind speed and direction [26]. Appearance of macroalgae on a beach is not a permanent or regular process, so the composition and amount of washed out biomass is not predictable [27]. The amount of washed out algae is not monitored in Latvia.

3. Characteristics of macroalgae

Macroalgae is an important key part of the ecosystem and abundance, distribution and structural characteristics display large variation both dimensional and temporal. The variation is important for ecosystem functions and may affect the dynamics of associated species [28]. In coastal ecosystems macroalgae is affected by multiple stressors like climate change, eutrophication and habitat destruction [29]. However these species that have managed to adapt to these disturbing factors also have developed thicker cell walls [17].

There are three types of seaweed abundant in the Baltic Sea. An important part of the seaweed is normally attached to the substrate, some of the seaweed are detached of substrate and some algae are distributed on the coastline and are called algae wreck [18]. In the Baltic Sea and in the Gulf of Riga substrate usually is sandy, muddy and stony, partially covered with seashells. Waves and streams can easily move the substrate around not letting seaweed to grow and form algal stands [17]. Growing and harvesting of macroalgae removes nutrients from a system, reducing nutrient loading and therefore the possibility of eutrophication [30].

Currently in Latvia there are no data available about macroalgal species composition. In Finland, in an area where the level of salinity is the same as the average salinity in Latvia (5.7 psu), there are 17 macroalgal species abundant in total [31]. Following species of algae have been evaluated as abundant in Latvia: 4 brown algae (*Phaeophyceae*), 9 red algae (*Rhodophyta*) and 4 green algae (*Chlorophyta*) species can be obtained on the coastline of Latvia. Most common seaweed species of these genera are: *Fucus vesiculosus*, *Furcellaria lumbricalis*, *Ulva intestinalis* [32]. On the coastline of open sea *Furcellaria sp.* is observed more often, but in the Gulf of Riga, the brown algae *Fucus vesiculosus* are more abundant. Green algae, forming filaments on rocks or other substrates are widely distributed on the coastline of Latvia [30].

Fucus vesiculosus also called bladder wrack is one of the most common seaweeds in the Baltic Sea [20]. In the last decades in some Baltic Sea regions, the amount of these algae has decreased. Disappearance of these algae can be explained with the increase of eutrophication, local leakages of toxic components and amount of herbivores using algae as feed [33].

F. vesiculosus is perennial brown algae in the Baltic Sea. In Latvia they grow in a depth 1.5–5.5 m. They can form new thalli from rhizoids. Thalli can live several years and when they die, rhizoid can form new thalli. The length of the *F. vesiculosus* life can reach up to even 50 years [19]. Bladder wrack genus has dichotomous forked ribbon thallus with well-marked central vein. It can be 50 cm high and it is held in a vertical position with the help of the air bladders [34]. In literature information about chemical composition in bladder wrack may differ. *F. vesiculosus* contains 70–85 % water and dry matter is only 15–30 %.

Furcellaria lumbricalis is one of few red algae members adapted to brackish water and is abundant in the Baltic Sea. Attached and unattached forms of *F. lumbricalis* exist. The attached form can be found practically throughout the photic range on hard substrates at salinities down to 3.6 psu in the Baltic Sea. In the Baltic Sea the unattached form is found only in few habitats near Estonia [35]. Unlike the common attached form, the unattached form of *F. lumbricalis* can reproduce only vegetative, mainly by thallus fragmentation [36].

Ulva intestinalis is a filament forming species of green algae. Filaments are typically unbranched and may be 10–30 cm or more in length. It is abundant in different levels of salinity and very common in brackish water areas, where there is appreciable fresh water runoff and in wet areas of the splash zone [37]. It can grow on different substrates: rocks, mud, sand and even on other algae and seashells. Detached from the substratum it can continue its growth in floating masses [38]. *U. intestinalis* is a summer annual, decaying and forming masses of bleached white fronds towards the end of the season [39].

4. Chemical composition

Macroalgae has very high water content. Water can make 70–90 % of macroalgal biomass. Compared to microalgae, macroalgae has lower level of proteins and lipids, but macroalgae contain more carbohydrates [40]. Red algae and brown algae have a lower lipid content than green algae [41]. Macroalgae are rich with carbohydrates, but amount of it may differ between species – red algae 30–60 %, green algae 25–50 %, brown algae 30–50 % [42, 43]. For the most part, carbohydrates in a form of starch and/or cellulose are found in the cell wall [41].

Algal biomass is rich with nutrients such as carbon, nitrogen and phosphorus. Algal chemical composition is one of the most important factors about algal use to produce bioenergy. Methane potential and ammonium yields can be calculated using chemical composition [44].

Current studies on macroalgae fermentation to ethanol show low yields [45] therefore this review is focusing on anaerobic digestion of macroalgae to produce biogas.

Important factor for biogas production is optimal C/N mass content, which should be between 20 and 30 [46]. If C/N ratio is lower than 20 too much ammonium is produced during anaerobic fermentation, which creates and unsuitable environment for methanogen bacteria and decreases the rate of methane production, so it is suggested to add some carbon-rich biomass such as straw or fallen leaves [47]. This ratio was taken as main aspect to analyse the suitability of marine macroalgae on coastline of Latvia.

Three main species of macroalgae were compared to get the view about use of these algae as feedstock for energy production (Table 1). Data obtained from research carried out in the Gulf of Gdansk [48] and results of analysis of *F. vesiculosus* were analysed.

Table 1. Characteristics of macroalgae abundant on Latvian coastline (a – Gulf of Gdansk, b – Gulf of Riga) [45].

Species	C, kg/t d.w.	N, kg/t d.w.	P, kg/t d.w.	Mass ratio		Atomic ratio	
				C/N	N/P	C/N	N/P
Ulva intestinalis (a)	264.33	19.73	1.60	13	12	18	32
Fucus vesiculosus (a)	365.67	14.17	1.57	26	9	34	23
Furcellaria lumbricalis (a)	314.75	24.13	1.23	13	20	17	51
Fucus vesiculosus (b)	369.81	20.16	-	18	-	24	-

5. Algae potential as a novel bioenergy source in Latvia

Nowadays the use of marine macroalgae biomass in Latvia is not exploited as a resource for energy purposes, this biomass is thus left or disposed of which presents an important waste problem on the shore. The potential use of washed ashore biomass in Latvia represent a potential bioenergy source even if the conversion of the most abundant macroalgae type identified within the Latvian territory (i.e. *Ulva intestinalis*, *Furcellaria lumbricalis*, *Fucus vesiculosus*) has been described only in a limited amount of literature as primary feedstock for digestion processes or other types of final energy usages. Even though the collection of natural growing marine macroalgae is restricted, washed out seaweed represent an extremely productive potential source of biomass (i.e. tonnes a day [49]).

The transformation routes of the marine macroalgae for energy can be different. Dębowski [50] identified the final algae-based fuels in terms of biogas, bioethanol, biodiesel and bio-oils specifically obtained from the conversion processes of anaerobic digestion, fermentation, transesterification, liquefaction and pyrolysis technique methods. These represent the most valuable and feasible conversion routes with some constraints related to algae biomass composition. Looking toward biogas production, there is vast literature about the yield and effectiveness of both anaerobic digestion (AD) processes [1, 41] and overall conversion for energetic purposes [3, 8].

The methane production in different algal species can vary from 100 ml CH_4 g^{-1} VS of *Ulva sp.* unwashed biomass [8] till the value for *Laminaria digitata* of 500 ml CH_4 g^{-1} VS [50]. There are several constraint factors undermining the AD conversion from algae biomass. Too high level of cellulose or hemicellulose can increase the resistance of cell wall. Different compounds (i.e. alkaline metals) cause inhibition of AD. Improper C:N ratio in the biomass have a negative impact on the fermentation processes [51, 52]. Normal C:N ratio is among 20–30. Usually too high nitrogen content in some algae is lowering the optimal ratio. Physical or chemical pre-treatments can be favourable in terms of breaking down of cell walls facilitating a more easy access to the algae matter from the methanogens bacteria; while co-digestion of macroalgae can correct the C:N ratios [53].

Montigelli [1] suggests a mild pre-treatment, due to the low content of lignin within the algal biomass if compared with other biomass feedstock for AD. In this perspective, the author suggests physical pre-treatments in terms of simplicity and effectiveness.

A potential energy conversion of macro algae biomass is fermentation to have bioethanol as transportation fuel [41]. Macroalgae presents a high level of carbohydrates and little lignin [54] thus can be considered a proper substrate in fermentation process prior hydrolysis process. Still, some concerns exist on the economic viability of the bioethanol production from macroalgae, since the technological processes can be expensive [45]. Nevertheless a different level of sugar in the type of seaweeds can affect the effectiveness within the transformation process.

Macroalgae is more beneficial for production routes involving biogas and bioethanol rather than biodiesel due to the low level of triglycerides [41] (i.e. microalgae lipids content range around 3–20 % d.w. [42, 55] with peaks of 90 % d.w., macroalgae lipids content range around 0.4 % and 3.5 % d.w. [1]), even though macroalgae biodiesel has been investigated with rather poor results if compared with the use of microalgae [56, 57]. Nevertheless there is experience of macroalgae conversion into bio-oil (lipids and free fatty acids) for biodiesel production [41].

It has been found that brown seaweeds have low levels of easily fermentable sugars [58], while green and red seaweeds have high levels of easily accessible sugars [58, 59].

Recently the potential use of pyrolysis for macroalgae bio-oil based production has gained attention within the scientific arena since it seems to be an acceptable method due to the high ash content of the algae compared to other biochemical conversion methods [41].

In addition to the previous mentioned final energy end-uses of algae biomass, there are other thermochemical roots for conversion of macroalgae like direct combustion [8] or even taking account the production of solid pellets. The principle bottleneck is the high value of the moisture of the raw biomass.

The overall economic feasibility of the previous identified bioenergy algae-based pathways should be investigated through a holistic perspective involving for example an LCA approach. Thanks to that would be possible to identify impacts and benefits related to the use of novel feedstocks and algae-based technologies from the cultivation and harvesting phase till the final end use. These aspects would be essential in order to evaluate the opportunity of a scaled-up system thus identifying economic and environmental sustainability benchmarks. This evaluation would be relevant as well for the Latvian context.

A preliminary sustainability analysis could be the evaluated according to the guidelines and approach proposed by McKendry [60]. The author identifies 3 macro indicators peculiar for the selection of potential novel energy crops: i) the value of the growing rate (thus the biomass yield), ii) the relative low cost (or competitive with respect to the fossil based fuel), iii) and the mechanical/physical property of the biomass. The use of the macroalgae represents a novel feedstock with a relative higher growing rate respect any other terrestrial plants, this is beneficial in reference to the first indicator identified by MacKendry. Nevertheless due to the not complete maturity of the bioenergy algae-based systems and due to a not favourable biofuel yields the second and third macro-indicators present more criticalities.

Cultivation is energy intensive and thus presents high costs. Marginal costs are also affected by the type of cultivation adopted. Thus the energy production from seaweed should be framed within a bio-refinery perspective in fact not considering only a final end product within the transformation of the initial feedstock but rather a gamma of specific valuable products – i.e. feed for animal, or alginate extraction. In this way the overall cost impact would be share on several final products or sub products. Energy inputs related to aeration of the ponds and/or feeding are also recognized as environmental hot spots within the whole biomass production phase. The study have shown that aeration can be reduced once the concentration of the nutrient is sufficient [8]. In a cost-benefit evaluation, the high level of moisture (i.e. 78 % and 90 % [58, 61]) which represents a negative impact on the overall economic and energetic transformation efficiency should also be considered. Recent challenges are looking toward pyrolysis, gasification as well as hydrothermal carbonisation.

Even if the effect of a high eutrophication of the sea produces an over accumulation of algae biomass on the shore, in an overall evaluation the loss of biodiversity and disturbance to the local flora and fauna should also be considered due the depletion of the resource in the harvesting phase.

6. Conclusions

Latvia currently does not have any industry or pilot scale project for macroalgae production and/or cultivation, but there are some species that are suitable for cultivation in the Baltic Sea and ready to be potentially exploited. The most abundant algae in the Baltic Sea are green algae *Ulva intestinalis*, brown algae *Fucus vesiculosus* and red algae *Furcellaria lumbricalis*. These types of macroalgae present a higher biomass yield and a high photosynthetic efficiency if compared to terrestrial crops.

AD technology is at a rather mature level on implementing seaweed biomass as possibility to optimize biogas and biomethane yields. Innovative conversion processes such as bioethanol, gasification, pyrolysis and hydrothermal carbonation from wet macroalgae are promising opportunities but still at an infancy level.

The washed ashore seaweed biomass collected during beach cleaning in Latvia can be used as energy feedstock even if there are important legal restrictions related to the protection of natural stocks. The distribution of seaweed and amount of seaweed washed ashore is not monitored in Latvia, but should be done to evaluate macroalgae as a potential source of energy in Latvia. The overall potential use of macroalgae from the bioenergy perspective must be evaluated from life cycle and sustainability perspectives and should take into account the beneficial effect of using algae and not only to focus on the sole energy purpose.

This study wanted to provide a preliminary overview about the marine macroalgae potential in Latvia and opportunities of final energy end usages from this biomass. The study underlined the principle and more common types of sewed available in Latvia and opportunity of their use in the Latvian content as potential alternative energy crops. The potential of algal biomass for bioenergy purpose in not yet at its mature phase even though several realities are recognized as already economically feasible and sustainable.

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References

[1] Montingelli ME, Tedesco S, Olabi AG. Biogas production from algal biomass: A review. Renew Sustain Energy Rev 2015;43:961–72.
[2] Bird MI, Wurster CM, De Paula Silva PH, Paul N a., De Nys R. Algal biochar: effects and applications. GCB Bioenergy 2012;4:61–9.
[3] Lauka D, Pastare L, Blumberga D, Romagnoli F. Preliminary Analysis of Anaerobic Digestion Process using Cerathophyllum demersum and Low Carbon Content Additives: A Batch Test Study. Energy Procedia 2015;72:142–7.
[4] Timilsina GR, Mevel S. Biofuels and Climate Change Mitigation: A CGE Analysis Incorporating Land-Use Change. Environ Resour Econ 2013;55:1–19.
[5] Sexton S, Rajagopal D, Zilberman D, Hochman G. Food versus fuel: how biofuels make food more costly and gasoline cheaper. ARE Update 2008;12(1):1–6.
[6] Pastare L, Romagnoli F, Rugele K, Dzene I, Blumberga D. Biochemical Methane Potential from Anaerobic Digestion of the Macrophyte Cerathophyllum Demersum: A Batch Test study for Latvian Conditions. Energy Procedia 2015;72:310–6.
[7] Gremillet D, Lewis S, Drapeau L, Van Der Lingen CD, Huggett JA, Coetzee JC, et al. Spatial match-mismatch in the Benguela upwelling zone: Should we expect chlorophyll and sea-surface temperature to predict marine predator distributions? J Appl Ecol 2008;45:610–21.
[8] Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, et al. Bioenergy potential of Ulva lactuca: biomass yield, methane production and combustion. Bioresour Technol 2011;102:2595–604.
[9] Gao Y, Skutsch M, Masera O, Pacheco P. A global analysis of deforestation due to biofuel development. Cent International For Res 2011:68.
[10] Chynoweth DP, Owens JM, Legrand R. Renewable methane from anaerobic digestion of biomass. Renew Energy 2001;22(1–3):1–8.
[11] Hughes AD, Kelly MS, Black KD, Stanley MS. Biogas from Macroalgae: is it time to revisit the idea? Biotechnol Biofuels 2012;5:86.
[12] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.
[13] Merceron M, Antoine V, Auby I, Morand P. In situ growth potential of the subtidal part of green tide forming Ulva spp. stocks. Sci Total Environ 2007;384:293–305.
[14] Cappelli A, Gigli E, Romagnoli F, Simoni S, Blumberga D, Palerno M, et al. Co-digestion of Macroalgae for Biogas Production: An LCA-based Environmental Evaluation. Energy Procedia 2015;72:3–10.
[15] Johannesson K, Johansson D, Larsson KH, Huenchuñir CJ, Perus J, Forslund H, et al. Frequent clonality in fucoids (Fucus radicans and Fucus vesiculosus; Fucales, phaeophyceae) in the Baltic sea. J Phycol 2011;47:990–8.
[16] Ojaveer H, Jaanus A, Mackenzie BR, Martin G, Olenin S, Radziejewska T, et al. Status of biodiversity in the Baltic Sea. PLoS One 2010;5(9).
[17] Znotiņa V. Juras biotope; 2014. Available: http://latvijas.daba.lv/biotopi/jura.shtml
[18] Suursaar U, Torn K, Martin G, Herkul K, Kullas T. Formation and species composition of stormcast beach wrack in the Gulf of Riga, Baltic Sea.The study was supported by the EU Life+project MARMONI. Innovative approaches for marine biodiversity monitoring and assessment of conservation status of nature v. Oceanologia 2014;56:673–95.
[19] Kautsky L, Svensson S. Life in the Baltic Sea. Environ Sci Understanding, Prot Manag Environ Balt Sea Reg 2003:148–81.
[20] Torn K, Krause-Jensen D, Martin G. Present and past depth distribution of bladderwrack (Fucus vesiculosus) in the Baltic Sea. Aquat Bot 2006;84:53–62.
[21] Seghetta M, Ostergard H, Bastianoni S. Energy analysis of using macroalgae from eutrophic waters as a bioethanol feedstock. Ecol Modell 2014;288:25–37.
[22] Berger R, Bergstrom L, Gran E, Kautsky L. How does eutrophication affect different life stages of Fucus vesiculosus in the Baltic Sea ? – a conceptual model 2004:243–8.
[23] Aleksandrov S V. Biological production and eutrophication of Baltic Sea estuarine ecosystems: the Curonian and Vistula Lagoons. Mar Pollut Bull 2010;61:205–10.

[24] Zbikowski R, Szefer P, Latała A. Comparison of green algae Cladophora sp. and Enteromorpha sp. as potential biomonitors of chemical elements in the southern Baltic. Sci Total Environ 2007;387:320–32.

[25] Eriksson BK, Bergstrom L. Local distribution patterns of macroalgae in relation to environmental variables in the northern Baltic Proper. Estuar Coast Shelf Sci 2005;62:109–17.

[26] Burkholder JM, Tomasko DA, Touchette BW. Seagrasses and eutrophication. J Exp Mar Bio Ecol 2007;350:46–72.

[27] Filipkowska A, Lubecki L, Szymczak-Zyla M, Lotocka M, Kowalewska G. Factors affecting the occurrence of algae on the Sopot beach (Baltic Sea). Oceanologia 2009;51:233–62.

[28] Kersen P, Orav-Kotta H, Kotta J, Kukk H. Effect of abiotic environment on the distribution of the attached and drifting red algae Furcellaria lumbricalis in the Estonian coastal sea. Est J Ecol 2009;58:245.

[29] Garden CJ, Smith AM. Voyages of seaweeds: The role of macroalgae in sediment transport. Sediment Geol 2015;318:1–9.

[30] Strunga A. Mikro un makro algu audzesanas un parstrades iespejas Baltijas juras regiona. Liepaja: Submariner; 2013.

[31] Schubert H, Feuerpfeil P, Marquardt R, Telesh I, Skarlato S. Macroalgal diversity along the Baltic Sea salinity gradient challenges Remane's species-minimum concept. Mar Pollut Bull 2011;62:1948–56.

[32] The Latvian Rural Advisory and Training Centre. Latvijas zivsaimniecības gadagrāmata; 2014. Available: http://www.laukutikls.lv/sites/laukutikls.lv/files/informativie_materiali/zivsaimniecibas_gadagramata_2014.pdf

[33] Wikstrom SA, Kautsky L. Structure and diversity of invertebrate communities in the presence and absence of canopy-forming Fucus vesiculosus in the Baltic Sea. Estuar Coast Shelf Sci 2007;72:168–76.

[34] Piterans A, Vimba E. Zemako augu sistematikas praktikums. Rīga: ZVAIGZNE; 1987.

[35] Malm T, Isæus M. Distribution of macroalgal communities in the central Baltic Sea 2005:257–66.

[36] Kersen P. Red seaweeds Furcellaria lumbricalis and Coccotylus truncatus: community structure, dynamics and growth in the northern Baltic Sea. Tallinn: Tallinn University; 2013.

[37] Alstrom-Rapaport C, Leskinen E, Pamilo P. Seasonal variation in the mode of reproduction of Ulva intestinalis in a brackish water environment. Aquat Bot 2010;93:244–9.

[38] Aguilera-Morales M, Casas-Valdez M, Carrillo-Domínguez S, Gonzalez-Acosta B, Perez-Gil F. Chemical composition and microbiological assays of marine algae Enteromorpha spp. as a potential food source. J Food Compos Anal 2005;18:79–88.

[39] Pihl L, Svenson A, Moksnes P-O, Wennhage H. Distribution of green algal mats throughout shallow soft bottoms of the Swedish Skagerrak archipelago in relation to nutrient sources and wave exposure. J Sea Res 1999;41:281–94.

[40] Jung KA, Lim S-R, Kim Y, Park JM. Potentials of macroalgae as feedstocks for biorefinery. Bioresour Technol 2013;135:182–90.

[41] Chen H, Zhou D, Luo G, Zhang S, Chen J. Macroalgae for biofuels production: Progress and perspectives. Renew Sustain Energy Rev 2015;47:427–37.

[42] Roesijadi G, Jones SB, Zhu Y. Macroalgae as a Biomass Feedstock : A Preliminary Analysis; 2010. Available: https://www.pnl.gov/main/publications/external/technical_reports/PNNL-19944.pdf

[43] Yazdani P, Zamani A, Karimi K, Taherzadeh MJ. Characterization of Nizimuddinia zanardini macroalgae biomass composition and its potential for biofuel production. Bioresour Technol 2015;176:196–202.

[44] Aitken D, Bulboa C, Godoy-Faundez A, Turrion-Gomez JL, Antizar-Ladislao B. Life cycle assessment of macroalgae cultivation and processing for biofuel production. J Clean Prod 2014;75:45–56.

[45] Isa A, Mishima Y, Takimura O, Minowa T. Preliminary Study on Ethanol Production by Using Macro Green Algae. J Japan Inst Energy 2009;88:912–7.

[46] Yen H-W, Hu I-C, Chen C-Y, Ho S-H, Lee D-J, Chang J-S. Microalgae-based biorefinery--from biofuels to natural products. Bioresour Technol 2013;135:166–74.

[47] McGinn PJ, Dickinson KE, Park KC, Whitney CG, MacQuarrie SP, Black FJ, et al. Assessment of the bioenergy and bioremediation potentials of the microalga Scenedesmus sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode. Algal Res 2012;1:155–65.

[48] Bucholc K, Szymczak-Zyla M, Lubecki L, Zamojska A, Hapter P, Tjernstrom E, et al. Nutrient content in macrophyta collected from southern Baltic Sea beaches in relation to eutrophication and biogas production. Sci Total Environ 2014;473–474:298–307.

[49] Zeng S, Yuan X, Shi X, Qiu Y. Effect of inoculum/substrate ratio on methane yield and orthophosphate release from anaerobic digestion of Microcystis spp. J Hazard Mater 2010;178:89–93.

[50] Dębowski M, Zieliński M, Grala A, Dudek M. Algae biomass as an alternative substrate in biogas production technologies—Review. Renew Sustain Energy Rev 2013;27:596–604.

[51] Mata-Alvarez J, Mace S, Llabres P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technol 2000;74:3–16.

[52] Wu X, Yao W, Zhu J, Miller C. Biogas and CH(4) productivity by co-digesting swine manure with three crop residues as an external carbon source. Bioresour Technol 2010;101:4042–7.

[53] Vivekanand V, Eijsink VGH, Horn SJ. Biogas production from the brown seaweed Saccharina latissima: Thermal pretreatment and codigestion with wheat straw. J Appl Phycol 2012;24:1295–301.

[54] John RP, Anisha GS, Nampoothiri KM, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 2011;102:186–93.

[55] Chiu S-Y, Kao C-Y, Chen T-Y, Chang Y-B, Kuo C-M, Lin C-S. Cultivation of Microalgal Chlorella for Biomass and Lipid Production Using Wastewater as Nutrient Resource. Bioresour Technol 2014;184:179–89.

[56] El-Moneim M. R. Afify A, Shalaby E a., Shanab SMM. Enhancement of biodiesel production from different species of algae. Grasas Y Aceites 2010;61:416–22.

[57] Maceiras R, Rodriguez M, Cancela A, Urrejola S, Sanchez A. Macroalgae: Raw material for biodiesel production. Appl Energy 2011;88:3318–23.
[58] Bruton T (BioXL), Lyons H, Lerat Y, Stanley M, Rasmussen MB. A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. Sustain Energy Irel 2009:92.
[59] Percival E. The polysaccharides of green, red and brown seaweeds: Their basic structure, biosynthesis and function. Br Phycol J 1979;14:103–17.
[60] McKendry P. Energy production from biomass (part 1): overview of biomass. Bioresour Technol 2002;83:37–46.
[61] Marinho-Soriano E, Fonseca PC, Carneiro MAA, Moreira WSC. Seasonal variation in the chemical composition of two tropical seaweeds. Bioresour Technol 2006;97:2402–6.

Paper II

Chemical Composition and Potential Use of Fucus Vesiculosus from Gulf of Riga.

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Chemical composition and potential use of *Fucus vesiculosus* from Gulf of Riga

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Abstract

Seaweed biomass is washed ashore on beaches causing recreational problems for local inhabitants and tourists. The management of marine waste in Latvia is not well developed. Brown algae *Fucus vesiculosus* is one of the most abundant seaweeds in Latvia. The chemical composition of brown algae *Fucus vesiculosus*, collected from the Gulf of Riga, was evaluated. Algae contain higher amounts of both macroelements (490 – 21500 ppm; P, K, Ca, Mg, Na, Fe, Mn) and trace elements (0.11 – 930 ppm; Zn, Cu, Cr, Pb, Sr, As, Cd, Se) than terrestrial plants. The obtained composition was used to describe the potential uses of seaweed. Food, pharmacy and bioenergy were considered as potentially the best sectors for macroalgae use. Due to high levels of heavy metals in seaweed, it is not recommended to use *F. vesiculosus* from Gulf of Riga as a food. The best potential use is found to be using it as biomass feed to obtain biogas.

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Keywords: macroalgae; marine biomass; heavy metals; mineral composition; edible seaweeds; biofuels; *Fucus vesiculosus*

1. Introduction

Seaweed aquaculture is popular in Asian countries, but the seaweed natural distribution area covers the whole world including Europe and the Gulf of Riga [1]. Seaweed cultivation industry and mass production of algae is not developed in Latvia. Seaweed in the Baltic Sea is abundant in three different forms. Firstly, an important part of

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seaweed is attached to the substrate and is protected, some part of seaweed are detached of substrate and drift subordinated by waves and currents [2]. Eventually seaweed is washed up on shore, waves and currents – respectively affected by wind speed and direction [3] – define their pathway on surface and finally to the shore, creating the third form. The appearance of macroalgae on a beach is not a permanent or regular process, thus the composition and amount of the biomass washed ashore is not predictable [4]. Although it is a natural coastal process, it can be an important problem during the tourism season, when decomposing algae decreases the recreational value of beaches [5]. Historically algae waste has been used as a fertilizer on agricultural lands by local inhabitants. According to EU Bathing Water Directive 2006/7/EC, during the beach season in the places where it might interfere with people, washed out algae is collected by local municipalities [6].

In regard to macroalgae in the Latvian context, *Fucus vesiculosus* L. is a dominant brown alga on the rocky bottoms of the Baltic Sea coastal areas [7, 8]. In the coastal waters *F. vesiculosus* forms dense canopies [9]. It is the most abundant marine brown algae in the Gulf of Riga.

The use of marine macroalgae could be wide. Raw seaweed and seaweed food products in Asian countries have been consumed for centuries. More recently seaweed products as a source of polysaccharides for food and pharmaceutical uses are becoming popular in Europe [10]. The seaweed mineral content is higher than mineral level in terrestrial plants and animal products. High mineral content and low fat content makes it a perfect food supplement [11]. Polysaccharides found in seaweed make it attractive to the pharmaceutical industry. Fucoidans found in brown algae exhibit various biological activities with potential health benefits [12]. Marine seaweed biomass can also be used as a feedstock for energetic purposes. Seaweed can be transformed in different types of biofuels such as biogas, bioethanol, and biodiesel [13, 14].

This study investigates the chemical element composition of brown algae *Fucus vesiculosus* collected on the coastal zone of the Gulf of Riga. Three different types of algae end uses are discussed. The results of analysing the chemical composition and the available information is taken into account when considering the most appropriate end uses. *F. vesiculosus* can be used as a food supplement, biomass for bioenergy and as a source of pharmaceutically important matters. Due to high content of heavy metals in the biomass of *F. vesiculosus* in the Gulf of Riga, it is suggested to use this algae as a feedstock of bioenergy. It is not suggested to be used in human consumption unless heavy metals are removed.

2. Materials and methods

2.1. Sample collection

Fucus vesiculosus was manually collected from the beach zone in Jūrmala, central Latvia (56°59′ N and 23°51′ E) in January 2015. The algae was identified using a taxonomical identification key [15]. The site is characterised by shallow depths (0.5 – 1 m) and high nutrient levels. At the laboratory the algae was rinsed with freshwater to remove sand. Additives like wooden pieces, grass and shells were separated from the sample manually. The collected algae samples were preserved in a plastic bags in a freezer (-18°C) and defrosted one day before analysis.

2.2. Ash content

The ash content of the seaweed was determined by heating the dried (at 105 °C) samples in a muffle furnace (at 550 °C) following the standard method LVS EN 14775.

2.3. Chemical composition

Chemical analysis was carried out in the Latvian Environment, Geology and Meteorology Centre following the Latvian standard methodologies (US EPA Method 7060A:1994, LVS EN ISO 15586:2003, LVS ISO 11047:1998, US EPA Method 7380:1986, LVS EN ISO 7980:2000, LVS EN 14672:2005, LVS ISO 11261:2002, LVS ISO 9964-3:1993, , LVS ISO 11466:1995, LVS ISO 11463:2006. Macroelements C, H, N, S were analysed in Latvian Institute of Organic Synthesis using atomic spectroscopy.

3. Results and discussion

3.1. Chemical composition of Fucus vesiculosus

The chemical composition of the brown algae *F. vesiculosus* analysed in this study is listed in Table 1. The organic elements, macroelements, microelements and metal contents are given.

Table 1. *Fucus vesiculosus* chemical composition.

Organic element	% TS	Macroelement	mg kg^{-1} TS	Microelements	mg kg^{-1} TS	Heavy metals	mg kg^{-1} TS
Carbon (C)	36.98	Potassium (K)	11000	Iron (Fe)	490	Selenium (Se)	0.11
Hydrogen (H)	5.12	Phosphorous (P)	1400	Manganese (Mn)	1680	Lead (Pb)	11
Oxygen (O)	34.66	Calcium (Ca)	21500	Chromium (Cr)	9.6	Zinc (Zn)	89
Nitrogen (N)	2.02	Magnesium (Mg)	9300	Strontium (Sr)	930	Copper (Cu)	12.7
Sulphur (S)	2.82	Sodium (Na)	6300			Arsenic (As)	13.5
Ash (inorganic elements)	18.40					Cadmium (Cd)	1.7

The chemical analysis indicates that *F. vesiculosus* is lower in C, H and O than terrestrial biomass and higher in N and S. Similar characteristics are also reported in the study by Ross [13, 16]. By contrast, the amount of macronutrients obtained in these laboratory tests is different from that in other publications. In the study by Ross, higher values of P and N and lower amounts of Ca and Mg were reported for *F. vesiculosus* collected on the south coast of England. This could be explained by the effect related to local harvesting conditions and seasonal variations as reported and confirmed by Allen [13]. The variation observed in microelement and heavy metal composition of *F. vesiculosus* at different places is considerable and could be attributed to different geographical locations. The amount of Fe in different publications has been reported 440 mg kg^{-1} TS in the North Sea near Scotland to 2860 mg kg^{-1} TS on the South coast of England. The amount of other microelements and heavy metals also show great fluctuations depending on the gathering place.

Seaweed is able to accumulate different metals. The ability of seaweed to absorb minerals and nutrients from the surrounding environment is greater than in terrestrial plants [12]. It has been reported that levels of heavy metals in the Baltic Sea water are up to 20 times higher compared to the North Atlantic [17] thus affecting the algal biomass composition. The polluted algal growth environment has resulted in increased amounts of some minerals in seaweed. Once pollution is released into the Baltic Sea, heavy metals can remain in the water for very long periods [17]. Chemical composition of seaweed is dependent on many different endogenous and exogenous factors. Endogenous factors like the cell wall structure and the seaweed type are components that display different mineral sorbent capacity. It has been reported that brown seaweed varieties have a higher capacity to absorb minerals, because of a larger amount of polysaccharides in their walls [18]. Chemical composition of the seaweed is also affected by the exogenous factors like geographic location, season, wave exposure, and sea water temperature, mineral levels in seawater, pH level and salinity. In order to identify the reason for these differences in element composition, an in-depth analysis of sediments, water and surrounding environment is necessary.

3.2. Potential use of Fucus vesiculosus

Brown algae is not properly used in Latvia. It is known that it has been used as a fertilizer - playing an important role on local agricultural land and contributing nutrients to the beach ecosystem preserving biodiversity. Because of the high capacity to absorb environmental pollution, it can be used as a biosorbent to remove heavy metals from the environment [19].

3.2.1. Food

The level of Na is dependent on the salinity of the sea. A higher level of sodium in *Fucus vesiculosus* is found closer to the Atlantic Ocean where the salinity is higher. A high salt level is also reported in the publication of Peinado [20] who found 49.8 – 51.2 mg/g NaCl in dry weight. *F. vesiculosus* presents a high level of lipids as well as high nucleotide content. The dominating fatty acid has been mentioned oleic acid, which is classified as a monounsaturated omega-9 fatty acid. High antioxidant activity supported by high levels of nucleotides and fatty acids makes it not only nutritional, but also healthy. In addition, using *F. vesiculosus* as a food supplement can increase the umami taste and reduce the need for additional salt [20]. Concerns persist because of level of heavy metals in algae. To evaluate the level of heavy metals in seaweed, current amount of As, Cd, Pb in *Fucus vesiculosus* was compared with the maximal levels applied to edible seaweeds in France (Regulation (EC) No 629/2008 on Edible Seaweed & French Regulation). Comparison is displayed in Table 2.

Table 2. Quality criteria applied to edible seaweeds sold in France.

		Maximal level (mg kg^{-1} TS)	Current amount (mg kg^{-1} TS)
Arsenic	As	3	13.5
Cadmium	Cd	0.5	1.7
Lead	Pb	5	11

The level of arsenic in the algae analysed was 13.5 mg kg^{-1} TS, while the amount of lead (11 mg kg^{-1} TS) and cadmium (1.7 mg kg^{-1} TS) were lower. These concentrations are higher than maximum levels allowed in edible seaweed for these contaminants. The allowed level of arsenic in edible seaweed is 3 mg kg-1 TS but in *Fucus* found in Gulf of Riga this level is almost four times higher. In seaweed cadmium is found 1.7 mg kg^{-1} TS, that is three times greater than the limited amount (0.5 mg kg^{-1} TS). Amount of lead in *Fucus* found in the Baltic Sea is 11 mg kg^{-1} TS, which is more than the maximal level (5 mg kg^{-1} TS).

Fucus vesiculosus could serve as a good protein source, but the utilization of algae as a food can be complicated because of the high level of heavy metals. This means that extraction of algal compounds, such as proteins, minerals and fatty acids, for use as ingredients in food production may be a better strategy.

3.2.2. Pharmacy

Medicinal benefits of *F. vesiculosus* have already been evaluated for centuries. Simulation of the thyroid gland as a treatment for problems like obesity and cellulite has been mentioned as the main benefit to medicine from the use of this plant. Seaweed is also well known for its high iodine content which is not detected in our analysis, but is reported in many publications [10, 20, 21] . The high iodine content of *F. vesiculosus* stimulates thyroid function which boosts metabolic processes and solves problems with lipid balance which may help to reduce weight [23]. It is reported that *Fucus* can solve hormonal problems, it has anti-cancer potential, it reduces blood sugar, and it works as an anticoagulant and can be used to treat high blood pressure. As shown in Table 1, *F. vesiculosus* is also rich with calcium, magnesium, potassium, sodium and it also contains essential elements like phosphorus, selenium, manganese and zinc which could be beneficial as a food supplement. It is also mentioned in literature that algae contain A, C, E, G and B complex vitamins. Algae is also rich in alginin and mannitol, carotene and zeaxantin [12].

As mentioned above, apart from the many positive traits associated with intake of algae, there are also concerns to take into account. It is suggested to use *Fucus* as a supplement but avoid consuming it in large amounts because of the high iodine level and heavy metal contamination that can cause negative side effects [24]. Heavy metals accumulated in algae may be detrimental to human health. The amount of heavy metals found in our analysis is higher and exceeds maximum levels (as mentioned in previous section).

3.2.3. Bioenergy

Fucus vesiculosus biomass washed ashore in Latvia could be a potential bioenergy source. The transformation routes of the seaweeds for energy can be different. Dębowski [25] identified the final algae-based fuels in terms of biogas, bioethanol, biodiesel and bio-oils specifically obtained from the conversion processes of anaerobic digestion, fermentation, transesterification, liquefaction and pyrolysis methods. These represent the most valuable and feasible conversion routes with some constraints related to the algae biomass composition.

Looking toward biogas production, there is a lot of literature about the yield and effectiveness of anaerobic digestion (AD) processes [26, 27]. Several studies confirm anaerobic digestion to be an effective technology for algae biomass conversion for energetic purposes [28–30].

The methane production in *Fucus vesiculosus* can vary from 47 mL CH_4 g^{-1} VS of *F. vesiculosus* unwashed biomass up to the value of 113 mL CH_4 g^{-1} VS of pre-treated biomass [31]. Even though there are several constraint factors that can include: the resistance of cell walls to be degraded during the AD processes due to a too high level of cellulose or hemicellulose, the capability of algae to release compounds inhibiting the activity of the anaerobic bacteria (i.e. alkaline metals), and the improper C:N ratio in the biomass subjected to the fermentation processes [32, 33] (in fact to get a higher yield of CH_4, the optimal C:N ratio is necessary – i.e. 20 – 30) meaning that the nitrogen content in some algae could be too high.

In this study C:N ratio varies between 18 and 19 and these numbers are higher than reported in other publications [13, 16]. Bucholc et al. reported C:N ratio 26 for *F. vesiculosus* and this value is in the range best for AD.

Table 3. Organic element content (% TS), C/N ratios in *Fucus vesiculosus*.

Location		C	H	O	N	C/N
Gulf of Riga	$January_1$	37.07	5.11	34.55	2.05	18
Gulf of Riga	$January_2$	36.89	5.14	34.76	1.98	19
South coast of England [16]	February	32.88	4.77	35.63	2.53	13
South coast of Ireland [13]	August	26.80	3.20	44.50	1.50	18

Some of these problems can be overcome by taking into account specific physical or chemical pre-treatment methods that can favour the breakdown of cell walls facilitating easier access to the algae matter from the methanogen bacteria or mixing of macroalgae in a co-digestion in order to re-establish a correct C:N ratios more beneficial for the biogas production [33, 34]. Mechanical chopping is one of the most common pre-treatment methods in order to favour methanogen bacteria activity.

As mentioned before, an important factor (to be considered while evaluating the economic feasibility) is related to the overall biogas/biomethane yield depending on the pre-treatment procedure [26]: a proper method can better enhance the biomethane fraction within the biogas mix. Montigelli [26] suggests a mild pre-treatment, due to the low content of lignin within the algal biomass if compared with other biomass feedstock for AD. In this perspective, the author suggests physical pre-treatments in terms of simplicity and effectiveness.

A potential method for energy conversion of macro algae biomass is fermentation to obtain bioethanol as transportation fuel [27]. Macroalgae presents a high level of carbohydrates and a small amount of lignin [36] thus can be considered a proper substrate in fermentation prior hydrolysis process. Although there are some concerns on the macroalgae bioethanol production yield [37].

Macroalgae is more beneficial for production routes involving biogas and bioethanol rather than biodiesel due to the low level of triglycerides [27] (i.e. the microalgae lipid content ranges from 3–20 % d.w. [38] with a peak of 90 % d.w., macroalgae lipid content range from 0.4 to 3.5 % d.w. [26]). Macroalgae biodiesel has been investigated with rather poor results if compared with the use of microalgae [39,40].

It has been found that brown seaweed has low levels of easily fermentable sugars [41] thus it would be more beneficial for standard AD that requests pre-treatment to break the polysaccharides into monomers prior to hydrolysis.

4. Conclusion

Fucus vesiculosus is a dominant brown alga growing on the rocky bottoms of the Baltic Sea coastal area. Algae washed up on shore can cause recreational problems and should be removed. Within this study, the chemical element composition was determined in order to find the potentially most appropriate use for such type of algal biomass.

F. vesiculosus chemical analysis showed a high level of different minerals. Algae could successfully be used as a food or pharmaceutical compound, but based on the analysis this final use pathway is not suggested due to high levels of heavy metals, which exceed permissible levels. Nevertheless, the potential use of *F. vesiculosus* biomass as a feedstock for bioenergy is proposed. In fact *F. vesiculosus* show chemical properties, suitable for use in anaerobic digestion.

Further research is necessary to evaluate the available algal biomass in the Gulf of Riga. More analysis is required to assess chemical composition in different seasons.

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References

[1] Eriksson BK, Bergström L. Local distribution patterns of macroalgae in relation to environmental variables in the northern Baltic Proper. Estuar Coast Shelf Sci 2005;62:109–17.
[2] Suursaar Ü, Torn K, Martin G, Herkül K, Kullas T. Formation and species composition of stormcast beach wrack in the Gulf of Riga, Baltic Sea **The study was supported by the EU Life+project MARMONI 'Innovative approaches for marine biodiversity monitoring and assessment of conservation status of nature v. Oceanologia 2014;56:673–95.
[3] Burkholder JM, Tomasko DA, Touchette BW. Seagrasses and eutrophication. J Exp Mar Bio Ecol 2007;350:46–72.
[4] Filipkowska A, Lubecki L, Szymczak-Żyła M, Łotocka M, Kowalewska G. Factors affecting the occurrence of algae on the Sopot beach (Baltic Sea). Oceanologia 2009;51:233–62.
[5] Burkholder JM, Tomasko DA, Touchette BW. Seagrasses and eutrophication. J Exp Mar Bio Ecol 2007;350:46–72.
[6] EU. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Off J Eur Union 2006;L 064:37–51.
[7] Lago-Lestón A, Mota C, Kautsky L, Pearson GA. Functional divergence in heat shock response following rapid speciation of *Fucus spp.* in the Baltic Sea. Mar Biol 2010;157:683–8.
[8] Torn K, Krause-Jensen D, Martin G. Present and past depth distribution of bladderwrack (*Fucus vesiculosus*) in the Baltic Sea. Aquat Bot 2006;84:53–62.
[9] Piazzi L, Balata D, Cinelli F. Species composition and morphological groups of macroalgal assemblages around Gorgona Island (north-western Mediterranean Sea). Cryptogam Algol 2004;25:19–38.
[10] Maehre HK, Malde MK, Eilertsen KE, Elvevoll EO. Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. J Sci Food Agric 2014;94:3281–90.
[11] RUPEREZ P. Mineral content of edible marine seaweeds. Food Chem 2002;79:23–6.
[12] Taylor S. Marine Medicinal Foods: Implications and Applications. Macro and Microalgae. Elsevier Inc; 2011.
[13] Allen E, Wall DM, Herrmann C, Xia A, Murphy JD. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed? Energy 2015;81:352–60.
[14] Pastare L, Romagnoli F, Rugele K, Dzene I, Blumberga D. Biochemical Methane Potential from Anaerobic Digestion of the Macrophyte Cerathophyllum Demersum: A Batch Test study for Latvian Conditions. Energy Procedia 2015;72:310–6.
[15] Rudzroga A. Izplatītāko Latvijas aļģu noteicējs. Rīga: Zinātne; 1995.
[16] Ross AB, Jones JM, Kubacki ML, Bridgeman T. Classification of macroalgae as fuel and its thermochemical behaviour. Bioresour Technol 2008;99:6494–504.
[17] HELCOM. Heavy Metal Pollution to the Baltic Sea in 2004. Balt Sea Environ Proceedings No 108; 2007.
[18] Wei N, Quarterman J, Jin Y-S. Marine macroalgae: an untapped resource for producing fuels and chemicals. Trends Biotechnol 2013;31:70–7.
[19] Murphy V, Hughes H, McLoughlin P. Comparative study of chromium biosorption by red, green and brown seaweed biomass. Chemosphere 2008;70:1128–34.
[20] Peinado I, Girón J, Koutsidis G, Ames JM. Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds. Food Res Int 2014;66:36–44.
[21] Burtin P. Nutritional Value of Seaweeds. Electron J Environ Agric Food Chem 2003;2:1579–4377.

[22] Truus K, Vaher M, Taure I. Algal biomass from *Fucus vesiculosus* (Phaeophyta): Investigation of the mineral and alginate components. Proc Est Acad Sci Chem 2001;50:95–103.
[23] Moro CO, Basile G. Obesity and medicinal plants. Fitoterapia 2000;71:73–82.
[24] Müssig K. Iodine-Induced Toxic Effects due to Seaweed Consumption. In: Preedy VR, Burrow GN, Watson RR, editors. Compr. Handb. Iodine Nutr. Biochem. Pathol. Ther. Asp., Elsevier Inc.; 2009;897–908.
[25] Dębowski M, Zieliński M, Grala A, Dudek M. Algae biomass as an alternative substrate in biogas production technologies—Review. Renew Sustain Energy Rev 2013;27:596–604.
[26] Montingelli ME, Tedesco S, Olabi AG. Biogas production from algal biomass: A review. Renew Sustain Energy Rev 2015;43:961–72.
[27] Chen H, Zhou D, Luo G, Zhang S, Chen J. Macroalgae for biofuels production: Progress and perspectives. Renew Sustain Energy Rev 2015;47:427–37.
[28] Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, et al. Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. Bioresour Technol 2011;102:2595–604.
[29] Pastare L, Romagnoli F, Lauka D, Dzene I, Kuznecova T. Sustainable use of Macro-Algae for biogas production inLatvian conditions: A preliminary study through an integrated MCA and LCA approach. Environ Clim Technol 2014;13:44–56.
[30] Pilicka I, Blumberga D, Romagnoli F. Life Cycle Assessment of Biogas Production from Marine Macroalgae : a Latvian Scenario. Environmental and Climate Technologies 2011;6:69–78.
[31] Barbot YN, Falk HM, Benz R. Thermo-acidic pretreatment of marine brown algae *Fucus vesiculosus* to increase methane production—a disposal principle for macroalgae waste from beaches. J Appl Phycol 2014;27:601–9.
[32] Mata-Alvarez J, Macé S, Llabrés P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technol 2000;74:3–16.
[33] Wu X, Yao W, Zhu J, Miller C. Biogas and CH(4) productivity by co-digesting swine manure with three crop residues as an external carbon source. Bioresour Technol 2010;101:4042–7.
[34] Vivekanand V, Eijsink VGH, Horn SJ. Biogas production from the brown seaweed *Saccharina latissima*: Thermal pretreatment and codigestion with wheat straw. J Appl Phycol 2012;24:1295–301.
[35] Romagnoli F, Systems E, Blumberga D. Biogas from Marine Macroalgae : a New Environmental Technology – Life Cycle Inventory for a Further LCA 2010;4:97–109.
[36] John RP, Anisha GS, Nampoothiri KM, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 2011;102:186–93.
[37] Isa A, Mishima Y, Takimura O, Minowa T. Preliminary Study on Ethanol Production by Using Macro Green Algae. J Japan Inst Energy 2009;88:912–7.
[38] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.
[39] El-Moneim MR, Afify A, Shalaby EA, Shanab SMM. Enhancement of biodiesel production from different species of algae. Grasas Y Aceites 2010;61:416–22.
[40] Maceiras R, Rodrı´guez M, Cancela A, Urréjola S, Sánchez A. Macroalgae: Raw material for biodiesel production. Appl Energy 2011;88:3318–23.
[41] Bruton T (BioXL), Lyons H, Lerat Y, Stanley M, Rasmussen MB. A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. Sustain Energy Irel 2009;92.

Paper III

Comprehensive literature review on valuable compounds and extraction technologies: the Eastern Baltic Sea seaweeds.

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Comprehensive Literature Review on Valuable Compounds and Extraction Technologies: The Eastern Baltic Sea Seaweeds

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Abstract **– Seaweed valuables have been researched a lot in the last decades but there is a lack of information on brackish seaweed at the eastern part of the Baltic Sea. Previous research shows that Baltic seaweed can be used as a source for phycocolloids as well as for bioenergy. The amount of available usable biomass is not clear, also seaweed in brackish seawater does not reach the dimensions such as the same species in Western parts of the Baltic Sea where the salinity is higher. Therefore, the use of this biomass must be smart to create economic benefit. Three abundant Baltic brackish seaweed species were chosen, to represent green, brown and red seaweed groups and an in-depth information analysis was made to clarify possible focus substances that could be extracted from these species. In this paper we summarize literature of common seaweed components, traditional extraction technology, and potential amount in seaweed and give an overview of novel methods for extraction of seaweed bioactive compounds.**

Keywords **– Bioeconomy; extraction; *Fucus vesiculosus; Furcellaria lumbricalis;* macroalgae; phytobenthos; *Ulva sp.***

1. INTRODUCTION

Biorefinery is an important part of the biobased economy and biotechonomy integrating different biomass conversion processes to produce energy and value-added products into a single facility. Biotechonomy is the sustainable conversion of a biomass to produce energy, food, feed, pharmaceuticals and other materials [1]–[3]. The production of these products through a biorefinery concept and in compliance with the biotechonomy approach make the cultivation and seaweed processing economically and environmentally feasible, respecting social and policy angles. Nowadays the global biorefinery concept mainly includes terrestrial biomass with plants and forest on top and only a small part has recently been devoted to algae [4].

Marine macroalgae or seaweed have the potential to partly replace terrestrial biomass. With current research going on in this field it is already declared that algae are a third generation bioresource and do not compete with food and feed plants, nor do they use resources for their growth. Valuable substances that can be found in algae can be a way to promising low-carbon

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economy. Seaweed aquaculture is already popular in Asian countries [5], but seaweed natural distribution area covers the world, including Europe and the Baltic Sea [6]. Recently seaweed products have become popular in Europe as a source of polysaccharides for food and pharmaceutical use [7], [8]. The seaweed mineral content is higher than the mineral level in terrestrial plants and animal products [9], [10]. High mineral and low-fat content makes seaweed a suitable feedstock for food and feed.

Even though seaweed compounds have recently been widely researched, there has been lack of information on brackish seaweed naturally growing on the eastern part of the Baltic Sea. In any case, the amount of available biomass is not clear, but it is known that specimens do not develop to a great size as the same species in the Western parts of the Baltic Sea. To gain the maximum benefit from a minimum amount of biomass, a smart biorefinery strategy has to be used.

In this review, a summary of seaweed biorefinery potential compiling the most common seaweed compounds and their contents have been developed. Three Baltic Sea brackish seaweed species were chosen to represent green, brown, and red seaweed groups and an in-depth information analysis was conducted to clarify possible focus substances that could be extracted from these species. Extraction techniques that would allow to use leftover biomass from extraction processes were summarized and discussed.

This review focuses on seaweeds abundant in Eastern Baltic Sea region, where salinity ranges from 5 % to 7 %. A comprehensive literature review was done to investigate the potential added value compounds contained in three Eastern Baltic typical seaweed species and their extraction technologies to build the analytical basis for prediction and planning of Baltic seaweed application pathways under the biorefinery concept. An in-depth literature search was done to summarize the research performed on seaweed extraction, and relevant quantitative and qualitative data on seaweed extraction was summarized and combined in tables.

2. Eastern Baltic Seaweed Potential

2.1. Seaweed Components

Seaweed is composed of a special composition of substances. Even though it is often considered as a close ancestor to terrestrial plants, substances found in seaweed are different [11]. Known for their high nutritional and pharmaceutical value, they are widely consumed as food and as herbal remedies to cure health problems like eczema, psoriasis, renal disorders, digestive system problems, heart and cardiovascular diseases and are even mentioned as a treatment for cancer [12]–[15]. Seaweed use as feed, food, fertilizer, fungicide, herbicide has developed a demand for seaweed as a valuable resource [15]–[17].

Nutrient 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 great differences in their nutritional composition.

Seaweed biomass has a high polysaccharide amount (Table 1) that exists in the cell wall structures and has numerous commercial applications in products such as stabilisers, thickeners, emulsifiers, food, feed, beverages, etc [18], [19]. The total amount of polysaccharides can range from 4 % to 76 % of dry weight.

TABLE 1. MAJOR SACCHARIDES AND POLYSACCHARIDES FOUND IN EACH OF THE THREE TYPES OF SEAWEED [20]

Green algae	Brown algae	Red algae
Cellulose Starch	Cellulose Laminarin starch	Cellulose Floridean starch
Mannan or galactan	Mannitol (monomer)	Agar
Heteroglycan	Alginic acid	Carrageenan
Ulvan	Fucoidans	Xylan
Xylan		Galactan

Structural features of polysaccharides give them the ability to bind water up to 20 times their weight to give hydrogel, which qualifies them to be referred to as hydrocolloids or phycocolloids. The formation of gel involves non-covalent interaction, such as hydrogen bonding, hydrophobic and ionic interaction among the constituents and are formed from cooling of heated solutions of polymers. Many polysaccharides can form hydrogels by either heating or cooling. The gel is composed of at least two components, where a polymer forms a three-dimensional network in a liquid medium such as water.

The amount of proteins in seaweed varies in relation to surrounding environmental factors and species [21]. Highest protein concentrations are reported in winter and early spring months and lowest concentrations regarding to nitrogen concentrations have been observed from July to October. In general, red and green seaweed have relatively high protein concentrations (10 %–30 % dry matter), while brown seaweed contains an average of 3 %–15 % of dry weight [22]. Brackish red seaweed *Furcellaria lumbricalis* sometimes is assimilated to *Palmaria palmata* for which protein content can represent even up to 35 % and 47 % of the dry mass. That is higher protein amount than legumes, like soybean with 35 % of protein in dry mass, meaning it can be alternative dietary addition for vegetarian and vegan diet. The amino acid composition of seaweeds can be compared to other protein sources such as eggs and soybean. For most seaweed, glutamic acid and aspartic acids together make a large part of the total amount of amino acids [21].

As photosynthetic organisms, seaweed contains pigments that are responsible for the variety of colours observed in brown, green and red seaweed. These pigments allow seaweed 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 (Table 2).

TABLE 2. DOMINANT PIGMENTS REPRESENTING THE THREE MACROALGAE GROUPS

Pigment Class	Green Algae	Brown Algae	Red Algae	Reference
Chlorophylls	Chlorophyll a, Chlorophyll b, derivatives	Chlorophyll a Chlorophyll b Chlorophyll c derivatives	Chlorophylls a Chlorophyll d derivatives	[23]
Carotenoids	α, β, γ-carotene, Xanthophylls	Fucoxanthin Xanthophylls β-carotene	Xanthophylls α, β-carotene	[20], [23]–[25]
Phycobiliproteins	–	–	Phycoerythrin Phycocyanin	[23], [24]

Chlorophyll and its derivatives are associated with a number of health benefits including antioxidant and anti-mutagenic activities which may help to prevent cancer [26]. Carotenoids found in seaweed include β-carotene, fucoxanthin, astaxanthin, violaxanthin, tocopherol, zeaxanthin and lutein [19]. Fucoxanthin is another carotenoid present in brown seaweed such as *Ascophyllum nodosum* and *Laminaria digitata* [27]. Phycobiliproteins are water-soluble pigments that are found in red seaweed and include phycoerythrin, phycocyanin and allo-phycocyanin. Previous scientific studies have reported that this group of proteins possess anti-inflammatory, liver protecting, anti-viral, anti-tumour, serum lipid reducing and anti-oxidant properties [14]. Phycobiliproteins are found in red seaweed such as *Chondrus chrispus* and *Furcellaria lumbricalis* and are responsible for the red-brown colour of these species [28].

Lipids represent only 1–5 % of seaweed dry matter and show a valuable polyunsaturated fatty acid (PUFA) composition particularly regarding with omega-3 and omega-6 acids which play a role in the prevention of cardio-vascular diseases, osteoarthritis and diabetes. The green algae show interesting levels of alpha linolenic acid. The lipid content in seaweed is very sensitive and has significant differences between species, it also varies by geographical location, season, temperature, salinity and light intensity [29]. Although oxidative stability of PUFAs in brown seaweed lipids is not clear yet, these lipids could be applied to nutraceuticals and functional foods as an oxidative stable omega-3 source.

The mineral composition varies according to genera as well as various other factors such as seasonal, environmental, geographical and physiological variations, as well as the seaweed type such as wild type and cultivated type [15]. Seaweed contains significant amounts of essential minerals (Na, K, Ca, and Mg) and trace elements (Fe, Zn, Mn, and Cu), which play an important role in building human tissues and regulating vital reactions as related elements of many metalloenzymes due to their cell surface polysaccharides (e.g., agar, carrageenan, alginic acid, alginate, salt of alginate acids, and cellulose), enabling them to absorb inorganic substances from the ambient environment [15]. The mineral content in the form of ash of the seaweed reaches levels of up to 55 % on a dry weight basis.

Phenolic compounds are a group of secondary metabolites comprising a wide variety of compounds produced by both terrestrial and aquatic plants, which include seaweed [30]. One of their most outstanding features is their antioxidant properties, as they prevent the formation of many free radicals because of their metal ion chelating capacity [20], [31]. Phenolic compounds include: flavonoids – that are associated with various bioactivities, including the antioxidant and radical scavenging activity, lignans, tannins, tocopherols, and phenolic acids [32]. Flavonoids that are known as safe and non-toxic antioxidants, have an important function to protect the plant against UV radiation [33]. The capacity of flavonoids to act as antioxidants depends on their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities [34].

2.2. Eastern Baltic Seaweed Biorefinery Potential

To estimate Baltic seaweed biorefinery potential, the most abundant species were chosen and in-depth literature research was carried out to seek for possible compositions. Findings from researched scientific literature were summarized in Table 3. It must be mentioned that data summarized in this table is not only from seaweed from the Baltic Sea but also from the same species of algae growing around the world. In this way, we can evaluate all potential quantities that could be extracted from these species of seaweed. As mentioned before, seaweed composition can change from season, location, depth and other factors both biotic and abiotic. This table shows all concentrations of the substances that can be expected from these types of biomass. Before commencing any kind of production, it is necessary to carry out in-depth composition analysis for locally available seaweed, and repeat analysis 2–4 times through the year to see the composition dynamics during the seasons.

TABLE 3. EASTERN BALTIC SEAWEED BIOREFINERY POTENTIAL

	Green algae (*Ulva intestinalis*)		**Brown algae (*Fucus vesiculosus*)**		**Red algae (*Furcellaria lumbricalis*)**	
Carbohydrates (% DW)	31.34–92	[35]–[39]	65.7	[7]	55.4	
Polysacchrides	4.9–59	[35], [38], [40]–[42]	2.31–22	[43]		
Agar					19–28	[44]
Alginate	2–59	[38]				
Furcellaran					40–50	
Cellulose					3.4–5.7	[28], [45]
Proteins (% DW)	9.49–20.60	[35], [37]–[39], [41], [42], [46], [47]	1.4–11.3	[9], [48]	13.1–28	[28], [49]–[51]
Pigments (% of total pigments)						
Chl a	0.394	[52]	0.157–5	[52], [53]	0.228	[52]
Chl b					0.078	
Chl c			0.035	[52]		
B carotenoids			0.2	[53]	13.3–28.6	[28], [52]
Fucoxantin			1			
R-phycoerythrin					0.1	[28]
Xantophyll (mg/kg)					32.8	[50]
Phenolic compounds (% ww water extracts)			18.4	[20], [53], [54]	2.25–4.6	[28], [52]
Lipids (% DW)	1.16–22.0	[9], [39], [47], [55]–[57]	3.95–4.8	[48], [58]	1 %	[49], [50]
Fatty acids (FA)		[9], [55], [56], [59]		[9], [48], [60]		[50], [51], [60]
SFA (% of total FA)	25.0–60.6		24.3		38	
C10:0			2.8–18.8			
C14:0	1.8–5.38		7.5–13.9		5.07	
C16:0	17.9–23.2		9.6–12.1		29.36	
MUFA (% of total FA)	21.81–24.8		47.1		28.80	
C16:1, n7	1.8–6.56		46.9–31.9		8.54	
C18:1, n7	7.6–15.2				4.80	
C18:1. n9	1.5–5.4		46.0		10.22	

PUFA (% of total FA)	14.8–37.1		25.8		14.45	
C16:4. n3	4.8–10.0					
C18:2. n6	4.6–5.8		7.5–10		2.48	
C18:3. n3	8.55–24.1		2.7–3.4		2.05	
C18:4. n3	4.39–14.4		2.2		0.92	
C20:4. n6	1.4–1.5		7.4		1.63	
C20:5. n3	0.8–5.43		3.7–6.7		3.26	
Minerals (mg/100g)		[47], [60]	[6], [7], [70], [76]			[60]
Mg	11		6.7		8.9	
K	12		25		42	
Ca	29		30		3.7	
Na	8.5		18		10	
P	1.7		1		1.2	
Cu	5.7		3.7		6.2	
Fe	5800		290		130	
I	130		260		84	
Mn	180		37		7.5	
Se	0.76		0.08		0.1	
Zn	21		28		23	
Total ASH (% DW)	5.42–29.4	[38], [42], [46], [47], [61]	18.74–30.30	[7], [9], [10], [54]	9–41	[45], [50], [51]

As illustrated in the table, green algae can be rich with carbohydrates, therefore can be used as a source for cellulose and alginate. Red algae are rich with pigments, that also are valuable antioxidants, therefore can be used for nutritional and pharmaceutical purposes. Values of minerals and phenolic compounds in brown algae show that those could be potential use pathways for these types of seaweed. The amount of the substances detected in the biomass depends mainly on the extraction technologies used.

3. Technological Scheme of Seaweed Extraction

3.1. Selection of Criteria for Seaweed Biomass Extraction

To determine extraction parameters for an application of seaweed extracts it is necessary to define its field of application before using the macroalgae. The degree of purity of the product and impurities are co-factors that determine the national economy sector in which the extract is to be used. In context of biorefinery, the field of application also determines the number of extraction steps, theoretical structure of the plant and technological steps [62], [63]. Seaweed composition varies significantly between species depending on nutrient availability, seasonality and other environmental factors [63], [64]. The choice of species of algae for the desired production is an important factor as it affects not only the ability to produce large-scale biomass but also the composition of valuable compounds under relevant environmental conditions. Although each species of algae offers a unique proportion of proteins, carbohydrates and lipids, some are high in lipids while others are high in protein or carbohydrates. Selection criteria should be based on their nutrient content as well as their specific use requirements [65].

The following criteria should be considered when selecting the appropriate algae for food, feed and fuel production:

- Constantly and steadily growing (open pond/sea);
- Produce large-scale biomass;
- Produce high quality and relatively constant ingredients of desirable nutritional value;
- Survive and grow seasonally and with daily climate change;
- Exhibit high photosynthesis efficiency and energy conversion rate;
- Provide minimal dirt from attachment to environment;
- Easy to collect and extract substances [66].

Selection of criteria also includes seaweed harvest, pre-treatment and storage methods [67]. According to the Baltic Marine Environment Protection Commission (HELCOM), the following seaweed species are available for biomass extraction in the Baltic Sea: *Furcellaria lumbricalis, Fucus vesiculosus, Cladophora aegagrophila, Laminaria digitata, Chorda filum, Fucus serratus, Chorda tomentosa, Fucus spiralis, Laminaria sacchari* [68]. This list includes two of the Eastern Baltic seaweed species used in this research: *Furcellaria lumbricalis* and *Fucus vesiculosus*.

In order to obtain the highest quality product, there are several steps to increase efficiency of seaweed extraction (Fig. 1).

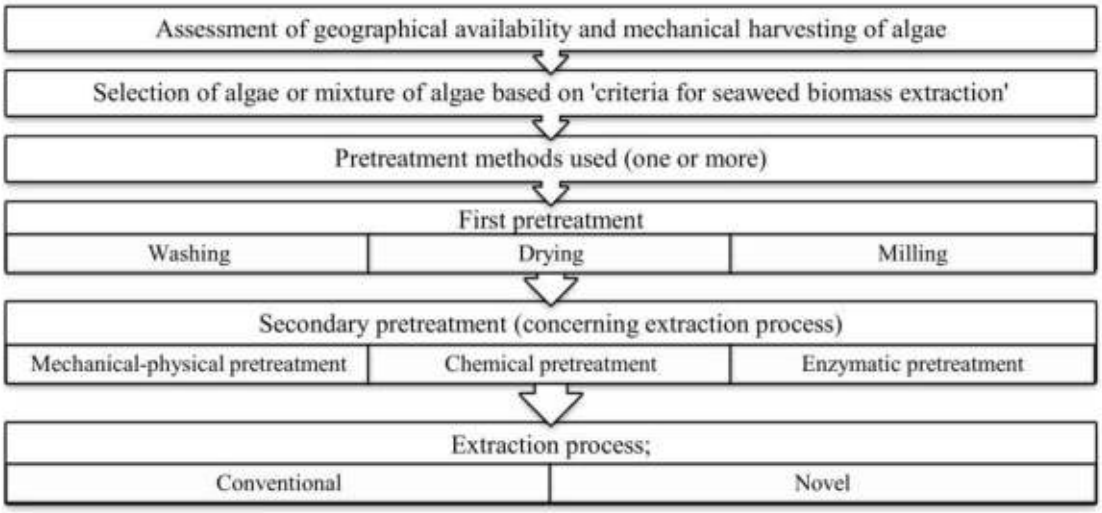

Fig. 1. Scheme of seaweed handling before extraction.

Extraction process of seaweed can be done in different ways depending on product quality parameters and specific biomolecules needed. Based on previous work [62], it is clear that the use of biorefinery principles is needed to ensure the economical and sustainable extraction of algae products. The conceptual model proposed in the previous work states that a high added value product is obtained and biomass is used with maximum efficiency meaning that physical, chemical and biological transformation processes must operate in a sequential system and in a symbiotic operation to ensure efficient, and hence more profitable, product production [62].

Existing scientific literature offers two perspectives on extraction. The first approach is: (a) based on the treatment of substrates under defined conditions with conventional extraction methods, in this case, seaweed extraction to obtain biomolecules, (b) second approach is based on novel extraction techniques and methods that reduce the cost of extraction, reduce the number of extraction steps and increase the yield of biomolecules.

Traditional and innovative methods can be combined to get the best extraction yield at the lowest cost and least impact on the environment. Traditional extraction methods are based on thermomechanical effects and chemical hydrolysis processes, while novel techniques are a significant improvement on existing technologies and are based on the use of physical phenomena (pressure, electric field, ultrasound, microwaves) and biological (enzymes) effects on the matrix [69], [70]. This review article does not address groups of substances or compounds that are relatively unexplored and commercially insignificant.

Just before the extraction of the bioactive substances, it is necessary to process the biomass in order to obtain maximum yield. Secondary pre-treatment methods are divided into three groups of methods that can be used to extract different bioactive substances – lipids, Pigments and sugars [71]:

- Mechanical-physical pre-treatment methods e.g. autoclaving, bead-beating, microwave, sonication, freeze-drying, mechanical crushing, lyophilization and pulsed electric field technology.
- Chemical pre-treatment methods e.g. liquid nitrogen, nitric acid, acetic acid, hydrolysis by NaOH, HCl, H_2SO_4, NaCl solution, nitrous acid.
- Enzymatic pre-treatment methods e.g. cellulase, protease K, driselase, alginate lyase S.

3.2. Conventional Extraction Techniques

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. The main purpose of these aggressive substances is to disrupt cell membranes and allow substances contained in the algae to enter the extraction matrix. According to current trends, the solvent used in the extraction process should be cheap and non-toxic [71].

Several types of extraction methods have been used based on the literature on extraction of bioactive compounds from various matrices. Existing conventional extraction methods include:

1. Hydrodistillation;
2. Soxhlet extraction;
3. Maceration;
4. Percolation;
5. Infusion;
6. Decoction; hot continuous extraction [72].

Effectiveness of these methods depends on various influencing parameters, such as solvent properties (polarity, toxicity, volatility, viscosity, and purity), sample size and concentration, particle size, time, polarity of extractant [73], [74]. Drawbacks of conventional techniques are long extraction time, need for very high purity solvents, energy consumption associated with evaporation of a large amount of solvent, relatively low extraction yield, selective and thermolabile degradation of the components used [75]. Traditional extraction methods are relatively well described in scientific literature (lab scale). Environmental policy and resource consumption, scientific research viewpoint has advanced green extraction methods (innovative - modern - non-conventional) [69], [70], [75], [76].

Seaweed carbohydrate extraction methods: 1) Food grade – agar, alginate, carrageenan, mannitol; 2) Nonfood grade polysaccharides – fucose-containing sulfated polysaccharides/fucoidan, laminaran, ulvan; their sources, structures and physical properties and uses are well described in Rioux and Turgeon, 2015 [77], in context of hydrocolloids [78] and dietary fibers [76]. Generally, seaweed carbohydrate compounds are extracted using the following methods: i) heating in water; ii) by heating in water with an alkali compound (e.g., sodium bicarbonate) followed by cooling, separation and purification. One of the major drawbacks of the current industrial extraction of seaweed hydrocolloids is the huge time, energy and water consumption. Extraction of seaweed hydrocolloids usually takes 3 hours to achieve optimum yield, depending on the type of hydrocolloids involved. Basically, agar, alginate, and carrageenan extraction should take 2 to 4 hours, but with green methods, it may take up to a few minutes [63], [77], [78]. Seaweed cellulose also belongs to this product group but is not mentioned because existing land-based biomass is a much more accessible and easily obtainable source of cellulose.

Extraction of seaweed proteins, peptides, and amino acids is mainly done on a laboratory scale. Main methods for extracting seaweed protein fractions in the context of traditional methods are solvent extraction, proteolytic hydrolysis (enzymes from microorganisms, plants), hydrolysis by proteolytic microorganisms during fermentation. The overall view of protein in seaweed and extraction methods, is well considered in Pangestuti and Kim, 2015; Bleakley and Hayes, 2017; Kazir *et al.*, 2019 [79]–[81]. Algae proteins are extracted by water, acid and alkali methods followed by several centrifugations, dialysis and recovery steps using methods such as ultrafiltration, precipitation or chromatography. Successful extraction of algae proteins can be greatly influenced by the availability of protein molecules, which are significantly inhibited by high viscosity and anion cell wall polysaccharides such as alginates and carrageenans [80].

Macroalgae are generally considered unsuitable for the production of oil-based products since most species have a low total lipid content <5 % by weight [64], [82]. Oils from algae, plant biomass are extracted through a variety of methods including organic solvents and water [83]. However, the green extraction process is better suited for low oil oxidation and high yield [84]. The most common traditional lipid extraction methods are water vapour extraction or solvent extraction, such as soxhlet [72].

Seaweed contains a large amount of minerals, up to 30 % of dry weight. Minerals include Na, Ca, Mg, K, Cl, S and P and trace elements (Fe, Zn, Mn, Cu). Mineral content of seaweed is generally high (8–40 %). Minerals and trace elements essential for human consumption are predominantly in brown and red algae [64], [82]. Part of the minerals from the algae biomass can be extracted by incineration and acid treatment of the resulting material [85].

3.3. Novel Extraction Techniques

Extraction of biologically active compounds from macroalgae can be conducted through novel methods. These methods are often qualified as green methods. Green methods have several advantages over conventional, including reduced amount of solvent used (including its recovery), shorter time of extraction, and technological performance at lower temperatures. These methods also include improved selectivity for isolation of the desired compounds while avoiding the formation of by-products during extraction and adverse reactions [86]. Most of the extraction methods listed below are considered "green" because they meet the standards that have crystallized in green extraction [87], [88]. Compared to conventional extraction methods, the main advantages of innovative extraction methods are higher efficiency, use of water, renewable raw materials, more environmentally friendly treatment conditions, significantly reduced use of hazardous chemicals, safer co-solvents, energy efficiency, reduced derivatives [72]. Based on the reviewed papers and others, there are six novel techniques for biomolecule extraction from seaweed [67], [71], [72], [74], [75], [86], [89]:

- Supercritical fluid extraction (SFE) – SC-CO_2;
- Microwave-assisted extraction (MAE);
- Ultrasound-assisted extraction (UAE);
- High-pressure methods (HPM);
- Ionic liquids extraction (ILE);
- Enzymes-assisted extraction (EAE);
- Pulsed electric field extraction (PEF) (see Annex Table 1).

Supercritical fluid extraction (SCF-CO_2) applies supercritical fluids to separate compound from matrix using SC-CO_2 as solvent. The most important factors affecting the extraction are pressure, temperature, time and SC-CO_2 flow rate. The prerequisite for the method is extraction in a dry environment where humidity is below 20 % in the extraction matrix. As a result, SCF-CO_2 extracts non-polar materials. The co-solvents used, such as methanol or ethanol, make the spectrum and method of extraction more efficient (for polar materials).

Microwave-assisted extraction (MAE) uses microwaves to warm the solvents in contact with solid matrix to extract contents from the solution. The solvents used, the temperature range, the time of extraction and the power used affect the MAE. This method makes it easier to obtain a spectrum of different polar compounds. The selectivity is affected by the solvent used.

Ultrasound-assisted extraction (UAE) utilizes ultrasound to penetrate solvents in contact with the solid matrix to extract content from the solution. The advantages of the UAE method are the low operating temperatures, efficient cell disruption and various extraction media. Disadvantages are high energy consumption and low extraction volumes, which significantly complicate the technology scale-up.

Enzymatic hydrolysis uses exogenous enzymes to digest material. The efficiency of the method is influenced by the enzyme used, its activity and concentration, temperature, pH. The method is ineffective at elevated temperatures due to enzyme denaturation. Hydrolysis is stopped by heating the material.

High-pressure methods use solvents under critical conditions (increased temperature and/or pressure) to speed up extraction rate of solvents used. There are different variations of high-pressure methods. For example, "Subcritical Water Extraction (SWE)" and "Accelerated Solvent Extraction (ASE)". The influencing parameters are pressure, extraction temperature, solvent concentration and time. In the case of water as a solvent and other solvents, these parameters differ significantly (see Annex Table A1).

Ionic liquid extraction uses specially designed ionic liquids to extract a wide range of compounds. Applied extraction conditions strongly depends on target compound. Pulsed electric field extraction utilizes an electric field to disintegrate cell matrix.

4. Conclusion

Literature analysis shows several reviews on extraction of biomolecules from biomass in different contexts, like conventional and novel extraction, as well as pre-treatment of algae biomass, compounds from other marine organisms such as fish and crustacean. Our review shows there are many differences in bioactive compounds between Baltic seaweed species. It is possible to extract main seaweed polysaccharides, proteins, lipids, pigments, minerals using novel methods. The studies referred to in this review show the possibility of using eastern Baltic seaweed biomass to extract different kinds of valuables. Even though the quantities of valuables can change a lot due to environmental parameters, this analysis can be used to predict and plan Baltic seaweed application pathways. Novel methods are characterized by more environmentally friendly extraction conditions, high power consumption, need for ongoing optimization of processes. Availability and quality of algae species play an important role in integrating these extraction methods (scale-up). Seaweed biorefinery focuses on single product extraction, newer literature shows increase in products and extraction techniques. For development of more than single phase extraction system, further research in different directions, regarding optimal process parameters, consumption of chemicals (co-solvents), biotechnology and extraction vessels is needed. Our analysis also shows that most extraction processes and results are obtained from laboratory-scale experiments and there is a need for industrial scale data. Limited technologies and unpredictable amounts and quality of seaweed biomass still could be serious problems to limit extraction. This review can be used as a tool to consider ways to apply cascade principle to extraction process.

Still many challenges remain with respect to use of Baltic seaweed for chemical production, such as seaweed availability and large seasonal variation in the chemical and nutritional composition of the seaweed. Seaweed biomass varies between species, locations, season and the yields and type of products obtained are highly dependent on the processing technologies. Further research is suggested to analyse seaweed biomass and change of biomass composition during the different seasons and locations.

REFERENCES

[1] Pastare L., Romagnoli F. Life Cycle Cost Analysis of Biogas Production from Cerathophyllum demersum, Fucus vesiculosus and Ulva intestinalis in Latvian Conditions. *Environmental and Climate Technologies* 2019:23(2):258–271. https://doi.org/10.2478/rtuect-2019-0067

[2] Zihare L., Gusca J., Spalvins K., Blumberga D. Priorities Determination of Using Bioresources. Case Study of *Heracleum sosnowskyi*. *Environmental and Climate Technologies* 2019:23(1):242–256. https://doi.org/10.2478/rtuect-2019-0016

[3] Blumberga D., *et al*. Energy, Bioeconomy, Climate Changes and Environment Nexus. *Environmental and Climate Technologies* 2019:23(3):370–392. https://doi.org/10.2478/rtuect-2019-0102

[4] Ree R., Zeeland A. IEA Bioenergy: Task 42 Biorefining. Netherlands: IEA Bioenergy, 2014.

[5] Food and Agriculture Organization of the United Nations. Fishery and Aquaculture Statistics. Rome: FAO, 2011.

[6] NetAlgae. Seaweed Industry in Europe. 2012:11 [Online]. [Accessed 15.04.2020]. Available: www.netalgae.eu

[7] Rioux L.-E., Turgeon S. L., Beaulieu M. Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers* 2007:69(3):530–537. https://doi.org/10.1016/j.carbpol.2007.01.009

[8] Li Y., *et al*. Optimization study on the hydrogen peroxide pretreatment and production of bioethanol from seaweed *Ulva prolifera* biomass. *Bioresource Technology* 2016:214:144–149. https://doi.org/10.1016/j.biortech.2016.04.090

[9] Maehre H. K., *et al*. Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *Journal of the Science of Food and Agriculture* 2014:94(15):3281–3290. https://doi.org/10.1002/jsfa.6681

[10] Rupe P., Ruperez P. Mineral content of edible marine seaweeds. *Food Chemistry* 2002:79(1):23–26. https://doi.org/10.1016/S0308-8146(02)00171-1

[11] Wijayanta A. T., Goto M., Kamiya N. Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid–ionic liquid pretreatment. *Biomass and Bioenergy* 2015:81:63–69. https://doi.org/10.1016/j.biombioe.2015.05.023

[12] Taylor S. *Marine Medicinal Foods, Volume 64, 1st ed. Implications and Applications, Macro and Microalgae*. Elsevier, Academic Press 2011.

[13] Senthilkumar N., *et al*. Further studies and biological activities of macromolecular protein R-Phycoerythrin from *Portieria hornemannii*. *International Journal of Biological Macromolecules* 2013:62:107–116. https://doi.org/10.1016/j.ijbiomac.2013.08.004

[14] Dumay J., *et al*. Phycoerythrins: Valuable proteinic pigments in red seaweeds. *Advances in Botanical Research* 2014:71:321–343. https://doi.org/10.1016/B978-0-12-408062-1.00011-1

[15] Tiwari B. K., Ed. *Seaweed Sustainability, 1st ed. Food and Non-Food Applications*. Elsevier, Academic Press 2015.

[16] Rajauria G. Seaweeds: A sustainable feed source for livestock and aquaculture. *Seaweed Sustainability: Food and Non-Food Applications* 2015:389–420. https://doi.org/10.1016/B978-0-12-418697-2.00015-5

[17] Bikker P., *et al*. Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology* 2016:28:3511–3525. https://doi.org/10.1007/s10811-016-0842-3

[18] Venkatesan J., Anil S., Kim S. K. Eds. *Seaweed Polysaccharides, 1st ed. Isolation, Biological and Biomedical Applications*. Elsevier, 2017.

[19] Holdt S. L., Kraan S. Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology* 2011:23(3):543–597. https://doi.org/10.1007/s10811-010-9632-5

[20] Spurr H. I. Extraction, separation and purification of polyphenols, polysaccharides and pigments from British seaweed for high-value applications. Thesis (PhD),White Rose, 2014.

[21] Fleurence J., Morançais M., Dumay J. Seaweed proteins. In *Proteins in Food Processing (Second Edition)*. Woodhead Publishing Series in Food Science, Technology and Nutrition 2018, 245–262.

[22] Burtin P. Nutritional Value of Seaweeds. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 2003:2(4):1579–4377.

[23] Werlinger C., Alveal K., Romo H. Biología marina y oceanografia: conceptos y procesos. (Marine biology and oceanography: Concepts and processes.) Chile: University of Consepcion, 2009 (in Spanish)

[24] Bonanno G., Orlando-Bonaca M. Chemical elements in Mediterranean macroalgae. A review. *Ecotoxicology and Environmental Safety* 2018:148:44–71. https://doi.org/10.1016/j.ecoenv.2017.10.013

[25] Leliaert F., *et al*. Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* 2012:31(1):1–46. https://doi.org/10.1080/07352689.2011.615705

[26] Nunes N., *et al*. Biochemical composition, nutritional value, and antioxidant properties of seven seaweed species from the Madeira Archipelago. *Journal of Applied Phycology* 2017:29:2427–2437. https://doi.org/10.1007/s10811-017-1074-x

[27] Milledge J. J., Nielsen B. V., Bailey D. High-value products from macroalgae: the potential uses of the invasive brown seaweed, Sargassum muticum. *Reviews in Environmental Science and Biotechnology* 2016:15:67–88. https://doi.org/10.1007/s11157-015-9381-7

[28] Kersen P., Paalme T., Pajusalu L., Martin G. Biotechnological applications of the red alga *Furcellaria lumbricalis* and its cultivation potential in the Baltic Sea. *Botanica Marina* 2017:60(2):207–218. https://doi.org/10.1515/bot-2016-0062

[29] Sánchez-Machado D. I., *et al*. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry* 2004:85(3):439–444. https://doi.org/10.1016/j.foodchem.2003.08.001

[30] Duan X. J., *et al*. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata. *Food Chemistry* 2006:95(1):37–43. https://doi.org/10.1016/j.foodchem.2004.12.015

[31] Yanik J., *et al*. Pyrolysis of algal biomass. *Journal of Analytical and Applied Pyrolysis* 2013:103:134–141. https://doi.org/10.1016/j.jaap.2012.08.016

[32] Gomez-Zavaglia A., *et al*. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. *Antioxidants* 2019:8(9):406. https://doi.org/10.3390/antiox8090406

[33] Bergeron C., Carrier D. J., Ramaswamy S. *Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing*. John Wiley and Sons, Inc., 2012

[34] Meenakshi S., *et al*. Total flavanoid and in vitro antioxidant activity of two seaweeds of Rameshwaram coast. *Global Journal of Pharmacology* 2009:3(2):59–66.

[35] Li X., *et al*. Total fractionation and characterization of the water-soluble polysaccharides isolated from Enteromorpha intestinalis. *International Journal of Biological Macromolecules* 2018:111:319–325. https://doi.org/10.1016/j.ijbiomac.2018.01.018

[36] Wright R. T., Boorse D. F. *Environmental Science: Toward a Sustainable Future*, 12th Edition. Upper Saddle River: Pearson Education Inc., 2014.

[37] Peasura N., Laohakunjit N., Kerdchoechuen O., Wanlapa S. Characteristics and antioxidant of Ulva intestinalis sulphated polysaccharides extracted with different solvents. *International Journal of Biological Macromolecules* 2015:81:912–919. https://doi.org/10.1016/j.ijbiomac.2015.09.030

[38] Kidgell J. T., Magnusson M., de Nys R., Glasson C. R. K. Ulvan: A systematic review of extraction, composition and function. *Algal Research* 2019:39:101422. https://doi.org/10.1016/j.algal.2019.101422

[39] Szaniawska A., Normant M. Szaniawska, Normant - 2000 - The biochemical composition of *Enteromorpha* spp. from the Gulf of Gdańsk coast on the southern Balt. *Oceanologia* 2000:42(1):19–28.

[40] de Reviers B., Leproux A. Characterization of polysaccharides from *Enteromorpha intestinalis* (L.) link, chlorophyta. *Carbohydrate Polymers* 1993:22(4):253–259. https://doi.org/10.1016/0144-8617(93)90128-Q
[41] Tabarsa M., *et al*. Molecular characteristics and immunomodulatory activities of water-soluble sulfated polysaccharides from *Ulva pertusa*. *Journal of Medicinal Food* 2012:15(2):135–144. https://doi.org/10.1089/jmf.2011.1716
[42] Tabarsa M., You S. G., Dabaghian E. H., Surayot U. Water-soluble polysaccharides from Ulva intestinalis: Molecular properties, structural elucidation and immunomodulatory activities. *Journal of Food and Drug Analysis* 2018:26(2):599–608. https://doi.org/10.1016/j.jfda.2017.07.016
[43] Graiff A., Liesner D., Karsten U., Bartsch I. Temperature tolerance of western Baltic Sea Fucus vesiculosus – growth, photosynthesis and survival. *Journal of Experimental Marine Biology and Ecology* 2015:471:8–16. https://doi.org/10.1016/j.jembe.2015.05.009
[44] Tuvikene A. R., Robal M. Valgulised pigmendid Läänemere punavetikast Furcellaria lumbricalis : sisaldus ja eraldamisvõimalused. (Proteinic pigments from Baltic red algae Furcellaria lumbricalis: content and isolation possibilities.) Tallin: Tallin University, 2015. (in Estonian)
[45] Bird C. J., Saunders G. W., McLachlan J. Biology of *Furcellaria lumbricalis* (Hudson) Lamouroux (Rhodophyta: Gigartinales), a commercial carrageenophyte. *Journal of Applied Phycology* 1991:3(1):61–82. https://doi.org/10.1007/BF00003920
[46] Rahimi F., Tabarsa M., Rezaei M. Ulvan from green algae *Ulva intestinalis*: optimization of ultrasound-assisted extraction and antioxidant activity. *Journal of Applied Phycology* 2016:28(5):2979–2990. https://doi.org/10.1007/s10811-016-0824-5
[47] Benjama O., Masniyom P. Nutritional composition and physicochemical properties of two green seaweeds (*Ulva pertusa* and *U. intestinalis*) from the Pattani Bay in Southern Thailand. *Songklanakarin Journal of Science and Technology* 2011:33(5):575–583.
[48] Peinado I., Girón J., Koutsidis G., Ames J. M. Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds. *Food Research International* 2014:66:36–44. https://doi.org/10.1016/j.foodres.2014.08.035
[49] Cherry P., *et al*. Risks and benefits of consuming edible seaweeds. *Nutrition Reviews* 2019:77(5):307–329. https://doi.org/10.1093/nutrit/nuy066
[50] Parjikolaei B. R., *et al*. Valuable Biomolecules from Nine North Atlantic Red Macroalgae: Amino Acids, Fatty Acids, Carotenoids, Minerals and Metals. *Natural Resources* 2016:7(4):157–183. https://doi.org/10.4236/nr.2016.74016
[51] Naseri A., Holdt S. L., Jacobsen C. Biochemical and Nutritional Composition of Industrial Red Seaweed Used in Carrageenan Production. *Journal of Aquatic Food Product Technology* 2019:28(9):967–973. https://doi.org/10.1080/10498850.2019.1664693
[52] Dumay J., Morançais M. Proteins and Pigments. In J. Fleurence and I. Levine (Eds.) *Seaweed in Health and Disease Prevention*, Elsevier Inc. 2016. https://doi.org/10.1016/B978-0-12-802772-1.00009-9
[53] Hermund D. B. Extraction, characterization and application of antioxidants from the Nordic brown alga Fucus vesiculosus. Lyngby: DTU, 2016:312.
[54] Truus K., *et al*. Analysis of bioactive ingredients in the brown alga Fucus vesiculosus by capillary electrophoresis and neutron activation analysis. *Analytical and Bioanalytical Chemistry* 2004:379:849–852. https://doi.org/10.1007/s00216-004-2666-2
[55] Rozentsvet O. A., Nesterov V. N. Lipids and fatty acids from *Ulva intestinalis* from estuaries of the Caspian basin (elton region). *Chemistry of Natural Compounds* 2012:48(4):544–547. https://doi.org/10.1007/s10600-012-0305-2
[56] Ragonese C., *et al*. Characterisation of lipid fraction of marine macroalgae by means of chromatography techniques coupled to mass spectrometry. *Food Chemistry* 2014:145:932–940. https://doi.org/10.1016/j.foodchem.2013.08.130
[57] Jeong G. T., Park D. H. Optimization of lipid extraction from marine green macro-algae as biofuel resources. *Korean Journal of Chemical Engineering* 2015:32(12):2463–2467. https://doi.org/10.1007/s11814-015-0083-1
[58] Alam M., Chakravarti A., Ikawa M. Lipid composition of the brown alga Fucus vesiculosus. *Journal of Phycology* 1971:7(3):267–268. https://doi.org/10.1111/j.1529-8817.1971.tb01515
[59] Rohani-Ghadikolaei K., Abdulalian E., Ng W. K. Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of Food Science and Technology* 2012:49(6):774–780. https://doi.org/10.1007/s13197-010-0220-0
[60] Biancarosa I., *et al*. Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed. *Journal of the Science of Food and Agriculture* 2018:98(5):2035–2042. https://doi.org/10.1002/jsfa.8798
[61] Yin-Hu W., *et al*. Biomass production of a Scenedesmus sp. under phosphorous-starvation cultivation condition. *Bioresource technology* 2012:112:193–8. https://doi.org/10.1016/j.biortech.2012.02.037
[62] Balina K., Romagnoli F., Blumberga D. Seaweed biorefinery concept for sustainable use of marine resources. *Energy Procedia* 2017:128:504–511. https://doi.org/10.1016/j.egypro.2017.09.067
[63] Zollmann M., *et al*. Green technology in green macroalgal biorefineries. *Phycologia* 2019:58(5):516–534. https://doi.org/10.1080/00318884.2019.1640516

[64] Peng Y., *et al*. Chemical composition of seaweeds. In *Seaweed Sustainability, Food and Non-Food Applications* 2015:79–124. Elsevier Inc., 2015. https://doi.org/10.1016/B978-0-12-418697-2.00005-2

[65] Rajauria G., *et al*. Identification and selection of algae for food, feed, and fuel applications. In *Seaweed Sustainability, Food and Non-Food Applications* 2015:315–345. https://doi.org/10.1016/B978-0-12-418697-2.00012-X

[66] Edwards M. Algal Species Selection. 2010.

[67] Kadam S. U., *et al*. Processing of seaweeds. In *Seaweed Sustainability, Food and Non-Food Applications* 2015:61–78, Elsevier Inc., 2015.

[68] HELCOM. Pearls of the Baltic Sea. Networking for life: Special nature in a special sea. Helsinki: HELCOM, 2007

[69] Ledesma-Escobar C. A., de Castro M. D. L. Coverage Exploitation of By-Products from the Agrofood Industry. In *Green Extraction of Natural Products: Theory and Practice* 2014:265–306. https://doi.org/10.1002/9783527676828.ch8

[70] Cravotto G., Binello A., Orio L. Green extraction techniques. *AgroFOOD industry hi-tech* 2011:22(6):57–59.

[71] Michalak I., Chojnacka K. Algal extracts: Technology and advances. *Engineering in Life Sciences* 2014:14(6):581–591. https://doi.org/10.1002/elsc.201400139

[72] Sosa-Hernández J. E., *et al*. State-of-the-art extraction methodologies for bioactive compounds from algal biome to meet bio-economy challenges and opportunities. *Molecules* 2018:23(11). https://doi.org/10.3390/molecules23112953

[73] Razi Parjikolaei B. *et al*. Process design and economic evaluation of green extraction methods for recovery of astaxanthin from shrimp waste. *Chemical Engineering Research and Design* 2017:117:73–82. https://doi.org/10.1016/j.cherd.2016.10.015

[74] Grosso C., Valentão P., Ferreres F., Andrade P. B. Alternative and efficient extraction methods for marine-derived compounds. *Marine Drugs* 2015:13(5):3182–3230. https://doi.org/10.3390/md1305318

[75] Azmir J., *et al*. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering* 2013:117(4):426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

[76] Praveen M. A., *et al*. An overview of extraction and purification techniques of seaweed dietary fibers for immunomodulation on gut microbiota. *Trends in Food Science and Technology* 2019:92:46–64. https://doi.org/10.1016/j.tifs.2019.08.011

[77] Rioux L., Turgeon S. L. Seaweed carbohydrates. In *Seaweed Sustainability, Food and Non-Food Applications* 2015:141–192. https://doi.org/10.1016/B978-0-12-418697-2.00007-6

[78] Abdul Khalil H. P. S., *et al*. A review of extractions of seaweed hydrocolloids: Properties and applications. *eExpress Polymer Letters* 2018:12(4):296–317. https://doi.org/10.3144/expresspolymlett.2018.27

[79] Kazir M., *et al*. Extraction of proteins from two marine macroalgae, Ulva sp. and Gracilaria sp., for food application, and evaluating digestibility, amino acid composition and antioxidant properties of the protein concentrates. *Food Hydrocolloids* 2019:87:194–203. https://doi.org/10.1016/j.foodhyd.2018.07.047

[80] Bleakley S., Hayes M. Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods* 2017:6(5):33. https://doi.org/10.3390/foods6050033

[81] Pangestuti R., Kim S. Seaweed proteins, peptides, and amino acids. In *Seaweed Sustainability, Food and Non-Food Applications* 2015:125–140. https://doi.org/10.1016/B978-0-12-418697-2.00006-4

[82] Hamid N., *et al*. Seaweed minor constituents. In *Seaweed Sustainability, Food and Non-Food Applications* 2015:193–242. https://doi.org/10.1016/B978-0-12-418697-2.00008-8

[83] Chemat F., Cravotto G. *Enhancing Extraction Processes in the Food Industry*. CRC Press, 2011.

[84] Ivanovs K., Blumberga D. Extraction of fish oil using green extraction methods: A short review. *Energy Procedia* 2017:128:477–483. https://doi.org/10.1016/j.egypro.2017.09.033

[85] Gribovskaya I. V., Gladchenko I. A., Zinenko G. K. Extraction of mineral elements from inedible wastes of biological components of a life-support system and their utilization for plant nutrition. *Advances in Space Research* 1996:18(4–5):93–97. https://doi.org/10.1016/0273-1177(95)00865-C

[86] Ciko A. M., Jokić S., Šubarić D., Jerković I. Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. *Marine Drugs* 2018:16(10):348. https://doi.org/10.3390/md16100348

[87] Chemat F., Vian M. A., Cravotto G. Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences* 2012:13(7):8615–8627. https://doi.org/10.3390/ijms1307861

[88] Allaf T., Allaf K. Fundamentals of Process-Intensification Strategy for Green Extraction Operations. In *Green Extraction of Natural Products: Theory and Practice* 2014:145–172. https://doi.org/10.1002/9783527676828.ch5

[89] Kadam S. U., *et al*. Extraction of biomolecules from seaweeds. *Seaweed Sustainability* 2015:243–269. https://doi.org/10.1016/B978-0-12-418697-2.00009-X

[90] Quitain A. T., Kai T., Sasaki M., Goto M. Supercritical carbon dioxide extraction of fucoxanthin from undaria pinnatifida. *Journal of Agricultural and Food Chemistry* 2013:61(24):5792–5797. https://doi.org/10.1021/jf400740p

[91] Heffernan N., *et al*. Comparison of extraction methods for selected carotenoids from macroalgae and the assessment of their seasonal/spatial variation. *Innovative Food Science and Emerging Technologies* 2016:37(B):221–228. https://doi.org/10.1016/j.ifset.2016.06.004

[92] Sivagnanam S. P., *et al*. Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO2 extraction. *Marine Drugs* 2015:13(6):3422–3442. https://doi.org/10.3390/md13063422

[93] Michalak I., *et al*. Supercritical fluid extraction of algae enhances levels of biologically active compounds promoting plant growth. *European Journal of Phycology* 2016:51(3):243–252. https://doi.org/10.1080/09670262.2015.1134813
[94] Zhang R., *et al*. A comparative assessment of the activity and structure of phlorotannins from the brown seaweed *Carpophyllum flexuosum*. *Algal Research* 2018:29:130–141. https://doi.org/10.1016/j.algal.2017.11.027
[95] Magnusson M., *et al*. A comparative assessment of microwave assisted (MAE) and conventional solid-liquid (SLE) techniques for the extraction of phloroglucinol from brown seaweed. *Algal Research* 2017:23:28–36. https://doi.org/10.1016/j.algal.2017.01.002
[96] Quitain A. T., Kai T., Sasaki M., Goto M. Microwave-hydrothermal extraction and degradation of fucoidan from supercritical carbon dioxide deoiled Undaria pinnatifida. *Industrial and Engineering Chemistry Research* 2013:52(23):7940–7946. https://doi.org/10.1021/ie400527b
[97] Yuan Y., *et al*. Microwave assisted hydrothermal extraction of polysaccharides from *Ulva prolifera*: Functional properties and bioactivities. *Carbohydrate Polymers* 2018:181:902–910. https://doi.org/10.1016/j.carbpol.2017.11.061
[98] Romarís-Hortas V., Moreda-Piñeiro A., Bermejo-Barrera P. Microwave assisted extraction of iodine and bromine from edible seaweed for inductively coupled plasma-mass spectrometry determination. *Talanta* 2009:79(3):947–952. https://doi.org/10.1016/j.talanta.2009.05.036
[99] Wang F., *et al*. Ultrasound-assisted extraction and purification of taurine from the red algae *Porphyra yezoensis*. *Ultrasonics Sonochemistry* 2015:24:36–42. https://doi.org/10.1016/j.ultsonch.2014.12.009
[100] Mittal R., *et al*. Ultrasound assisted methods for enhanced extraction of phycobiliproteins from marine macro-algae, *Gelidium pusillum* (Rhodophyta). *Ultrasonics Sonochemistry* 2017:38:92–103. https://doi.org/10.1016/j.ultsonch.2017.02.030
[101] Lee S. H., *et al*. Potential use of ultrasound in antioxidant extraction from Ecklonia cava. *Algae* 2013:28(4):371–378. https://doi.org/10.4490/algae.2013.28.4.371
[102] Kadam S. U., *et al*. Laminarin from Irish Brown Seaweeds *Ascophyllum nodosum* and *Laminaria hyperborea*: Ultrasound Assisted Extraction, Characterization and Bioactivity. *Marine Drugs* 2015:13(7):4270–4280. https://doi.org/10.3390/md13074270
[103] Dang T. T., *et al*. Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant activities of the alga Hormosira banksii using response surface methodology. *Journal of Applied Phycology* 2017:29(6):3161–3173. https://doi.org/10.1007/s10811-017-1162-y
[104] Saravana P. S., *et al*. Evaluation of the chemical composition of brown seaweed (*Saccharina japonica*) hydrolysate by pressurized hot water extraction. *Algal Research* 2016:13:246–254. https://doi.org/10.1016/j.algal.2015.12.004
[105] Plaza M., *et al*. Facts about the formation of new antioxidants in natural samples after subcritical water extraction. *Food Research International* 2010:43(10):2341–2348. https://doi.org/10.1016/j.foodres.2010.07.036
[106] Anaëlle T., *et al*. Green improved processes to extract bioactive phenolic compounds from brown macroalgae using Sargassum muticum as model. *Talanta* 2013:104:44–52. https://doi.org/10.1016/j.talanta.2012.10.088
[107] Sánchez-Camargo A. D. P., *et al*. Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae. *Food Chemistry* 2016:192:67–74. https://doi.org/10.1016/j.foodchem.2015.06.098
[108] Hammed A. M., *et al*. Enzyme aided extraction of sulfated polysaccharides from Turbinaria turbinata brown seaweed. *International Food Research Journal* 2017:24(4):1660–1666.
[109] Vo Dinh T., Saravana P. S., Woo H. C., Chun B. S. Ionic liquid-assisted subcritical water enhances the extraction of phenolics from brown seaweed and its antioxidant activity. *Separation and Purification Technology* 2018:196:287–299. https://doi.org/10.1016/j.seppur.2017.06.009
[110] Yi J., Dong B., Jin J., Dai X. Effect of increasing total solids contents on anaerobic digestion of food waste under mesophilic conditions: Performance and microbial characteristics analysis. *PLoS ONE* 2014:9(7):e102548. https://doi.org/10.1371/journal.pone.0102548
[111] Gereniu C. R. N., Saravana P. S., Chun B. S. Recovery of carrageenan from Solomon Islands red seaweed using ionic liquid-assisted subcritical water extraction. *Separation and Purification Technology* 2018:196:309–317. https://doi.org/10.1016/j.seppur.2017.06.055
[112] Bozinou E., *et al*. Pulsed Electric Field Extraction and Antioxidant Activity Determination of Moringa oleifera Dry Leaves: A Comparative Study with Other Extraction Techniques. *Beverages* 2019:5(1):8. https://doi.org/10.3390/beverages5010008

ANNEX

TABLE A1. OVERVIEW OF NOVEL METHODS FOR SEAWEED BIOACTIVE COMPOUND EXTRACTION

Extraction technique Conditions (C) and influencing parameters (IP)	Seaweed species under investigation	Extracted bioactive compounds	Application outlook	References
Supercritical CO_2 (SC-CO_2) (C) Pressure 9.1–40 MPa, Temp. 25–75 °C, Time 50–360 min, >2 mL CO_2/min Co-solvents: EtOH 0.5–15 % Sunflower, soybean, canola oil 0.5–2 %. (IP) Water %, T °C, pressure. Flow of CO_2; Extraction type: continuous, co-solvent, soaking.	*Cladophora glomerata, Chara fragilis, Chondrus crispus, Dictyopteris membranacea, Fucus serratus,* *Gracilaria mammillaris, Hypnea charoides,* *Hypnea spinella, Halopytis incurvus,* *Porphyra sp., Laminaria digitata,* *Sargassum muticum, Sargassum vulgare,* *Ulva clathrata* *Undaria pinnatifida, Polysiphoniucoides, Saccharina japonica, Sargassum horneri,* *Undaria pinnatifida, Ulva flexuosa,*	Fucoxanthin, polyphenols, phlorotannins, carotenoids, pigments, fatty acids, cytokinins, auxins, microelements, macroelements	High investment cost; Operates in elevated pressure (safety); High power consumption.	[84], [86], [90]–[93]
Microwave-assisted extraction (MAE) (C) Power 300–1000 W; Frequency – 2450 MHz; Temperature – 10–185 °C; Solvents – EtOH, H_2O, acetone, propanol, ethyl acetate, 0.1 M HCl, petroleum ether, ethyl acetate; Time – 2–30 min. (IP) Particle size, solvent used, time, capacity, and frequency of microwave	*Ascophyllum nodosum, Carpophyllum flexuosum, Carpophyllum plumosum, Caulerpa racemose, Carpophyllum flexuosum, Ecklonia radiata, Enteromorpha prolifera, Fucus vesiculosus, Padina pavonica, Sargassum thunbergii, Monostroma latissimum, Ulva meridionalis, Ulva ohnoi, Ulva prolifera, Undaria pinnatifida,*	Polysaccharides, alkaline, galactans, carrageenans, agar, phlorotannins, phloroglucinol, iodine, bromine, phenols, phytosterols, phytol	Hard to scale up; Generation of heat leads to degradation of thermolabile compounds; Low efficiency when using volatile solvents.	[86], [94]–[98]
Ultrasound-assisted extraction (UAE)	*Hormosira banksia, Ascophyllum nodosum, Ascophyllum nodosum, Laminaria hyperborean, Ecklonia cava, Gelidium*	Polyphenols, laminarin, phycobili-proteins, taurine,	High power consumption and difficult to scale up.	[99]–[103]

(C) Ultrasound Equipment – Ultrasonic bath, Ultrasound probe; Frequency – 20–60 kHz; Power – 100–750 W; Temperature – 20–60 °C; Time – 2–720 min; Solvents: ethanol, 0,03 M HCl, methanol, water; Small sample – 1–10 g. (IP) Ultrasonic frequency, power, time and medium.	*pusillum, Sargassum muticum, Osmundea pinnatifida, Codium tomentosum, Laurencia obtuse, Porphyra yezoensis*	fucose, uronic acid, antioxidants, prebiotic compounds		
High pressure methods "Subcritical Water Extraction (SWE)" "Pressurized liquid extraction (PLE)" "Accelerated solvent extraction (ASE)" (C) Water extraction: Pressure – 1.3–52 MPa; Temperature – 50–420 °C; Time – 5–25 min; Solvent Extraction: 50–200 °C; 3.5–20 MPa (IP) Temperature (°C), solvent concentration (%), static time (min), pressure (psi), weight of sample (g), and flush volume (%).	*Ascophyllum nodosum, Fucus spiralis, Codium fragile, Cystoseira abies-marina, Sargassum muticum, Padina pavonica, Fucus serratus, Laminaria digitata, Gracilaria gracilis, Porphyra spp., Sargassum vulgare, Undaria pinnatifida, Halopitys incurvus, Himanthalia elongate, Pelvetia canaliculata, Ulva intestinalis, Saccharina japonica, Ulva lactuca, Fucus vesiculosus, Dictyota dichotoma, Cystoseira baccata, Himanthalia elongate*	Polyphenols, phlorotannins, fucoidan, total organic carbon, minerals, monosaccharides, amino acids, polar compounds; fatty acids	Not suitable for thermolabile compounds; Less selective than SFE.	[86], [104]–[106]
Enzyme-assisted extraction (EAE) (C) Time 1–4 h Temperature 40–60 °C The ratio of enzyme to substrate ~ 0.5–5 % (IP) Type, activity and amount of enzyme used, pH. Absence of endogenous enzymes.	*Sargassum horneri,brown seaweeds, Undaria pinnatifida, Sargassum coreanum*	Antioxidants, fucoxanthin, fatty acids, polysaccharides	Costs of enzymes are very high; Selectivity of enzymes.	[107], [108]

Ionic liquids extraction (ILE) (C) Chemicals: For phenolic extraction 0.5 M [C4C1im][BF4], 1:32 w/v mixing ratio; time 24 h, stirring at 500 rpm; Optional extraction vessel and pressure. Extraction conditions (ionic liquids used) strongly depends on target compound. (IP) Chemicals, vessel, pressure used.	*Kappaphycus alvarezii,* *S. japonica*	Phenolic compounds, polysaccharides, carrageenan, terpenoids, alkaloids	Some ILEs require purification process	[109]–[111]
Pulsed electric fields (PEFs) (C) field strength of 0.5–1.0 kV/cm treatment time 100–10,000 µs or 1–10 kV/cm and shorter time (5–100 µs) (IP) Field strength, time, conductivity of intact and disintegrated cells	–	Phenols, proteins	Optimization of process by using different parameters is needed. These include pulse duration, pulse interval, electric field strength, or other electrical pulse shapes.	[74], [112]

Paper IV

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Seaweed biorefinery concept for sustainable use of marine resources

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Abstract

Seaweed, also called marine macroalgae, have a potential to be a valuable feedstock for biorefinery. Depending from seaweed type and species it is possible to extract different fatty acids, oils, natural pigments, antioxidants, high value biological components and other substances which can be potentially used in an industrial production system. The seaweed biorefinery framework presents a conceptual model for high value added product production along with production of biofuels either fluid or gaseous. This in turn reduces the cost of fuel production with maximum utilization of the biomass. The role of seaweed biorefinery concept is analysed in this paper under the perspective of bioeconomy principles and through a SWOT analysis was made to indicate the role biorefinery concept can play to support the development of sustainable bioeconomy.

Keywords: macroalgae; seaweed; biorefinery; bioeconomy

1. Introduction

Seaweed has been widely used already for centuries, but their role has emerged in last decades when the problem of global population and food amount has been becoming an issue [1]. Similar to algae [2−5], unique composition of seaweeds allows to use them as food, feed and energy source [6]. Seaweeds are used as a feedstock for different kind of materials including biopolymers, cosmetics, agrifood and food supplements with several benefits for health [7, 8]. Algae processing to any kind of products generates leftovers and waste products, which are usually disposed [9].

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Nowadays we have to focus on sustainable bioeconomy and a production system where no waste flow is created. Biorefinery is a way to decrease negative impact on environment and create products with higher added value to get more economical and environmental benefits.

The integration of different biomass conversion processes to produce energy and value added products into a single facility is called a biorefinery [10]. Advances of biorefinery – combinations of processes and technologies for conversion of biomass to food, feed, energy and other value added products with minimal amount of waste – offers a way to develop sustainable industrial economy and reduce impact on climate change. Biorefinery approach is considered sustainable because the processes produce minimal waste to the environment in fact decreasing the environmental burden and the pressure on ecosystem services. The biorefinery concept is a technical application of this principle in which exploitation of biomass is enhanced beyond bioenergy production. In the biorefinery, available compounds of the biomass ere exploited and every step of value chain is used as a way to add value. If all of the bioresources within the biorefinery structure are efficiently used, including the biological leftovers from a cascade transformation process approach, a nearly zero waste production is achieved.

Biorefinery International Energy Agency (IEA) Bioenergy task 42 has a task devoted to biorefineries. The main goal of the task is to contribute to the development and implementation of sustainable biorefineries – as a part of highly efficient, zero waste value chains – synergistically producing biobased food and non-food products [11]. Since 2007, when the task was set, European and the global view to the task has been changed. In fact the approach has been growing and been developed. Nowadays global biorefinery concept mainly includes terrestrial biomass with plants and forest on top and only small part has recently been devoted to algae [12]. Since mainly first and second generation biomass is used for liquid biofuels production, it is significantly affecting the economy due to the competition for food and energy.

Marine macroalgae or seaweed have a high potential to partly replace terrestrial biomass. With current research going on in this field it is already declared that algae is a 3rd generation bioresource and doesn't compete with food and feed plants, nor is using resources for their growth. Valuable substances that can be found in algae can be a way to promising low-carbon economy. It is necessary to create seaweed biorefinery concept that would display valorization strategy that would display seaweed as a valuable feedstock that can compete with terrestrial biomass. Development of a seaweed biorefinery concept has attracted lot of attention recently and many ongoing research are focused on developing the seaweed biorefinery concept [10, 13–16].

This paper would like to explore the state-of-art of the biorefinery concept implementation in bioeconomy. In fact the paper would like to create a baseline for potential biorefinery concept integration at commercial dimension evaluating where important synergies can be identified among both commercial actors, stakeholders and private investors. Moreover the paper looks toward the identification of prerequisites for better exploitation of local available (and valuable) biorefinery feedstocks within bioeconomy overarching perspective. This will be essential in the direction of reaching the set targets of low-carbon economy, higher sustainability and optimal bioresource use efficiency. This paper review current state of art and display the basic structure of seaweed biorefinery concept and analyse its role in sustainable bioeconomy while focusing on Baltic Sea region.

2. Methodology

2.1. Seaweed biorefinery concept

Seaweed aquaculture is already popular in Asian countries [17], but seaweed natural distribution area is covering all over the world, including Europe and the Baltic Sea [18]. Seaweed cultivation industry and mass production of algae is underdeveloped in Eastern Europe. Seaweed cultivation could represent a promising opportunity to obtain seaweed biomass meanwhile reducing eutrophication problems [19] The opportunities of the marine macroalgae use can thus be wide.

Raw seaweeds and seaweed-based food products in Asian countries have been consumed for centuries [20]. More recently seaweed products as a source of polysaccharides for food and pharmaceutical uses are becoming popular in Europe [21, 22]. The seaweed mineral content is higher than mineral level in terrestrial plants and animal products [23, 24]. High mineral content and low fat content represent a suitable feedstock for food and feed. Macroalgae contain unique composition of carbohydrates, which have different properties than those from terrestrial plants [25]. Laminaran and mannitol are energy storage units in algae, like starch in the plants, but alginates are structural compounds like cellulose and lignin in plants [26]. Polysaccharides found in seaweeds make them attractive not only to food industry, but also to the pharmaceutical industry. Fucoidans and laminaran found in brown algae exhibit various biological activities with potential health benefits [23, 27]. Marine macroalgae biomass can also be used as a feedstock for energy purposes. They can be transformed in different types of biofuels such as biogas, bioethanol, and biodiesel, replacing a part of fossil fuels [28].

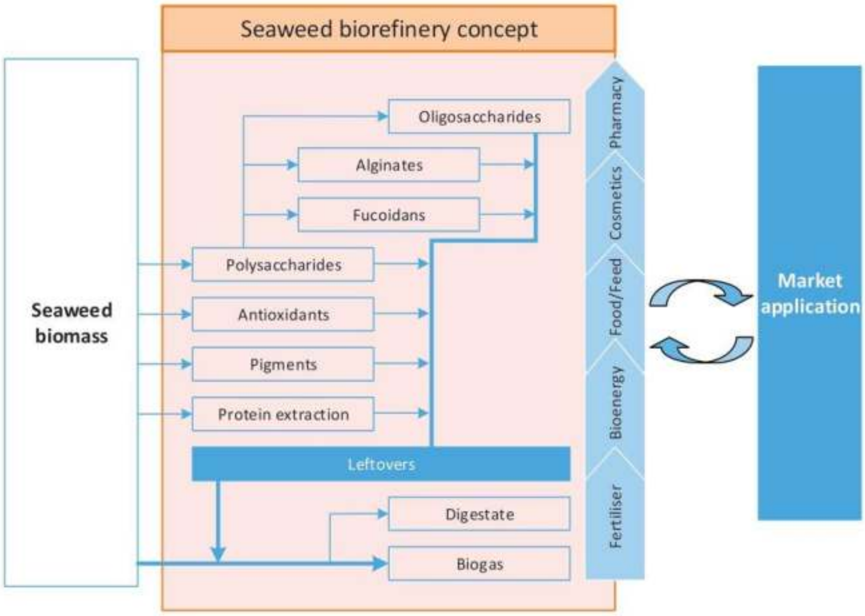

Fig. 1. Seaweed biorefinery concept.

The seaweed biorefinery concept presents a conceptual model for high value added product production along with production of biofuels. Biomass is used with maximum efficiency and expenses related to fuel production are reduced. It is important when planning production process and units, to use capable biorefinery concept where all biomass and energy from production processes would be used to the fullest extent. Conversion processes (physical, chemical, biological and thermal) used in production of products should work in symbiotic way, individually or in system to create economically sustainable products [28]. The various uses of products obtained from algal biorefineries include transport fuels, therapeutics, food additives and biofertilizers [28].

Products derived from feedstock can be separated in products which can be already used in market or they can be used as feedstock for further manufacturing operations to obtain value-added products. The waste and leftovers products obtained after each step of treatment are used as raw material inflows for a parallel production chains on cascading approach (see Fig. 1). Only leftovers which can't be utilized in further production processes with a 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. Seaweed biorefinery concept is mainly addressed to use particular seaweed species whose overgrowth, as a result of eutrophication, is causing ecological damages and affects the sustainability of coastal environments. Instead seaweeds can be used for remediation of environment since together with seaweed biomass certain amount of nitrogen and phosphorous is removed from water, nevertheless the identification of the thresholds affecting the overall ecosystem service provide by the depletion of a natural resources is a sensitive issue. Seaweed biorefinery concept can have a major impact by showing different aspects for utilizing seaweed to produce a wide range of value-added products, biofuels, and bioenergy within a concept, rather than focusing in a single product.

When developed and implemented in industry, practical impacts of biorefinery concept can be relevant at different levels: within the seaweed farming and industry, as employment increase in the sectors of the bioeconomy, triggering new investments, unlock incentive and favouring the integration of renewable energy within the overall energy systems [29]. 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 strengthening the competitiveness of biobased industries, green platform chemicals, materials and biopolymers, and both new and existing food and feed ingredients and processing industries.

2.2. Strengthening the role of biorefinery under bioeconomy principles

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 [30]. Bioeconomy key principles has 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 [31]. Seaweed biorefinery concept is following these principles and it has a significant role in strengthening the bioeconomy.

2.2.1. Food first

Food safety is set as a first principle and it is focusing on food quality and availability. Seaweed biorefinery concept is matching the aim of this principle. Nutritional value and health benefits of seaweeds are already well known for years. Seaweeds can provide human organism with necessary vitamins, minerals and antioxidants and is a valuable calorie source [32, 33]. Moreover, seaweeds are not competing for land with plants, and land can still be used for agriculture or forestry. Grown in the sea they do not require freshwater and additional nutrients, like terrestrial plants. It is predicted that in 2050, global population will reach 9 billion of people and with current food production it is possible to provide a food only for half of them [34], so it is necessary to find a new food resources or increase food quality. Valuable nutritional value of seaweeds matches with this demand for this additional food base.

2.2.2. Sustainable yields

Sustainable seaweed production currently might be the main challenging, since it is relatively young form of aquaculture. World production has exponentially increased during the last 50 years and it tripled between 1997 and 2012, from 7 million tons to 24 million tons and it is still increasing [35]. Seaweed yield sustainability is dependent of the region. Seaweed farming is rapidly increasing in some countries, while in others it is slowly gaining acceptance [36]. There are still more than 150 countries with coastal area which are not yet considering seaweed cultivation. Reasons are different: seasonality, lack of infrastructure and missing demand from society are the main reasons that hampers the development of the area. Also in Baltic region these are the main reasons that slows down the seaweed cultivation. In addition low level salinity sets some limitations regarding on cultivation species and yield sustainability.

2.2.3. Cascading approach

To ensure sustainable use of biomass, cascading approach has been developed which promotes that product with the highest value is made first, then the second highest is made and so on. The problem is that today large amount of valuable biomass which could be used for high value products is used to produce biofuels and bioenergy [37]. Seaweed biorefinery concept has been developed in a way that products with highest value are made first and products with lower value are made after. Bioenergy and biofuels are made using leftovers from other production processes. This approach increases resource efficiency and adds even higher value to used biomass, which is a part of circular economy. Increased resource efficiency also is saving the raw material supply, because biomass can be used repeatedly. With this principle usually the problem is that leftover biomass has to be transported from one location to another to continue with the next step of production, but biorefinery concept solves this problem since the concept is meant to be implemented in one location.

2.2.4. Circularity

Since cascading approach does not address the waste problem by itself, circularity principle has to be followed. The circular economy is built on tree basic rules: (1) there is no waste, since products are reusable and recyclable; (2) consumed materials should be returnable to the environment without any threats after cascading sequence of use, to provide their stock restoration, but durables should be reusable and upgradeable; and (3) and energy for all processes should be provided from renewable and sustainable sources. The same rules has been used in seaweed biorefinery concept and amount of waste is reduced to minimum and waste energy is used to supply energy to production processes [38].

2.2.5. Diversity

Seaweed biorefinery concept has been made to be diverse and it cover many sectors. Production systems has to be various and produce different outputs using different techniques. A diversity is a key to resilience, and bioeconomy require to develop more innovations to create diversity rather than limit it.

These five main properties does not give a limitation to this seaweed biorefinery concept, instead they are defining guidelines and basic rules biorefinery concept should follow to give the most benefit for bioeconomy. Not only all biorefinery concept together should be based on bioeconomy guidelines, 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.

3. Results and Discussion.

3.1. SWOT analysis

The perspective of a constant evolution of biorefineries will reveal new feedstock and increase their conversion efficiency. It will give opportunity to find new technologies and products. It will positively affect all existing sectors of bioeconomy and develop rural areas. New technologies with high production yield, low use of resources or waste use instead of valuable resources, gives more sustainable approach. Innovative technologies developed for biorefinery increases level of opportunities to all sectors. The creating of a biobased economy has the ability to not only move the world through current challenges on deficiency of biobased resources but will be also give benefits for production meaning reduced impact on environment. Specfically in this section we address the strengths, weaknesses, opportunities and threats of seaweed biorefinery concept and indicate the role biorefinery concept can play to support the development of sustainable bioeconomy within a SWOT analysis of a seaweed-based biorefinery concept.

Table 1. Pros and Cons of seaweed biorefinery concept: SWOT analysis.

	Positive	**Negative**
Internal	**Strengths**	**Weaknesses**
	• Adding value to the biomass; • Development of novel biotechnologies; • Environmentally friendly resource since it is not requiring drinking water and nutrients and it reduces eutrophication • Increase biomass conversion efficiency – reducing raw material requirements • Production of biobased products and bioenergy feeding the full bioeconomy • Circular approach with no waste principle	• Collaboration between representatives of different market sectors (agro, energy, chemical, ...) over full biomass value chain necessary; • Most encouraging biorefinery processes/concepts not clear; • Scientific and technological challenges in not mature technologies; • Studying and concept development instead of real market implementation; • Variability of quality and energy density of biomass.

External	Opportunities	Threats
	• Cascading approach gives more value to biomass • Strengthening of the economic position of numerous bioeconomy sectors (e.g. agriculture, fishery, chemical and energy) • Seaweed biorefinery can give significant input in sustainable bioeconomy • Popularization of sustainable use of seaweed for the production of energy is setting new goals for policy • International agreement that resource should be used as efficiently as possible, develops spreading of biorefinery concept in other sectors • International development of technological aspects of biorefinery concepts.	• Economic change and fluctuation of fossil fuel prices • Competition of other renewable energy technologies satisfying the market needs • Higher quality standards are applied to bioresource and bioenergy than to traditional products • Availability and quality of raw materials (e.g. climate change, policies, logistics) • Difficulties to find investment capital for pilot and demo refineries, and adjusting existing refineries • Changing governmental policies • No market demand for products • Increase of environmental pollution can limit range of products in biorefinery concept

3.1.1. Strenghts and weaknesses

Seaweeds are water organisms and comparing to land plants they do not require drinking water and nutrients. Since additional resource is not needed it reduce cost and energy related to growing. In the same time, since seaweed are consuming nutrients found in environment, eutrophication level can be reduced, meaning that there are both economic and environmental advantages. Seghetta et al. have obtained a reduction in marine eutrophication of 16.3 kg N eq./ha, thanks to bioextraction of nitrogen. It depends from type of seaweed. However seaweed biomass can differ not only in different species and regions, but also from season. That can cause some difficulties regarding to conversion efficiency [39, 40].

It is clear that additional value to biomass gives more economical benefits to involved sides. It is also agreed by Bruton who agrees that adding value to the biomass, which otherwise would be wasted, stimulates economics and raises innovations and investments [41]. Higher economic stability and innovations allows to develop novel biotechnologies. Number of technologies for algae processing has been increased because of growing interest in this field, which has also been reported in State of Technology Review – Algae Bioenergy [28].

Novel technologies and conversion methods allows to increase biomass conversion efficiency using cascading biorefinery approach for coproduction of high-value specialty and commodity chemicals. Huijgen et al. notes, that seaweed is still quite expensive feedstock and cascading approach allows to use biomass in full amount. However lots of concepts/approaches are still theoretical since technologies are not mature and some technical and scientific challenges still exist [13]. To make cascading approach to be the most profitable collaboration between representatives of different market sectors over full biomass value chain is necessary.

3.1.2. Opportunities and threats

Seaweed biorefinery could create a great opportunity to strengthen economic position of numerous bioeconomy sectors (e.g. agriculture, fishery, chemical and energy) if there will be enough political and social support. Valderrama et al. have analysed social and economic dimensions of seaweed farming and processing and they agree on economic profits but also accent that political support is very meaningful, saying that, political instability and insufficient governmental and external-aid-funded support for the sector are further risk factors for the industry's development potential [42].

While political support can be a threat to seaweed biorefinery concept, this concept can also set new political goals, which would promote a sustainable use of seaweed for the production.

Development of the concept would also increase international cooperation in resource management and technological development issues. It would also be necessary to avoid threats like fluctuating oil price and availability of resources that could affect economic feasibility of seaweed biorefinery concept.

4. Conclusions

Within a biotechonomy perspective the sustainable conversion of any biomass is a key aspect moving toward the implementation of novel technologies and integrated concepts in order to reach a sustainable economic growth and market development. This is properly reflected within a seaweed-based biorefinery concept as sustainable and potentially economically viable solution of producing both high value added products and bioenergy. Depending from seaweed types and species it is possible to extract different fatty acids, oils, natural pigments, antioxidants, high value biological components and other substances which can be used in production. This is the reason why seaweed can positively affects several biotechnology areas like biofuels, cosmetics, food and food supplement production and have an impact on aquacultures and pollution removal.

We conclude that seaweed biorefinery concept can make a significant contribution to sustainable development by adding value to the original biobased feedstock but it is crucial to undergo to a more clear understanding of the overall technological processes and pathways. Seaweed conversion into wide spectrum of products using cascade conversion should be adapted to local conditions. This concept allows maximizing the biomass conversion efficiency and reducing the amount of raw material needed within a nearly-zero waste approach. Biorefinery concept is thus merging different bioeconomy sectors to potentially create multi- (and inter-) disciplinary partnerships between stakeholders.

In the same time seaweed biorefinery concept satisfy bioeconomy principles. Seaweed biorefinery is taking care of food availability and quality and uses cascading approach in this way increasing resource efficiency. The described five main properties of biorefinery concept do should not be seen as limitation to this seaweed biorefinery concept itself, instead they are defining guidelines and basic rules on how get the biorefinery concept most beneficial for a more sustainable bioeconomy. Not only all biorefinery concept together should be based on bioeconomy guidelines, 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. Some concerns still exist with the sustainable yield principle. Seaweed availability is dependent on season, climate and nature conditions and this can be challenging factor regarding on sustainability.

Biorefinery concept presents a great way to efficient use of seaweeds. Some barriers like limited technologies and unpredictable amount and quality of seaweed biomass still exist. Once the difficulties are defeated, seaweeds have all necessary properties to significantly contribute to sustainable bioeconomy as a promising biomass.

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References

[1] Subba Rao PV, Mantri VA. Indian seaweed resources and sustainable utilization: Scenario at the dawn of a new century. Curr Sci 2006;91:164– 74.

[2] Elegbede I, Guerrero C. Algae Biofuel in the Nigerian Energy Context. Environmental and Climate Technologies 2016;17:44–60.

[3] Pastare L, Romagnoli F, Lauka D, et al. Sustainable Use Of Macro-Algae For Biogas Production In Latvian Conditions: A Preliminary Study Through An Integrated Mca And Lca Approach. Environmental and Climate Technologies 2014;13:44–56.

[4] Sabunas A, Romagnoli F, Pastare L, Balina K. Laboratory Algae Cultivation and BMP Tests with *Ulva intestinalis* from the Gulf of Riga. Energy Procedia 2017;113:277–284.

[5] Balina K, Balode M, Muzikante L, Blumberga D. Impact of synthetic hormone 17α-ethinylestradiol on growth of microalgae Desmodesmus communis. Agronomy Research 2015;13(2):445–454.

[6] Seghetta M, Romeo D, D'Este M, Alvarado-Morales M, Angelidaki I, Bastianoni S, et al. Seaweed as innovative feedstock for energy and feed – Evaluating the impacts through a Life Cycle Assessment. J Clean Prod 2017;150:1–15.

[7] Abenavoli LM, Cuzzupoli F, Chiaravalloti V, Proto AR. Traceability system of olive oil: a case study based on the performance of a new software cloud. Agronomy Research 2016;14(4):1247–1256.

[8] Davis TA, Volesky B, Mucci A. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 2003;37:4311–30.

[9] Uju, Wijayanta AT, Goto M, Kamiya N. Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid–ionic liquid pretreatment. Biomass and Bioenergy 2015;81:63–9.

[10] Bikker P, van Krimpen MM, van Wikselaar P, Houweling-Tan B, Scaccia N, van Hal JW, et al. Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. J Appl Phycol 2016:1–15.

[11] IEA. IEA bioenergy Task 42 on biorefineries: co-production of fuels, chemicals, power and materials from biomass. Minutes Third Task Meet; 2007.
[12] Ree R van, Zeeland A van. IEA Bioenergy: Task42 Biorefining 2014:1–66.
[13] Seghetta M, Hou X, Bastianoni S, Bjerre AB, Thomsen M. Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers ??? A step towards a regenerative bioeconomy. J Clean Prod 2016;137:1158–69.
[14] van Hal J, Huijgen W, Bjerre A-B, López Contreras A, Stanley M, Hreggvidsson G. Carbohydrate analysis of seaweed in the biorefinery to chemicals and fuel context. 22nd Int Seaweed Symp 2016 Acad Meets Ind Copenhagen, Denmark; 2016:54.
[15] Seghetta M, Marchi M, Thomsen M, Bjerre AB, Bastianoni S. Modelling biogenic carbon flow in a macroalgal biorefinery system. Algal Res 2016;18:144–155.
[16] [Ben Yahmed N, Jmel MA, Ben Alaya M, Bouallagui H, Marzouki MN, Smaali I. A biorefinery concept using the green macroalgae Chaetomorpha linum for the coproduction of bioethanol and biogas. Energy Convers Manag 2016;119:257–65.
[17] FAO (Food and Agriculture Organization of the United Nations). Fishery and Aquaculture Statistics; 2012. Available: http://ftp.fao.org/FI/CDrom/ CD_yearbook_2010/index.htm
[18] NetAlgae. Seaweed Industry in Europe 2012:11.
[19] Bruhn A, Torring D, Thomsen M, Canal-Vergés P, Nielsen M, Rasmussen M, et al. Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of Saccharina latissima. Aquac Environ Interact 2016;8:619–36.
[20] Little DC, Newton RW, Beveridge MCM. Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. Proc Nutr Soc 2016;75:274–86.
[21] Rioux L-E, Turgeon SL, Beaulieu M. Characterization of polysaccharides extracted from brown seaweeds. Carbohydr Polym 2007;69:530–7.
[22] Li Y, Cui J, Zhang G, Liu Z, Guan H, Hwang H, et al. Optimization study on the hydrogen peroxide pretreatment and production of bioethanol from seaweed Ulva prolifera biomass. Bioresour Technol 2016;214:144–9.
[23] Maehre HK, Malde MK, Eilertsen K-E, Elvevoll EO. Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. J Sci Food Agric 2014;94:3281–90.
[24] Rupe P, Ruperez P. Mineral content of edible marine seaweeds. Food Chem 2002;79:23–6.
[25] Roesijadi G, Jones SB, Zhu Y. Macroalgae as a Biomass Feedstock : A Preliminary Analysis. Analysis 2010:1–50.
[26] Baweja P, Kumar S, Sahoo D, Levine I. Seaweed in Health and Disease Prevention; 2016.
[27] Rioux LE, Turgeon SL, Beaulieu M. Structural characterization of laminaran and galactofucan extracted from the brown seaweed Saccharina longicruris. Phytochemistry 2010;71:1586–95.
[28] Laurens LML, McMillan JD, Baxter D, Cowie AL, Saddler J (John) N, Barbosa M, et al. State of Technology Review – Algae Bioenergy; 2017.
[29] Blumberga D, Muizniece I, Blumberga A, Baranenko D. Biotechonomy Framework for Bioenergy Use. Energy Procedia 2016;95:76–80.
[30] Muizniece I, Timma L, Blumberga A, Blumberga D. The Methodology for Assessment of Bioeconomy Efficiency. Energy Procedia 2016;95:482–6.
[31] Mathijs E, Brunori G, Carus M, Griffon M, Last L. Sustainable Agriculture, Forestry and Fisheries in the Bioeconomy -A Challenge for Europe; 2015.
[32] Kumar S, Sahoo D, Levine I. Assessment of nutritional value in a brown seaweed Sargassum wightii and their seasonal variations. Algal Res 2015;9:117–25.
[33] Mendis E. Chapter 1 – Present and Future Prospects of Seaweeds in Developing Functional Foods. Adv. Food Nutr. Res. 2011;64:1–15.
[34] FAO. How to Feed the World in 2050. Insights from an Expert Meet FAO 2009;2050:1–35.
[35] FAO Fisheries and Aquaculture Department. FAO yearbook of fishery and aquaculture statistics summary tables 2016;0:26–8.
[36] Tiwari BK, Troy DJ. Seaweed Sustainability: Food and Non-Food Applications; 2015.
[37] Geldermann J, Kolbe LM, Krause A, Mai C, Militz H, Osburg VS, et al. Improved resource efficiency and cascading utilisation of renewable materials. J Clean Prod 2016;110:1–8.
[38] Liguori R, Faraco V. Biological processes for advancing lignocellulosic waste biorefinery by advocating circular economy. Bioresour Technol 2016;215:13–20.
[39] Yue D, You F, Snyder SW. Biomass-to-bioenergy and biofuel supply chain optimization: Overview, key issues and challenges. Comput Chem Eng 2014;66:36–56.
[40] Briand X, Morand P. Anaerobic digestion of Ulva sp. 1. Relationship between Ulva composition and methanisation. J Appl Phycol 1997;9:511– 24.
[41] Bruton T (BioXL), Lyons H, Lerat Y, Stanley M, Rasmussen MB. A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. Sustain Energy Irel 2009:92.
[42] Valderrama D, Cai J, Hishamunda N, Ridler N. Social and economic dimensions of carrageenan seaweed farming: a global synthesis; 2013.

Paper V

Seaweed Cultivation Laboratory Testing: Effects of Nutrients on Growth Rate of Ulva intestinalis.

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Seaweed cultivation laboratory testing: effects of nutrients on growth rate of *Ulva intestinalis*

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Abstract

New seaweed cultivation laboratory was developed and primary laboratory bench was tested if the conditions are relevant for seaweed cultivation. Simple experiment was carried out using *Ulva intestinalis* as test organism. Different nutrient levels were provided to see, which amount of nutrient is the best for cultivation of U. intestinalis. Experiment results showed that best growth rate for *Ulva intestinalis* was on concentration 2 ml L^{-1} where growth rate reached on average 7.13 ± 3.44 % per day. Higher nutrient amounts slow down algae growth.

Peer-review under responsibility of the scientific committee of the International Scientific Conference "Environmental and Climate Technologies".

Keywords: macroalgae; cultivation; in vitro; *Ulva intestinalis*; Baltic Sea

1. Introduction

Energy demand has rapidly grown since the beginning of the Industrial Revolution and it is still increasing at a rapid pace [1]. In 2009 it was estimated that 86 % of world energy consumption is derived from fossil fuels such as oil, coal and gas. Furthermore, increasing population will increase demand of fossil fuels by 20 % in the next 20 years if a new cost effective and viable energy source is not found [2]. Excessive use of fossil fuels has a negative effect on both the environment and the economy. Overuse of fossil fuels leads to enormous amount of greenhouse gas emissions

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which contribute to global warming, rising sea levels and many other concerns such as loss of biodiversity or receding of glaciers. All of these factors contribute to the need for an alternative, sustainable, effective, cost effective and cleaner energy resource that would be able to meet future demand and needs [3–7].

Macroalgae are macroscopic multicellular organisms that, depending on their pigmentation, are divided into three groups: brown, red and green algae. While most of the species live in seawater, some species are found in freshwater as well. Macroalgae found in seawater are called seaweeds [8, 9]. Usually they are present near to the shore where they are attached to different types of substrates. Both microalgae and macroalgae are considered to be a third generation energy resource and scientists hope that this kind of bio energy will overcome imperfections of previous generations of bio energy [10, 11].

Bio energy is an excellent alternative to the traditional fossil fuels because instead of using gas, oil or coal to produce energy, for bio energy production a renewable resource – biomass is used. During the process of seaweed growth, light energy and CO_2 is absorbed to produce organic molecules such as carbohydrates and lipids. These molecules can then be used to produce a different kind of fuel [12, 13]. Seaweed cultivation is attractive with the fact that it does not require arable land and in the production process freshwater is not used so it does not compete with traditional crops. Cultivation process does not accelerate ongoing climate changes in any way because algae can absorb atmospheric CO_2. Furthermore, seaweed grow and multiply rapidly and their containing polysaccharides makes seaweed attractive not only for biofuel and biogas production, but for food, animal feed and various substance production [1, 3].

Seaweed use in Latvia is limited. Algae beds in the sea are protected, but washed out seaweed called "seawaste" are taken to landfill. Although due to the low salinity the size and biomass of seaweeds is not that big as in the southern Baltic Sea, seaweed biomass could give major input. The most effective way to increase available seaweed biomass would be to develop off shore seaweed cultivation systems. Seaweed consume various nutrients found in seawater and in such way cleans seawater and even could be a way to reduce eutrophication level in the Baltic Sea [14].

Since seaweed are sedentary organisms, they need to be resistant to a variety of physical conditions such as salinity, temperature, light intensity, water motion, nutrient level and CO_2 concentrations. For optimal growth, all of these factors should be in a certain, usually very narrow, range. For each of the species this range can vary so it is crucial to determine the best conditions for each species [3]. Although for many highly popular seaweed species all of these factors are studied and explored [15], studies on Latvian conditions are still scarce. One of the things that has to be done before starting seaweed cultivation for bio energy production, is to explore optimal growth conditions for the most popular seaweed species in Latvia *Ulva intestinalis*, *Furcellaria lumbricalis, Fucus vesiculosus* [7]. Seaweed cultivation laboratory is essential to prepare seeding material and identify the best parameters for algae growth in a laboratory environment. One of the most important parameters is nutrient level so the simple nutrient test was carried out to test seaweed cultivation laboratory using green seaweed *Ulva intestinalis* as a test organism.

2. Materials and Methods

Before building up the laboratory bench for seaweed cultivation, available literature was explored and the most essential parameters were identified and a lab scheme was developed (Fig. 1).

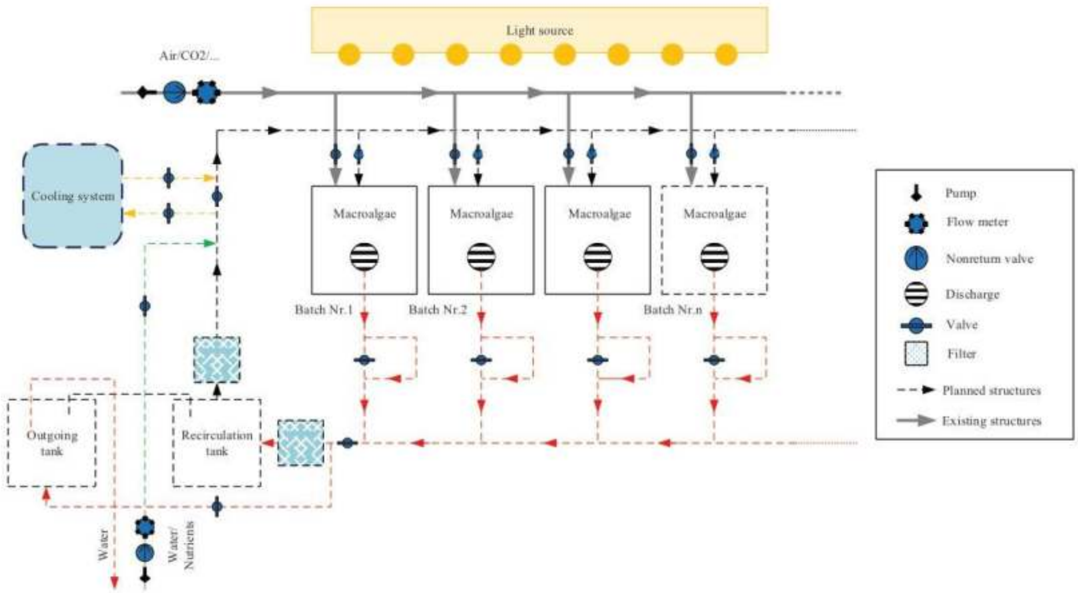

Fig. 1. Scheme of the laboratory testing bench.

The laboratory bench consists of different devices and equipment to provide water movement and light conditions. Water movement was ensured using air supply faucet, necessary light conditions were ensured by fluorescent tubes giving a light intensity of 75 µmol m^{-2} s^{-1} as described by Lignell [16]. The light regime was 16 h light followed by 8 h darkness. To gain the maximum seaweed growth all these parameters should be at the optimal level. Seaweed in different seasons from different geographical locations may require different slight changes in growth environment. Nutrient test is the simplest test to carry out to test the laboratory bench. *Ulva intestinalis* was used as a test organism. Algae growth inhibition test design was used as a base to perform a nutrient test.

2.1. Test organism and environment preparation

Green filamentous seaweed *Ulva intestinalis* was used as a test organism. The seaweed was collected from coastal water in the Riga Gulf on April 2016. Seaweed samples were washed to remove impurities. Seawater was collected using siphoning method to avoid contaminants from the water surface. Water was collected from the seashore into 20 L plastic buckets and delivered to the laboratory within an hour.

The water used in the experiment was filtered through filter paper to remove phytoplankton, zooplankton and debris. Water was sterilized in the microwave equipped with rotating base for 10 minutes in 700 W to sterilize the growth media [17]. 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 micro elements.

Conical glass flasks (250 mL) containing sterilized seawater with nutrients were inoculated with fresh seaweed thalli approximately 3 cm long. Nutrient concentrations 2, 5, 10, 50 ml L^{-1} were chosen based on the concentrations suggested in the literature [18] maximum and minimum was included. Flasks were placed under the light source with an irradiance of 1000 lux. Temperature remained constant during the experiment at 20 °C. Air was supplied with rubber tubes to provide constant water motion and gas exchange. Enriched growth media was changed once a week manually by pouring off most of the old media and replacing it with seawater enriched with nutrients. In case of evaporation, the water level was adjusted by pouring distilled water to maintain previous salinity.

2.2. Growth rate calculation methodology

Growth of *Ulva intestinalis* was measured every 7 days by placing them in a petri dish above millimeter paper and the growth rate was calculated using Eq. (1) [4]:

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

where W_t is length of specimen at the end of experiment, mm; W_0 is length of specimen at the beginning of experiment; mm; t is experiment time in days, d.

3. Results and discussion

The growth of *Ulva intestinalis* showed the specific growth pattern (Fig. 2). All of the specimens except two specimens from the highest concentration level have been growing in the first week of experiment. Few of the specimens have increased their length by 48 mm which is 10.58 % per day.

In the second week growing mostly stopped because of the plasmolysis caused by salinity. Added air supply system contributed evaporation which led to decreased water level in the flasks. Because water level was not adjusted as soon as needed, salinity level in the flasks increased and formed hypertonic solution. Water from the alga started to flow out leading to plasmolysis were cells of an alga starts to lift of from its membrane. Probably different species would be resistant against such salinity fluctuations but for the Latvian alga salinity that is two times higher than usual is fatal [19].

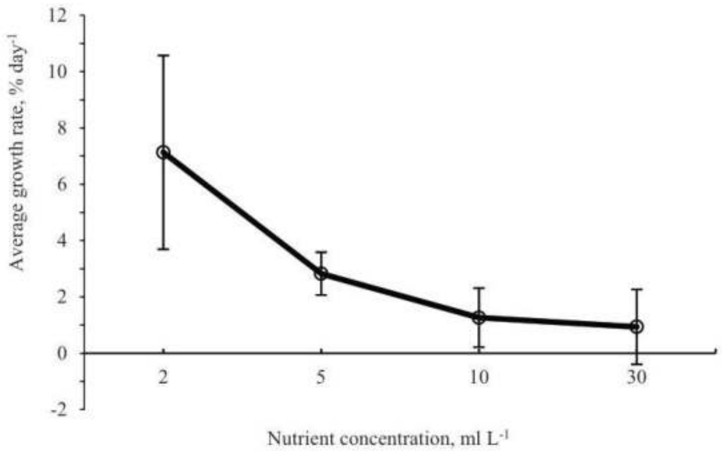

Fig. 2. Growth of *U. intestinalis* under different nutrient concentrations.

Fig. 2 shows the effect of different nutrient concentrations on algal growth. Data shows that nutrient concentrations significantly affected growth rate of *U. intestinalis*. Increasing concentration above 2 ml L^{-1} significantly slows down growth rate. Maximum growth rate was achieved with 2 ml L^{-1} high nutrient concentration and with such conditions growth rate reached in average 7.13 ± 3.44 % per day. Increasing concentration to 10 ml L^{-1}, *U. intestinalis* growth rate decreased and showed only 1.27 ± 1.05 % growth per day, which is less than the average growth rate for this species. Previous research with *U. intestinalis* showed that average growth rate for this type of alga is 0.15 – 0.25 cm per day which is 3.39 – 5.32 % per day [20]. These results were obtained with naturally occurring alga in the Black sea not with cultivated alga in laboratory and that could be the cause of better growth [15, 21].

The experimental laboratory provided sufficient conditions for algae cultivation. Main issues for algae cultivation lab would be the adjustment of the bubbling system to provide smooth bubbling. Current situation was that changing of water level, changed the strength of bubbling in many batches and evaporation happened in high rate. This situation could be solved using valves, which regulate air flow before every batch. Also temperature would be important factor that should be provided. Optimal water temperature for seaweed cultivation would be 10 °C – 15 °C [21]. This experiment was carried out in room temperature, which in this case was 20 °C. Necessary temperature should be provided using incubator or cooling system.

Next experiments should be carried out using nutrient concentrations, which exclude higher concentrations, to find limiting concentrations.

4. Conclusions

Experimental laboratory was provided with good conditions for algae cultivation and results from the first week showed high growth rate. The second week of experiment results showed slowing in the growth rate and death of specimens which was contributed to by extremely low water level which raised salinity level and caused plasmolysis. Nutrient concentration in the growth media significantly affects seaweed *U. intestinalis* growth rate. Increasing nutrient concentration above 2 ml L^{-1} *U. intestinalis* growth rate decreases. Too high nutrient content is toxic and dramatically slows down growth and leads to death of alga. Laboratory should be technically improved before carrying out the next tests, to avoid problems like evaporation and high temperature.

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References

[1] Wei N, Quarterman J, Jin Y-S. Marine macroalgae: an untapped resource for producing fuels and chemicals. Trends Biotechnol 2013;31:70–7.
[2] Taelman SE, Champenois J, Edwards MD, De Meester S, Dewulf J. Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe with a focus on quantifying sea surface occupation. Algal Res 2015;11:173–83.
[3] Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD. The cultivation of European kelp for bioenergy: Site and species selection. Biomass and Bioenergy 2015;80:229–42.
[4] Lauka D, Pastare L, Blumberga D, Romagnoli F. Preliminary Analysis of Anaerobic Digestion Process using Cerathophyllumdemersum and Low Carbon Content Additives: A Batch Test Study. Energy Procedia 2015;72:142–7.
[5] Pilicka I, Blumberga D, Romagnoli F. Life Cycle Assessment of Biogas Production from Marine Macroalgae : a Latvian Scenario. Environmental and Climate Technologies 2011;6:69–78.
[6] Cappelli A, Gigli E, Romagnoli F, Simoni S, Blumberga D, Palerno M, et al. Co-digestion of Macroalgae for Biogas Production: An LCA-based Environmental Evaluation. Energy Procedia 2015;72:3–10.
[7] Pastare L, Romagnoli F, Lauka D, Dzene I, Kuznecova T. Sustainable use of Macro-Algae for biogas production in latvian conditions: A preliminary study through an integrated MCA and LCA approach. Environ Clim Technol 2014;13:44–56.
[8] Maity JP, Bundschuh J, Chen C-Y, Bhattacharya P. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. Energy 2014;78:104–13.
[9] Chen H, Zhou D, Luo G, Zhang S, Chen J. Macroalgae for biofuels production: Progress and perspectives. Renew Sustain Energy Rev 2015;47:427–37.
[10] Van Hal JW, Huijgen WJJ, López-Contreras AM. Opportunities and challenges for seaweed in the biobased economy. Trends Biotechnol 2014;32:231–3.
[11] Herrmann C, FitzGerald J, O'Shea R, Xia A, O'Kiely P, Murphy JD. Ensiling of seaweed for a seaweed biofuel industry. Bioresour Technol 2015;196:301–13.
[12] Tan IS, Lee KT. Enzymatic hydrolysis and fermentation of seaweed solid wastes for bioethanol production: An optimization study. Energy 2014;78:53–62.
[13] Bucholc K, Szymczak-Zyla M, Lubecki L, Zamojska A, Hapter P, Tjernstrom E, et al. Nutrient content in macrophyta collected from southern Baltic Sea beaches in relation to eutrophication and biogas production. Sci Total Environ 2014;473–474:298–307.
[14] Burkholder JM, Tomasko DA, Touchette BW. Seagrasses and eutrophication. J Exp Mar Bio Ecol 2007;350:46–72.

[15] Lignell A, Ekman P, Pedersen M. Cultivation Technique for Marine Seaweeds Allowing Controlled and Optimized Conditions in the Laboratory and on a Pilotscale. Bot Mar 1987;30:417–24.
[16] Lignell A, Pedersen M. Spray Cultivation of Seaweeds with Emphasis on Their Light Requirements. Bot Mar 1986;29:509–16.
[17] Stengel DB, Connan S, Popper ZA. Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol Adv 2011;29:483–501.
[18] Anderson RA, Berges RA, Harrison PJ, Watanabe MM. Recipes for Freshwater and Seawater Media; Enriched Natural Seawater Media. Algal Cult. Tech. 2005;596.
[19] Fong P, Boyer KE, Desmond JS, Zedler JB. Salinity stress, nitrogen competition, and facilitation: What controls seasonal succession of two opportunistic green macroalgae? J Exp Mar Bio Ecol 1996;206:203–21.
[20] Zbikowski R, Szefer P, Latała A. Comparison of green algae Cladophora sp. and Enteromorpha sp. as potential biomonitors of chemical elements in the southern Baltic. Sci Total Environ 2007;387:320–32.
[21] Parchevskij V, Rabinovich M. Growth rate and harvest of the green alga Enteromorpha intestinalis on artificial substrata. Biol Morya 1991;2:56–60.

Paper VI

Laboratory Algae Cultivation and BMP Tests with Ulva intestinalis from the Gulf of Riga.

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Laboratory Algae cultivation and BMP tests with *Ulva intestinalis* from the Gulf of Riga

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Abstract

This study aims to quantitatively evaluate the biogas potential of the green alga *Ulva intestinalis* from the Gulf of Riga, using BMP test with sewage sludge as inoculum. Meantime the nutrients role in biomass growth has been explored.
The results from growing rate evaluation show that the growth of macroalgae was observed up to a certain point (typically 6–8 days of exposure) even within samples without any additional nutrients; on the contrary higher concentration has shown no growth effects. This could be explained with too high nutrient concentrations jeopardizing other conditions that are vital for macroalgae growth.
The cumulative CH_4 yields show an important spectrum of cumulative methane yields, with the highest one observed in 1:3 A/I (algae/inoculum ratio) in which the macroalgae were chopped (92.1 ± 33.5 mL CH_4/g VS on average), while the lowest one was observed in 1:5 A/I where the macroalgae had no pre-treatment (36.0 ± 10.5 mL CH_4/g VS). The results do not show a clear impact of the pre-treatments. The scenarios with no pre-treatment exhibited the effectiveness in a range of 9.7–24.6 %, while the chopped ones – 14.56–24.8 %, pre-treated with pestle – 12.9–24.13 %. The results of this study confirm the suitability of *U. intestinalis* for biogas production, especially in the Baltic region.

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Keywords: macroalgae growing; sustainability of algae fuel; gasification of algae; co-digestion; methane yield

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1. Introduction

With the effects of a dramatic CO_2 increase in the atmosphere due to carbon-intensive activities, such as continuous fossil fuel burning, threatening the sustainability and posing multiple risks to the environment, alternative fuel sources have become an important topic both for national leaders and the scientific community. In this direction biofuels, biodiesel and biogas, frequently evaluated as either carbon-neutral or even carbon-negative, when sustainable feedstock, such as Algae, are used represent a viable solution [1] and have gained considerable scientific interest. Nevertheless, not all species are studied sufficiently well, including the feasible and sustainable techniques for wide scale cultivation. Lack of cost-efficient mass scale production routes prevents this feedstock from being economically exploitable [2]; therefore, the use of algae for energy production is still far from being commercialized at large scale, despite the promising potential [3]. Seaweeds have drawn particular attention due to their capability to consume greenhouse gases (GHG) as feedstock, as recent studies have found [4], and many studies have been conducted, including industrial production of not only biogas, but also biodiesel [5, 6], bio-ethanol [7] and hydrogen [8], among others. As most macroalgae species perform photosynthesis, growing and harvesting them results in removal of nutrients from a system, and thus minimizing conditions for eutrophication [9]. This might contribute to solving the problem of high chemical oxygen demand (COD) levels in aquatic ecosystems rich in nutrients. The problem of eutrophication is particularly relevant in the case of the Baltic Sea, where COD level is one of the highest among the seas in the world [10]. Furthermore, growing seaweed does not require a land to grow, or a competition with food crops, while structural polysaccharides and low or no lignin content make macroalgae appropriate for biogas production via anaerobic digestion [11]. All these features make environmental performance of macroalgae significantly more favourable than natural gas from the Life Cycle Assessment (LCA) perspective [12].

Macroalgae using sludge as inoculum have a potential to be used as biogas feedstock and thus significantly minimize the waste products by re-utilizing them and closing the loop. As such, using Algae mixed with sewage sludge for energy recovery can also contribute to enhancing Circular economy, one of the strategies to counter depletion of resources and traditionally carbon-intense economy. Gasifying sewage sludge is proposed by D. Buchholz as one of the measures for achieving Circular economy [13]. Even more importantly, studies that have been conducted so far show that the mixture of sludge and Algae exhibits a better productivity of biogas than the sludge alone [14]. This can be explained by the optimization of C/N ratio by improving it to 20.0 or higher and thus preventing from otherwise [15] inhibiting environment for methanogenic bacteria [16, 17].

Biochemical methane potential (BMP) test is a frequently used method to evaluate the anaerobic biodegradability of a specific substrate and thus assess a specific methane yield [9]. Methane production curves are usually divided into three stages: Lag phase, decomposition phase, and flattening phase [18]. The lag phase is the time from the start of the experiment to the start of the methane production. While the results differ, depending on the experiments, there was no lag phase observed for the bottles with only sewage sludge and with lower (12 % or 25 %) concentrations of algae and only a short lag phase for the bottles with 37 % algae in the experiments, conducted by Olsson et al. [19]. Such behaviour of methane formation could be justified by the fact that microorganisms are disturbed and need time to adjust to the new environment, resulting in lack of methane peaks at the beginning of the incubation.

Conversely, the shorter the lag phase, the more easily microorganisms adapt to the conditions and utilize the substrate efficiently. Some pre-treatment methods, such as drying the cells, could prolong the lag phase, as observed with microalgae samples by Olsson et al. [19]. However, the findings by Wang et al. suggest the opposite tendency, as the lag phase of the microalgae slurry was 20 days [20].

This study is going to evaluate the feasibility of *U. intestinalis* for biogas production through BMP test and the amount of nutrients that it would be optimal for a more efficient biomass growth.

2. Methodology

2.1. Algae selection and primary pre-treatment

Ulva intestinalis samples were collected from the substrates in the Latvian beaches of Mersrags (56°59′ N and 23°51′ E) and Kesterciems (57°07′ N and 23°14′ E), the Western part of Riga Gulf on 26 July, 2016. The site is characterised by shallow depths (0.5–1 m), high nutrient levels and low salinity level (5.5–6 ‰).

The marine macroalgae sample was then transported to the laboratory. The first pre-treatment involved washing the samples for the removal of sediments, small crustaceans and traces of other Algae. 12 fragments were allocated for further Algae growing for an experiment where the nutrient concentration impact on Algae growth was evaluated. The rest of biomass was frozen at –18 °C and defrosted a day before the start of BMP experiments.

2.2. Nutrients usage and Algae growth

Three different concentrations of nutrient were tested, in addition to a sample with no nutrients. 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 micro elements. Three 250 mL flasks filled with sea water from Riga Gulf were used per each nutrient concentration, with a fragment of macroalga added to each flask and the whole chain connected to the aeration source, as depicted in Fig. 1. The experiment was screened for 21 days and area of the seaweed was measured manually using a millimeter paper, in square millimeters. The concentrations of nutrient were equal 0 mg/l (A1–A3), 2 mg/l (B1–B3), 10 mg/l (C1–C3) and 30 mg/l (D1–D3). 16/8 h day/night cycle was applied to loosely simulate the conditions of summer in Latvia.

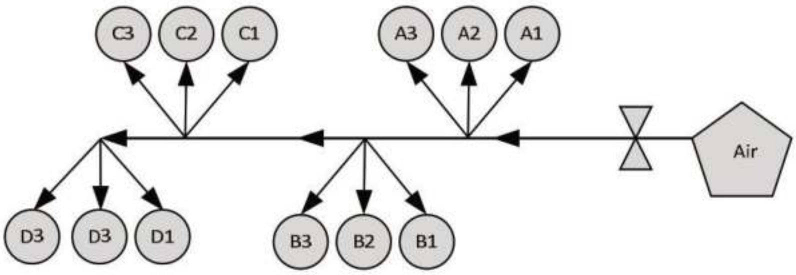

Fig. 1. The scheme depicting a placement of Algae samples in a chain.

Measurements were compared and the biomass growth was calculated in percentage using the Eq. (1):

$$\left[\left(\frac{W_t}{W_0}\right)^{\frac{1}{t}} - 1\right] \times 100\% \qquad (1)$$

where: W_t – the length of alga fragment at the end of the experiment, mm; W_o – the length of alga fragment in the beginning of the experiment, mm; t – number of days.

2.3. Biomethane potential (BMP) test

BMP test was conducted in a batch mode using 100 ml serum bottles with a working volume of 60 ml. Each bottle was filled with 30 ml of distilled water, 20 ml of inoculum with different amounts of *U. intestinalis* submerged in it. A buffer basal solution of 3 mole $NaHCO_3$ in a concentration of 3 g/L was also flushed for securing buffer capacity and avoiding fluctuation of pH (Fig. 2). pH values below 6.0–6.5 are known to inhibit methanogenic bacteria activity [22].

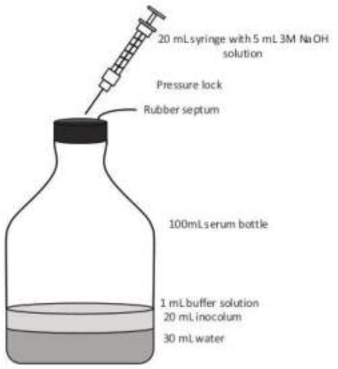

Fig. 2. The principal BMP scheme. Readapted from [21].

The samples were prepared simulating three conditions (algae/inoculum ratio, the type of sludge and pre-treatment, Table 1). Three replicates for a control assay (both for PFS and FS), containing only inoculum, were also set up in order to measure methane production of the residual substrate and thus to exclude this fraction.

The serum bottles were sealed and the headspace flushed with N_2 for approximately 2 min before sealing them with rubber stoppers and metal cramps. The test bottles were incubated in mesophilic conditions (37 °C) in the ECOCell © incubator for 30 days. The batched were manually shaken on average 1 time per day in order to maintain homogeneity in a substrate, preventing stratification of organic material and improving biogas production.

The Buswell equation can be interpreted as a stoichiometric value representing theoretical CH_4 and CO_2 volumes, produced in case the substrate is fully digested by the bacteria within the digester. The equation thus shows a theoretical CH_4 fraction in the theoretical BMP in volume is reported within Eq. (2):

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 \quad (2)$$

where: n – carbon atoms in biomass; a – hydrogen atoms in biomass; b – oxygen atoms in biomass.

Digested wastewater sludge collected from Wastewater treatment plant was used as inoculum for the BMP tests. 2 types of wastewater sludge samples were collected from Daugavgriva plant (Riga district, Latvia): the first just after the denitro/nitrification processes (PFS) and the second in the last sludge management process (FS). VS and TS content of *U. intestinalis* and both types of sludge was evaluated in accordance with EPA Standards [23, 24].

Prior to the BMP experiment the inoculum was put into a water-based incubator for 4 days at 37 °C and constantly degassed. BMP tests were used to define the amount of methane produced per gram of VS of the substrate function of different ratios among algae and inoculum (i. e. 1:3 and 1:5). This is significant to denote an optimal mix of sludge and Algae for biogas production.

Modified Gompertz model was used to analyse data accuracy and compare actual and predicted values of methane yield [24]. The Eq. (3) determines the maximum CH_4 production potential P, maximum CH_4 production rate R_{max}, as well as lag phase (time taken to start biogas production, or the acclimatization time for bacteria, expressed by λ):

$$M(t) = P.\exp\left\{-\exp\left[\frac{R_{\max}}{P}(\lambda - t) + 1\right]\right\} \quad (3)$$

where: P – the maximum CH_4 potential (mL CH_4/g $_{VS}$); R_{max} – CH_4 production rate (mL CH_4/g $_{VS\text{-}d}$); λ – lag phase (day).

$$rRMSE = \left(\frac{1}{m}\sum_{j=1}^{m}\left(\frac{d_j}{Y_j}\right)^2\right)^{\frac{1}{2}} \quad (4)$$

where: d_j – the deviation between the j_{th} measured and the predicted values; m – the number of experimental values; Yj – the j_{th} measured value.

The three parameters P, R_{max} and λ were estimated by curve-fitting using the Solver program in MS Excel 2007. The coefficient of determination for determining how close the data are to the fitted regression line is calculated using the Eq. (3). The reliability and preciseness of the acquired data are determined by the scope of deviations.

3. Case study

The green alga *Ulva*, formerly classified under the genus of *Enteromorpha* [26], is a green alga genus with up to 392 taxa attributable to it [27]. Despite having a wide range of species, *Ulva lactuca* appears to be one of the best-studied macroalgae for methane production, with its practical methane yield observed up to 371 L CH_4/kg $_{VS}$ [28]. Meanwhile, its relative *U. intestinalis* is a commonly found macroalgae species on the coast of the Baltic Sea [29] and is also potentially feasible feedstock for biogas generation.

Even though several studies have been made about the efficiency of *U. intestinalis* for biofuels, this one focuses both on its cultivation and BMP. Furthermore, as the biomass of algae differs depending on the location and such factors as temperature, salinity, sunshine hours and nutrients, this case study aims at better understanding macroalgae potential in the Gulf of Riga, as well as *U. intestinalis* methane potential. Conditions in the Gulf of Riga are peculiar due to low salinity which might result in poorer biomass growth [30]. Another unfavourable condition that macroalgae face in this location are waves and streams that can easily move the substrate around and thus not letting seaweed to form algal strands [31]. Despite that, 17 species of macroalgae have been evaluated as abundant on the coastline of Latvia, including the Gulf of Riga for some of them [29]. Consequently, this study serves as a contribution to the macroalgae potential evaluation in the Gulf of Riga and thus the Baltic Region as such. Furthermore, the data collected about the impact of nutrient concentration on the biomass growth might suggest more efficient set up of *U. intestinalis* energy plantations.

4. Results and Conclusions

4.1. Inoculum and substrate characterization

Volatile solids (VS) and total solids (TS) values were determined prior to the BMP test experiments. The results showed almost the same values of TS content in both PFS and FS (2.77 %) and similar value of VS (60.4 % and 61.3 % respectively). These values were higher for *U. intestinalis* (78.5 % of VS and 21.3 % of TS).

The chemical composition of the green alga *U. intestinalis*, was determined in a laboratory at the Latvian Institute of Organic Synthesis, LIOS. A stoichiometric equation was $C_{31.8}H_{5.2}O_{28.1}N$. Additionally, VS constituted an important part of the macroalgal biomass with a value of almost 79 %. Following the equation 1), this corresponds to a maximum theoretical yield of 371 L CH_4/kg $_{VS}$, with a theoretical share of 33.52 % volume of CH_4 and 66.48 % volume of CO_2, or 24.46 m^3 of CH_4 and 17.64 m^3 of CO_2 per 1 t of feedstock. This maximum theoretical amount of methane is further compared with the real outcomes from the BMP test in the following sections.

In comparison, the theoretical biogas potential from *Fucus vesiculosus*, also picked from the Bay of Riga and measured at the LIOS, was lower, showing 277.05 L CH_4/kg $_{VS}$ of theoretical yield and stoichiometric formula less favourable for bio-methane production than in *U. intestinalis* case ($C_{20.3}H_{33}O_{17.6}N$).

4.2. Algae growth with supplemented nutrients

While the average data showed the Algae growth peak of 3.78 % on the 3rd day, compared to the start of experiment, for 2 mL/L fertilizer samples, the peaks were even smaller for A and C samples. However, the biomass growing curves were much more diverse when comparing all 12 flasks (Fig. 3(a)). The highest peak reached in the sample B3, with 24 % of biomass increase on the 8th day. Overall, the growth of Algae was observed in all samples but D, where the fragments started to perish on the 2nd day on average. 30 mL/L can be described as an inhibitive concentration for the Alga cells. No growth can also be partially attributed to the drowsiness (and consequently lack of light) which occurred due to the accumulation of nutrients and crystal formation, which was over time the case for all other samples, where fertilizers were used.

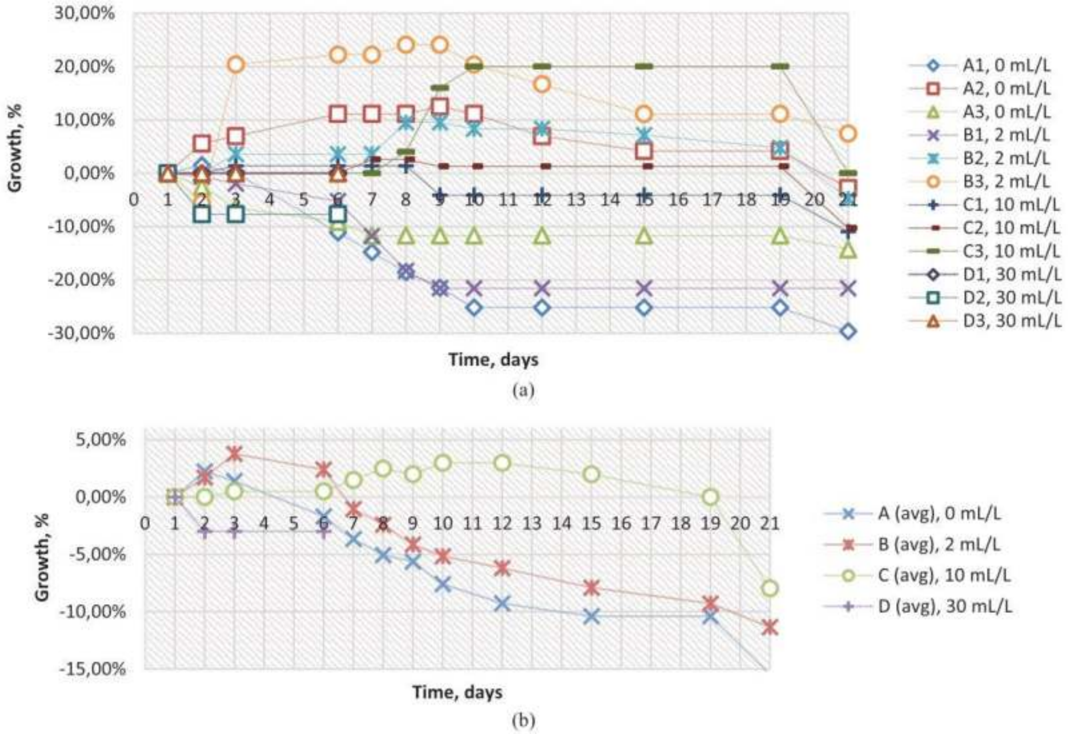

Fig. 3. Graphs showing the change in filament area in 21 days of screening, for all samples (a) and for the average (b).

While most of the screened samples had an increase in biomass in the beginning, 6th–8th days were crucial for A and B samples, as these were the days when the biomass decrease is being observed. This can also be attributed to generally undesirable conditions for Algae, as the water temperature was 21 °C and the salinity level fluctuating significantly during the night, as a result of evaporation. The optimal temperature and salinity values for *Ulva* are, however, documented to be 15 °C and 24 ‰ respectively, while 20 °C is deemed critical [32].

4.3. BMP yield results

Table 1. A table with a set of batch experimental conditions, containing predicted and practical methane yields (in CH_4/g VS), lag phase (λ, in days) and R^2. The batch number consists of the numeral, inoculum type, A/I ratio and a pre-treatment method.

Experimental condition	*Ulva intestinalis*		Inoculum	Pre-treatment method	Total CH_4 yield	λ (days)	R^2	Predicted CH_4 yield
Batch no.	Wet weight, g	Volume, ml	TS, g					
1_FS (sludge only)	-	20	0.556	-	23.6 ± 10.2	0.00	0.9392	29.9
2_PFS (sludge only)	-	20	0.554	-	21.5 ± 4.4	0.00	0.9529	24.4
3_FS, 1:3, 0	0.872	20	0.556	non-treated	37.1 ± 17.5	3.66	0.7891	35.2
4_PFS, 1:3, 0	0.869	20	0.554	non-treated	91.1 ± 15.4	0.80	0.6879	84.4
5_FS, 1:3, 1	0.872	20	0.556	chopped	78.7 ± 55	0.55	0.7828	75.6
6_PFS, 1:3, 1	0.869	20	0.554	chopped	54.0 ± 4.6	1.01	0.6734	49.4
7_FS, 1:3, 2	0.872	20	0.556	pestled	89.5 ± 11.8	0.40	0.8046	87.2
8_PFS, 1:3, 2	0.869	20	0.554	pestled	51.5 ± 37.5	0.11	0.8409	51.0
9_FS, 1:5, 0	0.523	20	0.556	non-treated	42.8 ± 0.4	0.37	0.7699	36.7
10_PFS, 1:5, 0	0.521	20	0.554	non-treated	36.0 ± 10.5	1.97	0.7389	30.6
11_FS, 1:5, 1	0.523	20	0.556	chopped	77.7 ± 15.5	1.77	0.8525	76.2
12_PFS, 1:5, 1	0.521	20	0.554	chopped	92.1 ± 33.5	2.12	0.9192	98.5
13_FS, 1:5, 2	0.523	20	0.556	pestled	47.8 ± 2.4	1.25	0.8022	45.0
14_PFS, 1:5, 2	0.521	20	0.554	pestled	67.1 ± 5.7	0.19	0.8876	69.1

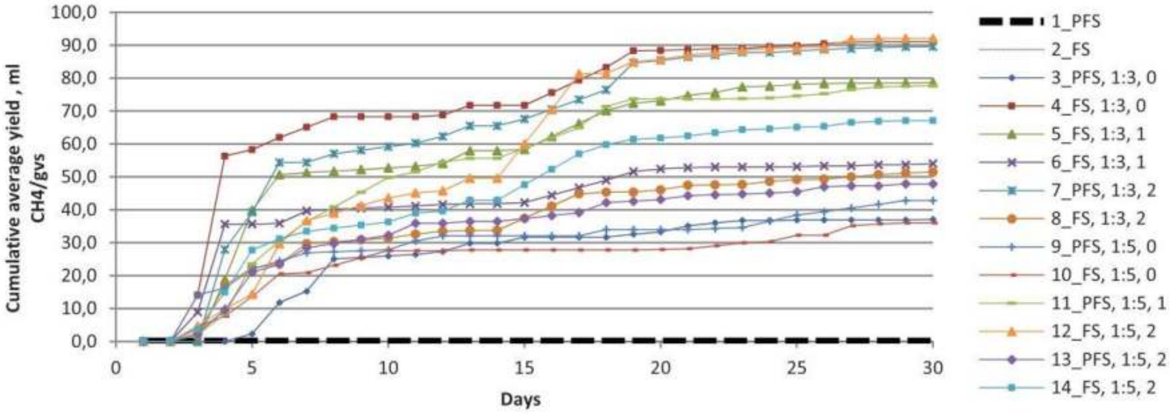

Fig. 4. Cumulative bio-methane yields for all batch trials.

BMP test with *U. intestinalis* batches showed a wide range of data (Fig. 4). While cumulative average yields were the lowest in control assays (21.47 mL CH_4/g_{VS} and 23.6 mL CH_4/g_{VS} on the average), four sample sets did not exceed 50 mL CH_4/g_{VS}, even though some non-uniform behaviour for belated and non-typical flattening phase was observed in many batches, including the ones with low yields (3_PFS, 1:3, 0; 7_PFS, 1:3, 2; 8_PFS, 1:3, 2; 13_PFS, 1:5, 2). Conversely, the highest cumulative CH_4 yield is observed in 12_PFS_1:5, 1 sample (92.1±33.5 mL CH_4/g_{VS} on average). Overall, 13.3 % of batches exceeded 100.0 mL CH_4/g_{VS} cumulative yield, with a peak value of 133.7 mL CH_4/g_{VS} (or 36 % of the theoretical potential according to Buswell equation) observed in a slightly mechanically pre-treated 1:3 PFS batch. Control assays exhibited zero lag phase, while in the case of 1:3 and 1:5 ratios the lag phase was minimal, amounting to 1.09 and 1.28 days on average and 0.11–3.66 individually (Table 1), supporting the findings by Olsson et al. [19], but conflicting with the ones by Wang et al. [20].

Another non-uniform behaviour, common for all batches, including control assays, was double methane peaks, occurring during the 3rd–4th days and a smaller one on the 14th to 15th day. This instance of non-uniformity can be explained by the issue of changing syringes regularly, as once syringes get older their performance at collecting methane gas becomes poorer, resulting in the shift of actual peak days.

All sets of batches had cumulative methane yields, accounting to not more than a quarter of the theoretical yield for *U. intestinalis*. The scenarios with no pre-treatment exhibited the effectiveness in a range of 9.7–24.6 %, while the chopped ones – 14.56–24.8 %, pre-treated with pestle – 12.9–24.13 %. In 53 % of the batches, gas fraction was 25 % or lower, but only in 20 % this value was 20 % or lower. Such relatively significant fractions from inoculum suggest lack of efficiency in pre-treatment, as increasing C/N ratio should result in more efficient methane production due to improved conditions for methanogenic bacteria [17].

Gompertz models show rMSE for the algae substrate in the range of 0.9–14.88 %. On the other hand, the range showed even less uniformity for inoculum-only standard samples (13.6 and 26.9 %). While most samples had obtained deviations lower than 8.5 %, a higher deviation in the other 4 suggests the presence of deviations in the application of methodology, namely the issue of regular change of syringes. A non-uniform behaviour of Algae fragments in the flasks with sea water and nutrients can also be attributed to poorly simulated conditions of their habitat, with too high water temperature, stress due to high salinity fluctuations during the day and while taking the measurements every 1–2 days. The scope should be increased for more data for analogical experiments to compare with. The outcome of this study, however, suggests the possibility for the Baltic States to develop advanced biofuel methods, using Algae which have not been commercially implemented so far.

Acknowledgements

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References

[1] Taylor B, Xiao N, Sikorski J, Yong M, Harris T, Helme T, Smallbone A, Bhave A, Kraft M. Techno-economic assessment of carbon-negative algal biodiesel for transport solutions. Applied Energy 2013;106:262–274.
[2] Jones CS, Mayfield SP. Algae biofuels: Versatility for the future of bioenergy. Current Opinion in Biotechnology 2012;23(3)346–351.
[3] Chiaramonti D, Prussi M, Buffi M, Casini D, Rizzo AM. Thermochemical Conversion of Microalgae: Challenges and Opportunities. Energy Procedia 75;2015:819–826.
[4] Ahmad AL, Mat Yasin NH, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: a review. Renew Sustain Energy Rev 2011;15(1):584–593.
[5] Andersson V, Viklund SB, Hackla R, Karlssonb M, Berntsson T. Algae-based biofuel production as part of an industrial cluster. Biomass and Bioenergy 2014;71:113–124.
[6] Panayiotis T, Wang Y, Roskilly AP, Caldwell GS. Algae to Energy: Engine Performance Using Raw Algal Oil. Energy Procedia 2014;61:656–659.
[7] Firoz A, Mobin S, Chowdhury H. Third Generation Biofuel from Algae. Procedia Engineering 2015;105:763–768.
[8] Azadi P, Brownbridge GPE, Mosbach S, Inderwildi OR, Kraft M. Production of Biorenewable Hydrogen and Syngas via Algae Gasification: A Sensitivity Analysis. Energy Procedia 2014;61:2767–2770.
[9] Pugliese A, Bidini G, Fantozzi F. Anaerobic Digestion of Macrophytes Algae for Eutrophication Mitigation and Biogas Production. Energy Procedia 2015;82:366–373.
[10] Ronnberg C, Bonsdorff E. Baltic Sea eutrophication: area-specific ecological consequences. Hydrobiologia 2004;514:227–241.
[11] Dave A, Huang Y, Rezvani S, McIlveen-Wright D, Novaes M, Hewitt N. Technoeconomic assessment of biofuel development by anaerobic digestion of European marine cold-water seaweeds. Bioresour Technol 2013;135:120–127.
[12] Cappelli A, Gigli E, Romagnoli F, Simonia S, Blumberga D, Palerno M, Guerriero E. Co-digestion of Macroalgae for Biogas Production: an LCA-based Environmental Evaluation. Energy Procedia 2015;72:3–10.
[13] Buchholz D. Gasifying sludge and slurry: Europe's circular economy in action. Renewable Energy Focus 2015;16:147–149.
[14] Panpong K, Nuithitikul K, O-thong S, Kongjan P. Anaerobic Co-Digestion Biomethanation of Cannery Seafood Wastewater with Microcystis SP; Blue Green Algae with/without Glycerol Waste. Energy Procedia 2015;79:103–110.
[15] Kabbashi N. Sewage sludge composting simulation as carbon/nitrogen concentration change. J Environ Sci 2011;23(11):1925–28.
[16] Balina K, Romagnoli F, Blumberga D. Chemical composition and potential use of Fucus vesiculosus from Gulf of Riga. Energy Procedia 2016;95:43–49.
[17] Wang X, Lu X, Li F, Yang G. Effects of Temperature and Carbon-Nitrogen (C/N) Ratio on the Performance of Anaerobic Co-Digestion of Dairy Manure, Chicken Manure and Rice Straw: Focusing on Ammonia Inhibition. PLoS One 2014; 9(5).
[18] Carlsson M, Schnurer A. RAPPORT U2011:12 – Handbok metanpotential. Avfall Sverige utveckling; 2011.
[19] Olsson J, Feng XM, Ascue J, Gentili FG, Shabiimam MA, Nehrenheima E, Thorin E. Co-digestion of Cultivated Microalgae and Sewage Sludge From Municipal Waste Water Treatment. Bioresource Technology 2014; 171:203–210.
[20] Wang H, Hu Z, Xiao B, Cheng QFL. Ammonium Nitrogen Removal in Batch Cultures Treating Digested Piggery Wastewater With Microalgae Oedogonium sp. Water Sci. Technol. 2013;68:269–275.
[21] Esposito G, Frunzo L, Panico A, Pirozzi F. Bio-Methane Potential Tests To Measure The Biogas Production From The Digestion and Co-Digestion of Complex Organic Substrates. The Open Environmental Engineering Journal 2012;5:1–8.
[22] Hansen TL, Schmidt JE, Angelidaki I, Marca E, Jansenb JC, Mosbæka H, Christensen TH. Method for determination of methane potentials of solid organic waste. Waste Manag. 2004;24(4):393–400.
[23] Regulations of the United States. EPA Method 24 – Determination of Volatile Matter Content, Water Content, Density, Volume Solids and Weight Solids of Surface Coatings, Appendix A-7, Part 60, Chapter 1 of Title 40 of the Code of Federal; 1991. Available: http://www.deq.state.or.us/aq/forms/sourcetest/appendix_a1.pdf
[24] Apha, Awwa, Wef. Standard Methods for the Examination of Water and Wastewater; 2005.
[25] Gompertz B. On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies. Philos. Trans. R. Soc. London 1825;115:513–583.
[26] Hayden HS, Blomster J, Maggs CA, Silva PC, Stanhope MJ, Waaland JR. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. European Journal of Phycology 2003;38:277–294.
[27] Guiry MD, Guiry GM. *Ulva Linnaeus*, 1753; AphiaID: 145984. AlgaeBase. National University of Ireland, Galway. World Register of Marine Species.
[28] Bruhn A, Dahl J, Nielsene HB, Nikolaisen L, Rasmussen MB, Markagerb S, Olesen B, Arias C, Jensen PD. Bioenergy potential of *Ulva lactuca:* Biomass yield, methane production and combustion. Bioresource Technology 2011;102:2595–2604.
[29] Strunga A. Mikro un makro algu audzesanas un parstrades iespejas Baltijas juras regiona. SUBMARINER, 2013.
[30] Ichihara K, Miyaji K, Shimada S. Comparing the low-salinity tolerance of *Ulva* species distributed in different environments. Phycological Research 2013;61(1):52–57.
[31] Ojaveer H, Jaanus A, Mackenzie BR, Martin G, Olenin S, Radziejewska T, Telesh I, Zettler ML, Zaiko A. Status of biodiversity in the Baltic sea. PLoS One 2010;5(9):1–19.
[32] Kang EJ, Kim JH, Kim K, Kim KY. Adaptations of a green tide forming *Ulva linza* (*Ulvophyceae, Chlorophyta*) to selected salinity and nutrients conditions mimicking representative environments in the Yellow Sea. Phycologia 2016;55(2):210–218.

Paper VII

Lab scale cultivation of Baltic Ulva intestinalis in different light and nutrient conditions: Effects on growth and morphology

LAB SCALE CULTIVATION OF BALTIC *ULVA INTESTINALIS* IN DIFFERENT LIGHT AND NUTRIENT CONDITIONS: EFFECTS ON GROWTH AND MORPHOLOGY

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ABSTRACT: Seaweed cultivation is underdeveloped in Baltic countries because low salinity reduce seaweed growing efficiency. Seaweed grown in brackish water have adapted to low salinity and their physical demands are different than seaweed growing in ocean water. Therefore, there is a need to determine best cultivation parameters that would favour brackish seaweed cultivation. The effects of nutrient loading and different lighting colours were assessed on the growth and morphology of the abundant filamentous green algae *Ulva intestinalis*, collected in coast of Latvia. Conventional fertiliser and special algae growth media was used in different concentrations and growth rate was measured. Different lighting was tested: normal lighting, blue, red, partial lighting and no lighting was compared, and growth rate was measured. Results show that plant fertilizer gives highest growth rate 3.4 %/day in concentration 2.5 mL·L^{-1}comparing to Provasoli Enriched seawater medium which gave highest yield in 5 mL·L^{-1}(1.70 %/day). Full light conditions show highest growth, other researched light regimes cause fragmentations of algae material and leads to loss of viability.
Keywords: macroalgae, *Ulva intestinalis*, laboratory scale, nutrient change, light impact.

1 INTRODUCTION

Even thoughglobal seaweed production is increasing brackish marine environment is not well known source for seaweed cultivation[9]. The Baltic Sea is known as a unique brackish environmentand it holds great amount of seaweed biomass which could be used for different kind of product production [3].*Ulva intestinalis* is a marine macrophytic green alga which is widely distributed in Baltic Sea and could be exploited with gaining economic benefits[5].Despite the fact that brackish seaweed holds commercial potential, they are not studied sufficiently well, and cultivation techniques for wide scale cultivation are not developed. Cultivation of seaweeds brings not only economic benefits but they contribute to solving the problem of eutrophication by removing nutrients from aquatic environment [20].

To initiate production on larger scale, it is important to establish feasible and sustainable techniques for seaweed cultivation[16].Most important parameters for seaweed cultivation are salinity, nutrients, temperature, light intensity water motion and CO_2 concentration.

Seaweeds are photosynthetic organisms and their growth is supported by light and CO_2[8]. Demand for lighting conditions vary between different species and diverse environmental conditions. There are species which need only30 – 70 µmol m^{-2} s^{-1} light intensity, but some require much higher level of light intensity 200 – 250 µmol m^{-2} s^{-1}[6,11,12]. In comparison with other seaweed green algae are the most similar to vascular plants and photosynthetic processes are similar.

Photosynthesis is carried out by chlorophyll a and chlorophyll b[17]. These pigments hold responsibility for the green colour of the algae. Chlorophyll captures the red light,abundant in shallow water[13]. *Ulva lactuca* is abundant in depth 2 – 5 m and it needs at least180 µmol m^{-2} s^{-1} light intensity, but research on *Ulva intestinalis* cultivation in laboratory environment showed that maximum growth yield is reached on light intensity 250 µmol m^{-2} s^{-1}[14]. This is very high light intensity and usually is reached only in tropical regions. Green algae growing in Latvia has adapted for lower light intensity.

Growth rate of seaweed is also influenced by different chemicals, their concentrations and their mutual ratios. In laboratory conditions phosphorous and nitrogen are the most used nutrient element. Several other research show that these are the elements that have the most positive impact on seaweed growth, but mainly it depends on seaweed natural habitat[10,19]. Nutrient concentrations can differ from one place to another depending from river inflows which usually contains nutrient rich water from agricultural lands. Nutrient concentrations vary in different water bodies and it affects number of algae there.

As soon as nutrient concentration reaches the optimum they start to reproduce in high rate [21]. Nygård in his research tells about increased photosynthetic activity in areas with higher nutrient concentrations[18]. These results suggest that higher nutrient concentrations should increase seaweed growing. *Ulva intestinalis* is green algae inhabiting coastline of the Baltic Sea. The Baltic Sea is a partly closed Sea and it is eutrophicated and rich with nutrients, chemicals and pharmaceuticals[7]. Nitrogen and phosphorous are the most important elements supporting algae growth and development.Short-term lack of nitrogen in the seaweed is also reducing amount of it in seaweed cells. Critical amount of N varies from 0.7 to 3.2% of dry mass[4].

Variations in light and nutrient concentration can have a significant impact on algae growing, therefore it is necessary to determine precise range of these parameters to enhance the productivity.

Algal productivity and morphological changes have been studied in different light and nutrient conditions in laboratory batch scale. The objective of the study was to determine optimal nutrient and light regimes for growing of *Ulva intestinalis*in laboratory.

2 MATERIALS AND METHODS

2.1 Algae selection and primary pre-treatment

Ulva intestinalis samples were collected from theirnatural habitat – stones of Tuja jetty in the eastern coast of the Riga Gulf(57°29′ N and 24°22′ E) on 25May 2017. They were collected in shallow zones where it can be done by hands. The site is characterised by shallow depths (0.5–1 m), high nutrient levels and low salinity level (5.5–6 ‰). Water temperature 13.7°C.

The seaweed was transported to the laboratory. The first pre-treatment involved washing the samples in clean seawater for the removal of sediments, small crustaceans and traces of other algae. Cleaned biomass was allocated for further maintenance in thermal chamber with temperature 13.7°C, artificial light 16:8 light : dark and medium bubbling for a week to adapt to experiment conditions.Only visually green and healthy specimens were chosen to be used in experiments. Seawater was filtered and microwaved in 700W every litre for 10 minutes to kill fungi and bacteria living in seawater [1].

2.2 Experiment design

Both tests were carried out in sterile 250 mL glass conical flasks in three replicates. Green and healthy algae specimens were selected, photographed and put in the flasks with growth medium. The flasks were placed in thermal chamber in 13.5°C and 16:8 light : darkcycle was applied to loosely simulate the conditions of summer in Latvia. Air output channel was usedto provide moderate bubbling, valves were used to adjust even bubbling in all batches.

The experiment was screened for 21 days. Every seven days growth medium and flask was changed, and seaweed samples were photographed. Pictures were analysed and surface area was measured using an image processing software "ImageJ" [22]. Growth of *Ulva intestinalis* was measured every 7 days by placing them in a petri dish above millimetre paper and growth rate was calculated using formula [15]:

$$\left|\left(\frac{W_t}{W_0}\right)^{\frac{1}{t}} - 1\right| * 100\%$$

where W_t is surface area of specimen at the end of experiment (mm), W_0 issurface area of specimen at the beginning of experiment (mm); t is experiment time in days (d).

2.3 Nutrient test

Two types of nutrients in five different concentrations of nutrient were tested, in addition to a sample with no nutrients.

As first 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 micro elements. Universal fertilizer was diluted with water to make stock solution with relevant N ion concentrations as special seaweed growing medium stock solution.As second nutrient source was usedProvasoli Enriched SeawaterStock Solution purposed for seaweed cultivation[1].

Both types of nutrients were tested in concentrations 0 mL·L^{-1}, for control, 2.5 mL·L^{-1}, 5 mL·L^{-1}, 10 mL·L^{-1}, 15 mL·L^{-1} and 20 mL·L^{-1}.

2.4 Light test

Five light regimes were tested during the experiment. Plastic screen was used to make light filters to provide different lighting conditions (Table I).

Table I: Experiment light conditions

Light condition	Filter used	Light intensity (μmol photons m^{-2} s^{-1})
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

3 RESULTS AND DISCUSSION

3.1 Effects of nutrients

The growth response over two weeks acclimatised*Ulva intestinalis* was tested using two types of nutrients at nutrient concentrations: 0 mL·L^{-1}, for control, 2.5 mL·L^{-1}, 5 mL·L^{-1}, 10 mL·L^{-1}, 15 mL·L^{-1} and 20 mL·L^{-1}.

Each of these nutrient sources show different impact on growth yield Figure 1.

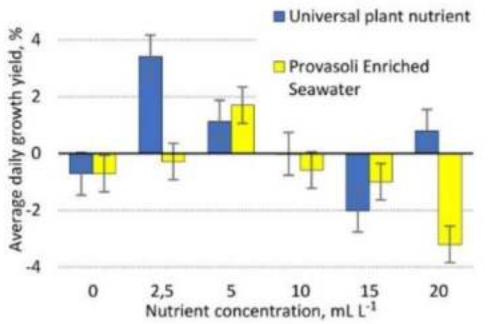

Figure 1: *Ulva intestinalis* average daily growth yield in two types of nutrients in five different concentrations.

Using universal plant fertilizer in concentrations that are relevant to special growth medium gives higher growth results. Best daily average growth yield was reached on concentration 2. where average daily growth was more than 3%. Increasing nutrient concentrationto 5 mL·L^{-1}reduced growth of algaeto 1% per day but concentrations 10 and 15 mLL^{-1}not only stopped seaweed growing but also caused degradation of *U. intestinalis* tissue. That could be caused by proliferation by microscopic algae which consumed nutrients necessary for growth of *Ulva* (Figure 2).

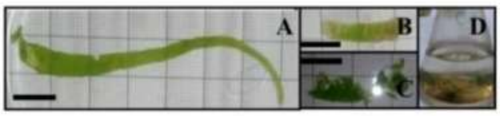

Figure 2: *Ulva intestinalis* development in 21 days in plant fertilizer concentration15 mLL^{-1} A Start of the experiment B 7 days C 14 days D 21 day

It was also observed that inthese concentrations algae material started to fragmentate.

Highest concentration used in this experiment was 20 mL·L^{-1}and it did have a positive impact on algal growth and fragmentation did not occur comparing to lower concentrations (Figure 3).It was also noticed that algae thalli were dark green and healthy.

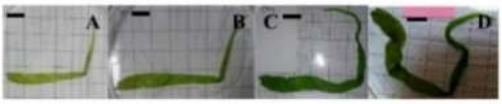

Figure 3: Ulva intestinalis development in 21 days in plant fertilizer concentration 20 mL·L^{-1} A. Start of the experiment B. 7 days C. 14 days D. 21 day

Higher growth in 20 mL·L^{-1}could be successful because there were no microalgal pollution and algal material saved its condition.

Results of using special Provasoli Enriched seawater show that highest growth was achieved in concentration 5 mL·L^{-1}and other concentrations was notnot giving positive results. These results show that *Ulva* growing in brackish Baltic Sea environment prefer lower medium concentration than suggested in literature [1].

For both nutrient sources lower concentrations showed better growth results. Highest growth can be achieved when universal plant fertilizer was used as a nutrient source. Highest biomass yield was achieved with concentration with 2.5 mL plant fertilizer stock solution to a litre filtered seawater. More than 3.4% daily growth was registered in this concentration. This trend is like results of previous research in which also plant fertiliser was used as a nutrient source. Concentration of 2 mL L^{-1} allowed to reach concentration 7% per day [2]. Average growth rate for the *Ulva intestinalis* is 0.15 and 0.25 cm.Special growth media often contain lot of metals, vitamins and stabilizers, that should provide metabolic processes and support algae with necessary diet. Results achieved in this research shows, that it is possible to grow algae using plant fertiliser and it even gives higher growth rate.

From morphological point of view there were no big differences observed between types of nutrients. Between concentrations proliferation of microscopic algae was observed and some unidentified thalli forming algae was noticed. Further microscopic analysis should be made to get more clear results.

3.2 Effects of lighting colour

Results of the experimentwith different lighting colours can be seen in Figure 4.

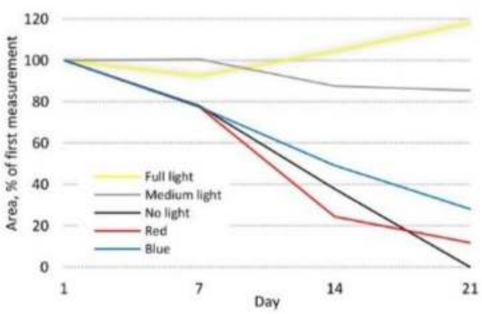

Figure 4: Effects of different lighting conditions on*Ulva intestinalis* size.

As it can be seen in the graph, the biggest growth was reached in full light without any filters. Even though degradation of seaweed material was observed after first week of experiment it might be caused by the stress of changing growth environment. During the next weeks algae growing was observed and total growth during the experiment was 18% (Figure 5).

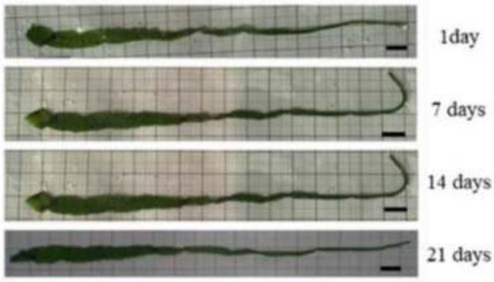

Figure 5: Growing of *U. intestinalis* in full light conditions in 21 days.

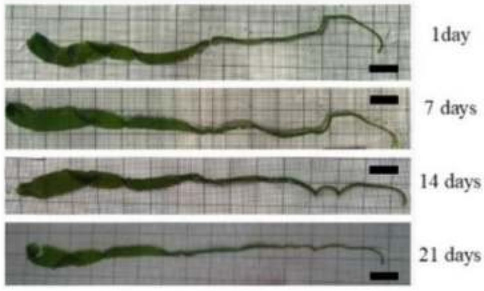

Figure 6: Growing of *U. intestinalis* in part light conditions in 21 days.

Reduced light conditions did not have any significant changes in size in first week, but in next week size of the seaweed reduced and in the end of the experiment it was 85% of the initial size. Algae material was fresh and green giving us insight that reduced light conditions did not affect viability but only growing (Figure 6).

When coloured lights were used, reduction of initial algae material was observed. Red light initiated 12% and blue light 28% reduction comparing to initial sample size.

These shades caused insufficient light conditions for algae life and growth (Figure 7). The specific filters did not provide enough light to continue photosynthetic processes.

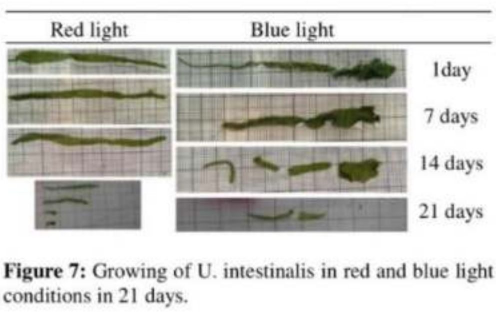

Figure 7: Growing of U. intestinalis in red and blue light conditions in 21 days.

Seaweed which were kept in dark showed no growing signs and after 14 days 38% reduction was observed and in 21 days all *U. intestinalis* material was pale coloured and decayed with no signs of life (Figure 8).

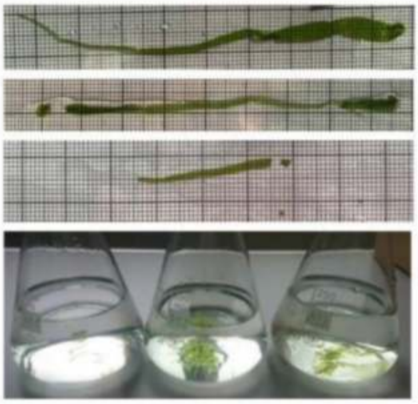

Figure 8: *U. intestinalis* in dark conditions in 21 days.

Photosynthesis is vital process to support metabolism in algae. Lack of light inhibits photosynthesis and cause algae death and decaying. Blue and red coloured lights caused algae material fragmentation, but material still saved its colour and firm condition.It could be that these colours stopped some photosynthetic processes but did not caused complete death of algae. These colours should be researched more to see the full impact on photosynthesis of seaweed.It is known, that for process of photosynthesis red light is absorbed by Photosystem II. Red light (540nm-630nm) activates the photosynthesis even though blue light is absorbed highest More precise data on light properties and interactions is needed to get more precise insight in light impact on growth of *Ulva intestinalis*.

4 CONCLUSIONS

In conclusion, both nutrient sources gave better results in lower concentrations. highest growth can be achieved when universal plant fertilizer was used as a nutrient source. Highest biomass yield was achieved with concentration with 2.5 mL plant fertilizer stock solution to a litre filtered seawater. More than 3.4% daily growth was registered in this concentration. Full light conditions have a positive impact on seaweed growing, but reduced light have a negative impact on growing and even cause degradation and full loss of viability. More research with more emphasis on photosynthetic activity is needed.

5 REFERENCES

[1] R.A. Andersen, Algal Culturing Techniques, Elsevier, 2005.

[2] K. Balina, A. Lika, F. Romagnoli, D. Blumberga, Seaweed Cultivation Laboratory Testing: Effects of Nutrients on Growth Rate of Ulva intestinalis, Energy Procedia, 113 (2017) pag. 454.

[3] K. Balina, F. Romagnoli, D. Blumberga, Seaweed biorefinery concept for sustainable use of marine resources, Energy Procedia, 128 (2017) pag. 504.

[4] J.A. Berges, D.J. Franklin, P.J. Harrison, Evolution of an artificial seawater medium: Improvements in enriched seawater, artificial water over the last two decades, Journal of Phycology, 37 (2001) pag. 1138.

[5] P. Bikker, M.M. van Krimpen, P. van Wikselaar, B. Houweling-Tan, N. Scaccia, J.W. van Hal, W.J.J. Huijgen, J.W. Cone, A.M. López-Contreras, Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels, Journal of Applied Phycology, (2016) pag. 1.

[6] N.L. Bird, L.C.-M.C.-M. Chen, J. McLachlan, J. McLachla, Effects of Temperature, Light and Salinity on Growth in Culture of Chondrus crispus. Furcellaria lumbricalis, Gracilaria tikvahiae (Gigartinales, Rhodophyta), and Fucus serratus (Fucales, Phaeophyta), Botanica Marina, XXII (1978) pag. 521.

[7] B. Björlenius, M. Ripszám, P. Haglund, R.H. Lindberg, M. Tysklind, J. Fick, Science of the Total Environment Pharmaceutical residues are widespread in Baltic Sea coastal and offshore waters – Screening for pharmaceuticals and modelling of environmental concentrations of carbamazepine, Science of the Total Environment, 633 (2018) pag. 1496.

[8] C.M. Buchholz, G. Krause, B.H. Buck, Seaweed Biology, Seaweed Biology, 219 (2012) pag. 471.

[9] FAO Fisheries and Aquaculture Department, FAO yearbook of fishery and aquaculture statistics summary tables, 0 (2016) pag. 26.

[10] F. Fernand, A. Israel, J. Skjermo, T. Wichard, K.R. Timmermans, A. Golberg, Offshore macroalgae biomass for bioenergy production: Environmental aspects, technological achievements and challenges, Renewable and Sustainable Energy Reviews, 75 (2017).

[11] D.C.M. Gattuso J.P., Gentili B., Light availability in the coastal ocean: impacts on the distribution of benthic photosynthetic organisms and contribution to primary production., Biogeosciences, (2006) pag. 489.

[12] B. George, I. Pancha, C. Desai, K. Chokshi, C. Paliwal, T. Ghosh, S. Mishra, Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae Ankistrodesmus falcatus - A potential strain for bio-fuel production., Bioresource Technology, 171 (2014) pag. 367.

[13] C.L. Hurd, P.J. Harrison, K. Bischof, C.S. Lobban, Seaweed Ecology and Physiology, 2014.

[14] K.Y. Kim, I.K. Lee, The germling growth of Enteromorpha intestinalis (Chlorophyta) in laboratory culture under different combinations of irradiance and salinity and temperature and salinity, Phycologia, 35 (1996) pag. 327.

[15] D. Lauka, L. Pastare, D. Blumberga, F. Romagnoli, Preliminary Analysis of Anaerobic Digestion Process using Cerathophyllum demersum and Low Carbon Content Additives: A Batch Test Study, Energy Procedia, 72 (2015) pag. 142.

[16] Y. Lehahn, K.N. Ingle, A. Golberg, Global potential of offshore and shallow waters macroalgal biorefineries to provide for food, chemicals and energy: Feasibility and sustainability, Algal Research, 17 (2016) pag. 150.

[17] M. Merceron, V. Antoine, I. Auby, P. Morand, In situ growth potential of the subtidal part of green tide

forming Ulva spp. stocks., The Science of the Total Environment, 384 (2007) pag. 293.
[18] C.A. Nygård, M.J. Dring, Influence of salinity, temperature, dissolved inorganic carbon and nutrient concentration on the photosynthesis and growth of Fucus vesiculosus from the Baltic and Irish Seas, European Journal of Phycology, 43 (2008) pag. 253.
[19] T.A. V. Rees, Safety factors and nutrient uptake by seaweeds, Marine Ecology Progress Series, 263 (2003) pag. 29.
[20] M. Seghetta, X. Hou, S. Bastianoni, A.B. Bjerre, M. Thomsen, Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers ??? A step towards a regenerative bioeconomy, Journal of Cleaner Production, 137 (2016) pag. 1158.
[21] Y. Yang, Z. Chai, Q. Wang, W. Chen, Z. He, S. Jiang, Cultivation of seaweed Gracilaria in Chinese coastal waters and its contribution to environmental improvements, Algal Research, 9 (2015) pag. 236.
[22] ImageJ, (n.d.).

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7 LOGO SPACE

LATENERGI PHYCOMORPH cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY