Journal of Applied Phycology
https://doi.org/10.1007/s10811-020-02044-0

23RD INTERNATIONAL SEAWEED SYMPOSIUM, JEJU

Integrated biorefinery process for sustainable fractionation of *Ulva ohnoi* (Chlorophyta): process optimization and revenue analysis

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Received: 24 July 2019 / Revised and accepted: 15 January 2020

Abstract

Blue economy, the sustainable and effective use of ocean resources for economic growth, is a major challenge to coastal communities. Marine macroalgae are potentially sustainable feedstock for future food, materials, chemicals, and fuels. For seaweed biorefinery, the fractionation of the biomass to co-produce multiple products is crucial in the efficient valorization of the marine biomass. In this work, we developed a protocol for co-production of six different products from the green macroalga *Ulva ohnoi* using green extraction methods. A total of 90.31 ± 1.94% of the initial biomass was recovered in separated products. The fraction of the recovered products from initial dry weight biomass was 45.42 ± 1.91% salts, 3.67 ± 1.38% starch, 3.81 ± 1.26% lipids, 13.88 ± 0.40% ulvan, 14.83 ± 1.06% proteins, and 8.70 ± 1.87% cellulose. A potential revenue analyses, based on these experimental data and current market prices, suggests that total the revenue fluctuates between US\$1.56 and US\$3.93 kg^{-1} of dry biomass and depends on recovered products fraction in the seaweed biomass and products market value.

Keywords Biorefinery · Blue economy · Co-production · Macroalgae · Marine biorefinery · *Ulva* · Chlorophyta

Introduction

For many coastal communities, extension of the economic activities to the seas and oceans provides an exciting opportunity for growth and development. Sustainable use and management of the marine areas, a blue economy, could provide novel tools to combat poverty while protecting the ecosystems (Olteanu A and Stinga V 2019). Marine macroalgae (seaweeds) are primary producers in the seas and oceans and thus can power marine aquaculture and marine biorefineries (Balina et al. 2017) as terrestrial crops power agriculture and land plant–based bioeconomy (Bikker et al. 2016; Magnusson et al. 2016; Singh et al. 2016; Gajaria et al. 2017; Sillanpää and Ncibi 2017; Prabhu et al. 2019). Production of terrestrial biomass requires freshwater and arable land. Macroalgae chemical composition suggests that if fractionated, their components can supply basic chemicals such as carbohydrates, lipids, starch, proteins, vitamins and minerals (Suutari et al. 2015; Wells et al. 2017) used in multiple bio-industries, contributing to the terrestrial plant biomass. Moreover, macroalgae also are a source of unique carbohydrates such as carrageenan, alginate, ulvan, mannitol and agar that have numerous medicinal and therapeutic applications (Thangavel and Sridevi 2015). Fractionation of seaweed biomass to

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10811-020-02044-0) contains supplementary material, which is available to authorized users.

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Published online: 17 February 2020

several useful and valuable components would maximize the biorefinery's overall economic, energetic, and environmental footprint performance of such supply chain (Golberg 2015; Laurens et al. 2017). The development of macroalgae-based biorefinery is still challenging because of multiple uncertainties in these new supply chains.

One source of this uncertainty is in the production process, namely which products can be produced and what product quantities are technologically and economically feasible? Another source is in the market value of the produced products and their production costs. To address the question of the production, several studies published in recent years showed that it is possible to fractionate seaweed biomass to protein, carbohydrates, lipids, pigments, salts and minerals, solvents, fertilizer, animal feed and fuel (van der Wal et al. 2013; Bikker et al. 2016; Magnusson et al. 2016; Ben Yahmed et al. 2016; Gajaria et al. 2017; Postma et al. 2018; Mhatre et al. 2018; Safavi et al. 2019). The question of the market value of seaweed-derived chemical is more complex, as current market exists mainly for raw seaweed biomass or unique chemicals such as carrageenan and alginate (Valderrama et al. 2013). The production of bulk commodity chemicals such as proteins, starch, and cellulose has not yet been demonstrated on a large scale to achieve commodity markets (Bleakley and Hayes 2017). Therefore, in this early stage of this technology, there is still a need for the development of new products and processes for seaweed-based biorefinery.

The goal of this study was to develop an integrated process for seaweed biomass fractionation and show a proof of concept for recovering six major components: starch, mineral salts, lipid, ulvan, protein, and cellulose from the green macroalga *Ulva ohnoi* (Chlorophyta). *Ulva* species are cosmopolitan (Bruhn et al. 2011), have high growth rates and thrive in diverse climate zones, and are suggested to be less sensitive to global warming (van den Burg et al. 2013; Gao et al. 2017). In addition, they have a wide tolerance to variable salinities (FAO 2003), low incident light intensities, and low temperatures (Kim and Chojnacka 2015). Given such ecological traits, green macroalgae have become one of the most promising alternative biorefinery feedstocks globally (Table 1).

In previous studies, we modeled and measured productivities and biomass yields in several *Ulva* species (Lehahn et al. 2016; Chemodanov et al. 2017; Zollmann et al. 2018; Habiby et al. 2018) and established protocols for "de-ashing," that is moving salts out of *Ulva* cells using pulsed electric fields (PEF) (Robin et al. 2018b), and single product extraction such as starch and protein (Robin et al. 2018a; Prabhu et al. 2019). We have also shown the economic advantage of *Ulva* over other typically Eastern Mediterranean seaweeds in, for example, the large number of monosaccharides that can be generated with acid hydrolysis (Robin et al. 2017; Qarri and Israel 2020). However, to further estimate the economics of *Ulva* biorefinery, new processes for the co-production of additional molecules are needed. One of such processes was proposed by Gajaria et al. (2017), who demonstrated a full cascading biorefinery process on *Ulva lactuca*, to extract five different chemical products: minerals, lipids, ulvan, protein, and cellulose. However, in their study, the extraction of lipids and cellulose required the use of environmentally hazardous organic

Table 1 *Ulva* (Chlorophyta) based macroalgae biorefinery (MAB) studies carried out for production of various products

Algae species	Biorefinery products	Technologies/methods	Reference
Ulva lactuca	Protein, carbohydrates	Osmotic shock, enzymatic hydrolysis, pulsed electric field, and high shear homogenization	(Postma et al. 2017)
Ulva lactuca	Animal feed, acetone, butanol, ethanol, and 1,2-propanediol	Thermal and enzymatic hydrolysis, fermentation	(Bikker et al. 2016b)
Chaetomorpha linum	Bioethanol and biogas	Thermochemical hydrolysis, enzymatic hydrolysis, fermentation	(Ben Yahmed et al. 2016)
Ulva fasciata	Mineral-rich liquid extract (MRLE), lipid, ulvan, and cellulose.	Mechanical grinding, thermal and chemical extraction, fermentation	(Trivedi et al. 2016)
Ulva ohnoi and *Ulva tepida*	Mainly salt (demonstrating the use of leftover biomass for protein, fertilizer, animal feed and fuel)	Aqueous washing and drying	(Magnusson et al. 2016)
Ulva lactuca	Mineral extract, lipid, ulvan, protein, cellulose	Mechanical pressing and crushing, heat treatment, organic solvent extraction, alkali extraction, chemical extraction	(Gajaria et al. 2017)
Ulva lactuca	ABE (acetone, butanol, ethanol)	Pre-treatment, enzymatic saccharification, fermentation	(van der Wal et al. 2013)
Ulva rigida	Liquid stream with carbohydrate and salt; a remaining stream with concentrated protein	Ionic liquid deconstruction	(Pezoa-Conte et al. 2015)
Ulva ohnoi	Salt, ulvan, pigment, and protein	Aqueous, thermal, and chemical extraction	(Glasson et al. 2017)
Ulva lactuca	Sap, ulvan, protein, methane	Aqueous, thermal, chemical extraction, and anaerobic fermentation	(Mhatre et al. 2018)

solvents (chloroform and methanol) and chemicals (sodium chlorite and hydrochloric acid). In addition, Gajaria et al. (2017) did not include *Ulva* starch in the cascade co-production. Starch is an essential commodity chemical produced today from the terrestrial plants that use large areas of land and vast volumes of fresh water (International Starch Institute 2006; Spiertz and Ewert 2009). In accordance, in this work, using sustainable green chemistry extraction methods (Chemat et al. 2012; Rombaut et al. 2014), we developed a protocol for co-production of six products: starch, mineral salts, lipid, ulvan, protein, and cellulose, from the same initial starting biomass. To the best of our knowledge, this is the first study to show the co-production of six products from *Ulva* sp. by using green extraction methods. In addition, based on the recently introduced economic model on feedstock farming and processing into multiple outputs (Palatnik et al. 2018), we performed a sensitivity analysis of total revenue for a seaweed biomass sequentially processed and biorefined into six co-products.

Material and methods

Materials

Materials such as chemicals and solvents used in this study were from Sigma-Aldrich and were of analytical grade. Nylon filter bags were from Sinun Tech. Ltd., Israel.

Cultivation and molecular characterization of *Ulva ohnoi*

A culture of *Ulva ohnoi* (see Krupnik et al. 2018; Prabhu et al. 2019) was taken from our outdoors seaweed cultivation system at Israel Oceanographic and Limnological Research, Ltd. (IOLR), Haifa, Israel. *Ulva ohnoi* culture was from several haphazard collections conducted along the costs of Israeli Mediterranean Sea. The cultivation system was made up of 1000 L, square, opaque fiberglass tanks containing 600 L working volume, equipped with aeration and running seawater pumped from a nearby shore site at a flow rate of 9 m^3 $tank^{-1}$ day^{-1}. Seawater was filtered through a 200-μm filter before it was added into cultivation tanks. The algae were fertilized once a week with a total concentration of 0.057 mM sodium *di*-hydrogen phosphate (NaH_2PO_4) and 0.59 mM ammonium chloride (NH_4Cl) (Chemodanov et al. 2017). For molecular identification, genomic DNA was isolated from the above *Ulva* culture using cetyl trimethylammonium bromide (CTAB) extraction method (Prabhu et al. 2019). BLAST (Basic Local alignment search tool) search was carried out and the gene sequence from the *Ulva* cultivated at IOLR, matched 98% with *Ulva ohnoi* (Hiraoka et al. 2004).

Harvesting and measurement of dry mass and ash of *Ulva ohnoi* biomass

Following 4 weeks of cultivation, *U. ohnoi* biomass was harvested. Remains of water on the thalli were removed using a portable laundry spin dryer and the biomass regarded as fresh mass (FM). Sub-samples were taken to determine dry mass (DM) and ash content. DM was calculated using a moisture analyzer (BM-50-5, Biobase Biodustry (Shandong) Co. Ltd., China) after drying the FM at 105 °C until the constant weight was obtained. Ash content was analyzed by burning the dry sample to constant weight at 550 °C (ISO-5984 2009).

Biorefinery design for co-production of starch, salt, lipids, ulvan, protein, and cellulose

Different treatment strategies were applied to fresh *U. ohnoi* biomass for the extraction of different products in a marine biorefinery as below.

Step 1: Starch and mineral salt extraction. Starch was extracted as previously reported (Prabhu et al. 2019). In brief, 200 g FM *U. ohnoi* was mixed with approximately 2800 mL distilled water and homogenized to obtain slurry with the help of a homogenizer (HG-300, Hasigtai Machinery Industry Co., Ltd., Taiwan). The slurry was sequentially filtered through nylon filters having pore size of 200, 50, and 10 μm, to obtain the filtrate. The filtrate was centrifuged at 5000 rpm for 10 min. The supernatant was dried at 105 °C to recover the salt fraction. The lipids and pigments in the starch pellet were removed by washing three times with excess absolute ethanol (total 600 mL). The off-white pellet left behind was dried at 40 °C until the constant mass was recorded.

Step 2: Lipid extraction. Lipid extraction was carried using the absolute ethanol method (Glasson et al. 2017). Solid *U. ohnoi* biomass after starch extraction (residue left in the 200-, 50-, and 10-μm nylon filter) in step 1 was suspended in absolute ethanol (500 mL) at room temperature and mixed using magnetic stirrer. After 3 h of mixing, the mixture was filtered through 50-μm pore size nylon filters using a glass vacuum filtration unit. The above extraction procedure using ethanol was repeated thrice. The ethanol fraction was combined, concentrated, and dried using a rotary evaporator. The ethanol fraction recovered in starch purification step was added to this fraction before it was concentrated. The fraction was finally dried at 40 ° until constant mass was reached and the final mass was recorded.

Step 3: Ulvan extraction. Ulvan was extracted using the oxalate salt method as described by Robic et al. (2009). Solid biomass left after filtration in step 2 was dried at 23 °C for 5 h and then suspended in 1 L $(NH_4)_2C_2O_4$ (0.05 M). The mixture was incubated at 80 °C for 2 h with gentle mixing every 15 min. After the incubation the mixture, while it was still warm (~45 °), was subjected to centrifugation for

Fig. 1 Image of the end products (dry) extracted in an integrated biorefinery. F1-Salt fraction, F2-starch fraction, F3-lipid fraction, F4-ulvan fraction, F5-protein fraction, and F6-cellulose fraction

10 min, at 1811×*g*. The supernatant was concentrated to about 300 mL and then dialyzed using 8-kDa MWCO dialysis membrane for 24 h, with 3 changes of distilled water. The dialysate was finally freeze-dried and weighed to estimate the mass of the ulvan fraction.

Step 4: Protein extraction. Solid biomass residue remaining after centrifugation in step 3 was used for protein extraction using alkaline treatment method with 1 L of a 0.25 M NaOH solution at 80 ° for 2 h (Mhatre et al. 2018). Then the mixture was cooled at room temperature and then centrifuged for 10 min at 1811×*g*. The supernatant was collected and neutralized using 6 N HCl. The neutralized liquid was concentrated to 300 mL, dialyzed, lyophilized, and weight was measured to record the mass of protein fraction.

Step 5: Cellulose extraction. The residue left at the end of protein extraction (step 4) was washed with excess deionized water to attain the neutral pH, followed by filtration through a nylon filter of 200-µm pore size. The solid residue was dried at 40 °C and the mass of the cellulose fraction was recorded.

Moisture content in all six fractions was determined at 105 °C using a moisture analyzer as mentioned above. The percentage of each fraction extracted was calculated with respect to the initial dry biomass of *U. ohnoi* used. The total recovered biomass in the form of all the products was calculated using Eq. **1.**

$$\text{Total recovered biomass} = \frac{(\mathrm{DW}_{\mathrm{f}_1} + \mathrm{DW}_{\mathrm{f}_2} + \ldots \mathrm{DW}_{\mathrm{f}_6})}{\mathrm{DW}} \quad (1)$$

where DW_{f_1} to DW_{f_6} are the dry weights of the six different products and DW is the dry weight of the initial *U. ohnoi* used for extraction.

The total salt content in the salt fraction was analyzed by burning the fraction at 550 °C in a muffle furnace, whereas purity of starch fraction was analyzed using total starch assay (Prabhu et al. 2019). Lipid content in the fraction was measured by following the protocol of Bligh and Dyer (1959). Total protein content in the fraction was determined by Lowry method (Lowry et al. 1951; Kazir et al. 2019). Cellulose content was measured by enzymatic method following the protocol of Matsura (2017).

Analysis of macroalgae biorefinery products market value

The profitability of marine biorefinery is subject to various sources of uncertainty such as of feedstock supply, processing technology, investment, contracting, and demand (Palatnik and Zilberman 2017). We denote the market price for the specific product I as p_i (US\$ kg^{-1}). Then the total revenue products (TR, US\$ kg^{-1}) for products co-produced from 1 kg of the biomass would be

$$\mathrm{TR} = \sum_{i=1}^{N} q_i \mathrm{p}_i \quad (2)$$

where q_i is the quantity (kg) of the extracted product *i*. All the potential products were considered when applying the value range.

Statistical analysis

A data analysis package in Excel program (ver. 13, Microsoft, USA) was used for data analysis and for calculating the mean and standard deviation. All the experiments were prepared and measured at least in triplicates and the standard deviation was calculated.

Results

Dry mass and ash content in *Ulva ohnoi*

Dewatered, fresh *U. ohnoi* biomass was characterized by dry mass and ash content. The dry matter content at 105 °C was 23.80 ± 0.31% of the FM and ash content was 37.39 ± 0.82% of the DM.

Biorefinery products and their market value

Six different products were sequentially extracted from *U. ohnoi* biomass (Fig. 1). In this study, an average of 45.42 ± 1.91% of DM salt–rich fraction, 3.67 ± 1.38% of DM

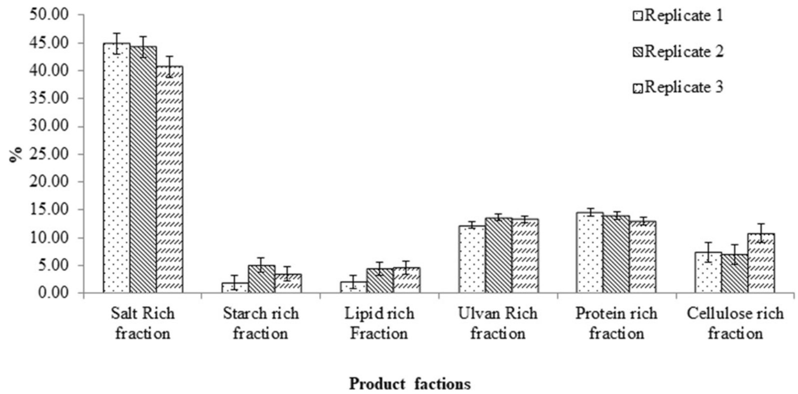

Fig. 2 Extractable yield (in %) of six different marine macroalgae biorefinery products extracted from *U. ohnoi* biomass

starch–rich fraction, 3.81 ± 1.26% of DM lipid–rich fraction, 13.88 ± 0.40% of DM ulvan–rich fraction, 14.83 ± 1.06% of DM protein–rich fraction, and 8.70 ± 1.87% of DM cellulose–rich fraction were obtained. The total recovery of all the products was 90.31 ± 1.94% of initial DM used, reducing significantly the wastes of the process. The percentage of extractable yield of each product is given in Fig. 2. The variations in each product quantity were due to the losses occurred during the extraction process.

Various technologies can convert algal biomass into starch, salts, lipids, sulfated polymers, proteins, and cellulose that can yield food, chemicals and biofuels. Total salt in the salt fraction was 81.25%, total starch content in starch fraction was 78.31%, total lipid content in lipid fraction was 75.14%, and total cellulose content in cellulose fraction was 80.19%. These results when compared with the data mentioned in the literature (products extracted using similar the extraction protocol followed) showed that the various product qualities were at least 75% pure. From our previously reported study, it is seen that the protein fraction is rich in essential (as well as non-essential) amino acids such as histidine, isoleucine, leucine, lysine, phenylalanine, threonine, valine and many other (Kazir et al. 2019). This certainly indicates that the protein fraction from *Ulva* is an important fraction in biorefiney.

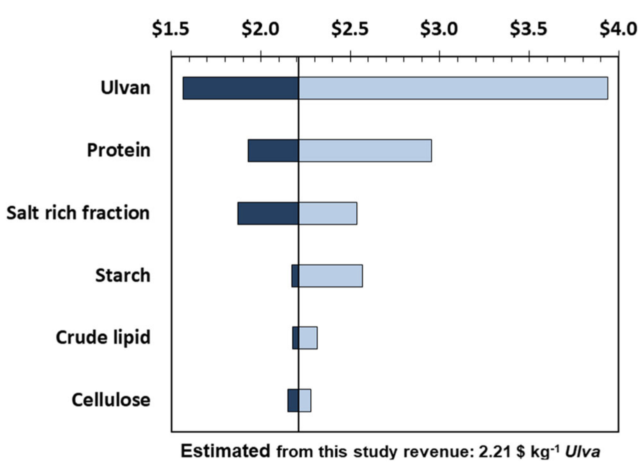

Fig. 3 One-way sensitivity analysis of total revenue from *Ulva* biomass when sold as fractionated ingredients

The entrepreneur, at each stage of the extraction process, should decide between different options, which ultimately affect the irreversible (sunk) and variable production costs such as productivity, and the output, which ultimately have an effect on the profitability. Yet, both the composition of the biomass the biorefinery yields and extracted ingredients are highly uncertain (Lehahn et al. 2016), signaling the immaturity of the technology (Fig. 3 and Table S1). The difference can be up to ten times between the upper value and the lower one. For example, our recent work on starch production in *Ulva* sp. showed variation in the starch content from 1.59 to 21.44%, depending on the cultivation conditions (Prabhu et al. 2019). Such variability significantly affects the potential profitability of the biorefinery (Fig.3). Because of the variations in the products yields and market prices (Table S1), the total revenue (TR) for the green macroalgae biorefinery process developed in this work could vary between US\$1.56 and US\$3.93 kg^{-1} *Ulva* DW when sold fractionated to ingredients (Table S2, Fig.3). It is important to emphasize that the exact prices to *Ulva*-derived products sold in the large scale are not yet available, as these products are not in the market yet and their unique properties are still not known. The current work showed the TR is most sensitive to ulvan content and market prices, followed by proteins, salt, starch, crude lipids and cellulose.

Discussion

In order to understand the economic potential of the biorefinery under investigation, the commercial applications of the co-outputs extracted in this work is reviewed below.

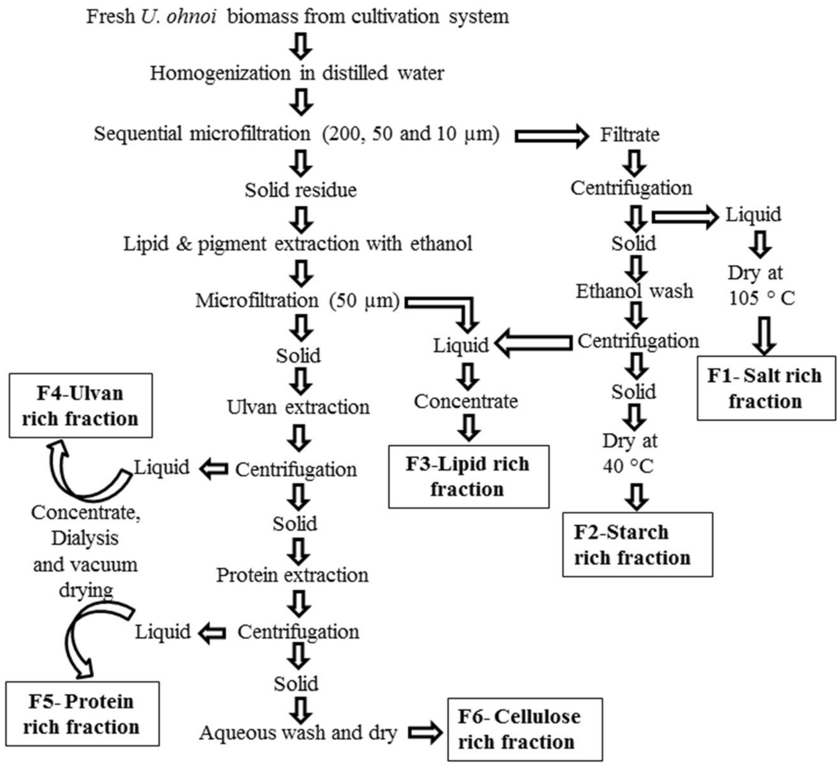

Fig. 4 The scheme of *U. ohnoi* biorefinery process. The integrated production of a wide range of valuable products, fractions F1–F6

The marine biorefinery design and approach

A sustainable biorefinery design should enable industrial production with minimum environmental impacts. Therefore, extraction procedures should be chosen and integrated wisely. The important factor in the biorefineries is the use of versatile, robust technologies for processing the biomass. The process design itself is important in solving some of these challenges, particularly the selection of processing methods so that the structural and functional properties of different products are maintained. Thus, specific and non-destructive processes must be applied first for the extraction of highly sensitive products (Balina et al. 2017). Once the sensitive molecules are recovered, more severe and destructive methods can be used on the leftover biomass for the recovery of other products such as cellulose. The sequential reduction of the residual biomass after each step reduces reagent demand and improves the yield of downstream extraction, thus increasing process sustainability and efficiency.

The improvement of modern biorefineries depends on the synergetic combination of process technologies, converting non-food biomass into a range of bio-based chemicals, e.g. materials, chemicals, and energy by a combination of, chemical, thermochemical, mechanical and biochemical processes. Thus, the maximum value from each feedstock is achieved (De Jong and Jungmeier 2015). By considering the above-mentioned factors, we carefully designed an integrated process to successfully extract the maximum yield of different valuable macromolecules (see Fig. 4).

The salt fraction containing inorganic salts and cations are accumulated from the local seawater environment and partly held in the intracellular fluid inside the vacuoles of the *Ulva* cells. The seaweed salt is of particular interest as it contains important minerals such as iodine along with dietary Na, K, Mg and Ca (Magnusson et al. 2016). Seaweed salt is an existing product. Seaweed-infused sea salts such as Moshio are known for unique color and delicious flavor. They have been produced since 2500 years ago in Japan, China, and Korea and have been used as a substitute for regular table salt (Moshio). This fraction also constitutes several types of bioactive including soluble sulfated fibers, macro- and micro-elements, and phytohormones that may be important for human and plant growth (Magnusson et al. 2016; Trivedi et al. 2016; Robin et al. 2018b). Previous studies have proposed the use of green seaweed extract that is rich in salt as biostimulant and fertilizer for various agronomical important crops (Mohanty et al. 2013; Magnusson et al. 2016). Ganapathy Selvam and Sivakumar 2013) reported that the use of at 2% salt-rich seaweed extract (containing 3.22 g L^{-1} of salts) on *Vigna mungo* results in improved yield and nutritional quality, leading to reduced use of recommended dose of chemical fertilizers. Similar extracts from the brown seaweed *Kappaphycus* and

Table 2 The range of biorefinery products produced from *Ulva ohnoi* biomass and their potential applications

Products	Applications	Reference
Raw biomass	Bioenergy (methane, etc.), food and animal feed, metal chelating agent, biomaterials	(Smitha et al. 2010; Garcia-Vaquero and Hayes 2016; 2018)
Salt	Superior table salt, biofertilizer	(Selvam and Sivakumar 2013; Magnusson et al. 2016)
Starch	Food and animal feed, biofuel and fermentation industry, biochemical industry	(Holdt and Kraan 2011; Korzen et al. 2016)
Pigments	Nutraceutical, food additives (coloring agent)	(Mohanty et al. 2013)
Lipids	Food supplement, animal feed, biofuel	(Gajaria et al. 2017; Balina et al. 2017)
Ulvan	Biofertilizer, biomedical and pharmaceutical application, nutraceutical, food additives	(Lahaye and Robic 2007; Cardoso et al. 2014; Wang et al. 2014; Manivasagan and Oh 2016)
Protein	Food & animal feed, food additives	(Bikker et al. 2016; Gajaria et al. 2017)
Cellulose	Biofuel and fermentation industry, biochemical industry, biomaterials, paper industry	(Mihranyan et al. 2004; Castelló et al. 2016)

the red seaweed *Gracilaria* containing ions such as Na^+, K^+, Ca_2^+, Mg_2^+, Zn_2^+, Mn_2^+, Fe_2^+, Cr_3^+, Cu_2^+, Ni_3^+ and P_3^+ were also shown to increase rice yield and maize yield at the field scale when applied at 15% concentration (Singh et al. 2016; Sharma et al. 2017).

Starch in *U. ohnoi* is stored intracellularly in the form of 5—7-μm size granules of various shapes from round, oval, spherical, to irregular shapes. This starch, like other starches, can be used in food, fermentation, textile, cosmetics, pharmaceutical, packaging, synthetic polymer industries and in biotechnological applications in various industries (Santana et al. 2014). It can further be used for the generation of energy by converting it to biofuel using the fermentation process.

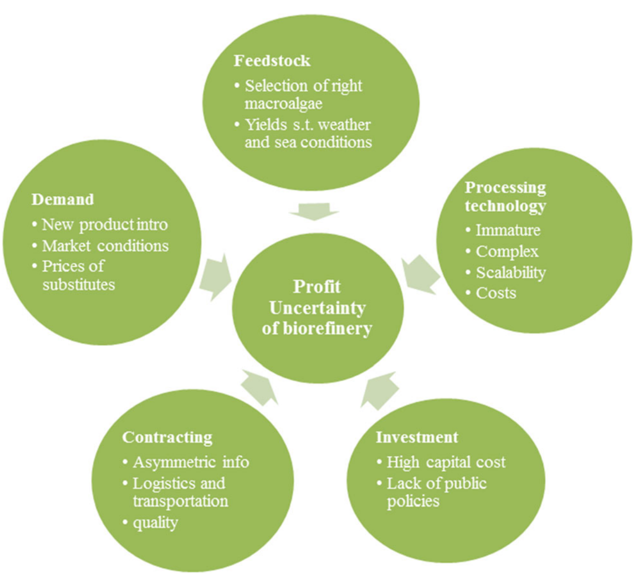

Fig. 5 Major sources of uncertainty for marine offshore marine biorefinery. Feedstock, processing technology, investment, contracting, and demand are the challenges associated with the successful deployment of marine biorefinery operations

Lipids, including their fatty acids, are vital and fundamental molecules for human nutrition. These include saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Seaweeds are comprised of relatively low lipid contents than microalgae but have higher proportions of C18 (linoleic and alpha-linolenic) fatty acids and low proportions of C20 PUFAs. Such combination could contribute to the prevention of cardiac diseases, osteoarthritis, diabetes, and have anti-inflammatory activities (Rodrigues et al. 2015; Gajaria et al. 2017). Thus, seaweed lipids can be of significant importance over microalgae. Alternatively, these lipids can be converted to biodiesel (Balina et al. 2017). This fraction along with lipids usually contains pigments. The pigments in *U. ohnoi* include chlorophyll *a* and *b*, and carotenoids and can vary in concentration based on the water quality and the quantity and quality of light where the organism is grown. In an earlier study, the liquid extract of *U. fasciata* containing pigment (along with other macromolecules), when applied to the soil, showed an increase in photosynthetic pigment composition, soluble protein, and starch, amino acid content in various crops (Mohanty et al. 2013).

Ulvan is the sulfated polysaccharide of the genus *Ulva* (Lahaye and Robic 2007). It is the main water-soluble, sulfur-containing polysaccharides (SPs) present in *Ulva* spp. Several potential applications have been investigated for such SPs: animal feed, antioxidant, antitumor, anticoagulant, immune modulator and biomedical applications such as drug delivery and tissue engineering (Lahaye and Robic 2007; Cardoso et al. 2014; Wang et al. 2014; Manivasagan and Oh 2016).

Protein concentration is found different in different seaweed species and in general green seaweeds have intermediate (18.8 g $(100\ g)^{-1}$ dry seaweed) amount of protein concentration when compared with red (20.2 to 23.8 g $(100\ g)^{-1}$ dry seaweed) and brown (14.4 to 16.9 g $(100\ g)^{-1}$ dry seaweed) seaweeds (Rodrigues et al. 2015). Proteins are highly significant in terms of both industrially valued product and as a dietary add-on in demand (Parthiban et al. 2013). The protein content of macroalgae contains all essential amino acids (EAA) with seasonal disparities in their concentrations (Bikker et al. 2016; Gajaria et al. 2017). Hence, macroalgae proteins are considered as an alternative protein source in human nutrition. However, a recent study on *Ulva* proteins (Polikovsky et al. 2019) extracted using PEF, osmotic shock, and thermochemical method contained food allergens such as superoxide dismutase (SOD), thioredoxin-h, aldolase A and troponin C. Alternatively, *Ulva* proteins can be used as an attractive protein source in animal and fish feed supplement after careful consideration Bikker et al. 2016; Seghetta et al. 2017).

As the cellulose is a strong polymer in macroalgae (Abdul Khalil et al. 2017) and is least affected by the upstream extraction treatments in comparison with any other components in *U. ohnoi*, it can be extracted as the final product in an integrated marine MAB approach. Cellulose has potential applications as a feedstock for the rapidly emerging era of biofuels and in paper industries. In addition, cellulose has been a preferred polymer as the basic raw material for various other industrial applications including the synthesis of nanocomposites, microcrystals and nanocrystals reinforcing material, bioplastic and more (Klemm et al. 2005; Ng et al. 2015). Researchers have extracted cellulose from *Ulva* (Trivedi et al. 2016) and *Cladophora* (Mihranyan et al. 2004). Mihranyan et al. (2004) also reported that the cellulose from *Cladophora* sp. harvested from the Baltic Sea has a crystallinity of 95.2%, thus absorbing much less moisture and making it a very attractive agent as a tableting excipient. Applications of various different materials for *U. ohnoi* are listed in Table 2.

Economic uncertainty and challenges of offshore marine macroalgae biorefineries

Macroalgae biomass can be a part of a human diet, or processed into pure ingredients, as shown in this study and in Table 1. There are numerous challenges associated with the successful deployment of marine biorefinery operations as summarized in Fig. 5. The profitability of marine MAB is subject to various sources of uncertainty such as that of feedstock supply, processing technology, investment, contracting, and demand (Palatnik and Zilberman 2017).

The entrepreneur should determine which algae-based activities are profitable under multi-dimensional uncertainty outlined below. The rate of feedstock growth shows a varied range of values. In the previous study for example, with offshore cultivation of *Ulva* growth rate, fluctuations from − 14 to +26% daily growth rate were shown (Chemodanov et al. 2017). This uncertainty in feedstock yield has a major impact on the economics of technology. Feedstock development relies on saturation kinetics by ambient dissolved inorganic nutrient concentrations, light intensity and optimum temperature (Lehahn et al. 2016). Environmental conditions such as stochastic weather, seasonal variability between regions and within and between years aggravate cultivation. Biomass fractionation to produce important chemicals in seaweed biorefinery requires quality raw material to maximize benefits. Sustainable fresh biomass of *Ulva* with similar chemical constituents will be needed for such demands. Nevertheless, seasonal effects and growth conditions such as light and nutrient availability, reproductive stages (e.g., gametophytes vs. sporophytes), and thallus age are well known to affect the chemical constituents in seaweeds (Ashkenazi et al. 2019). Further, biochemical profile in *Ulva* and other seaweeds, especially the carbon content of the biomass, namely carbohydrates, protein, and lipids, can be enhanced as these are dependent on environmental growth conditions (Lawton et al. 2013; Malta and De Nys 2016). This can be explained by the fact that changes in environmental conditions (including nutrients and light) inflame a condition of disturbed growth in algae (Israel et al. 1995), which results in photo-acclimation processes together with the reorganization of carbon resources. The content of carbon in algae is primarily determined by photosynthesis, which, in turn, is mediated by the availability of nutrients and light (Ashkenazi et al. 2019 and references therein). In practice and for biorefinery purposes, the need for large biomass yields in a sustainable manner will eventually prevail over traits of the chemical composition in the algal tissues. The above arguments can be supported by the work of Friedlander (2008) on *Ulva* biomass produced in short time periods at the local conditions. Studies point at biomass productivity and its fluctuation as the key limitation against being competitive with other potential protein and energy-producing technologies (Seghetta et al. 2016).

The design and construction of a new biorefinery require significant funds (Stichnothe et al. 2016). The strategy about the capacity of the biorefinery may change over time. It depends on prediction of market conditions, technology development, risks, and credit availability, as well organizational design (partnerships with potential users or sources of inputs, etc.) (Du et al. 2016). The entrepreneur may experiment by beginning at a small scale and continuously adapt as new information is revealed.

The beginning of new innovation and taking it to perfection depends on long and complex processes in which economics along with technology changes with time. Learning comes slowly as time progresses. The timing of introduction of innovations, their refinement, and their commercialization affect the dynamics of knowledge accumulation. For example, once a production system is established, the innovator may have to decide about structure of the organizations that implement the commercialization strategy (Zilberman et al. 2017). The decision concerning both the ability of innovation and the extent of dependence on exterior sources is also affected by the timing. Lack of public policies supporting biorefinery sector limits the long-term investment decision required. Although there are different strategies, there are no separate policy drivers for the exploitation of bio-based chemicals, in direct contrast to the biofuels industry where various national regulations are accelerating the rapid growth.

The impact of price variation should be examined in several aspects that include price uncertainties of feedstock for the biorefinery, competitive outputs (backstop technology), and the economic constraints faced by the aquafarmer. A seaweed industry containing many small-scale price-takers is particularly prone to boom-bust cycles. For example, in the Philippines, the price of dry cottonni went up from US\$ 900 t^{-1} in 2007 to almost US\$ 3000 t^{-1} in 2008 due to the high demand for cottonii from China. This caused the Philippines production to double from 1.5 million t (wet weight) in 2007 to 3.3 million t in 2008. However, the "seaweed rush" lasted only 1 year and the price dropped to US\$ 1300 t^{-1} in 2009 (Ricardo et al. 2015).

The economics of biorefinery-based products depends heavily on on-drop-in versus non-drop-in that is standing demand and infrastructure, which are very uncertain. The economics of bio-based supply chain and its design are very challenging, with high degree of uncertainty about the pricing and premia for bio-based, and their profitability implications (IEA Bioenergy 2017).

However, the major uncertainties are usually high and rely on the biomass market policy measures and on long-term policies regarding the biomass (Zhou et al. 2010; Kim et al. 2011; Yaich et al. 2011; Choi et al. 2013; Neveux et al. 2014; Rodrigues et al. 2015).

In addition, the expenses on research and development (R&D), formulation, marketing, etc. are also affected by the production cost of the biorefinery products, and their value, reaching end-users (Ricardo et al. 2015). In general, such exact information on these aspects is lacking. The blue bioeconomy is in its infancy. As Zilberman et al. (2013) suggest, establishing new bioeconomy ventures requires identifying feedstocks products derived from these feedstocks and then assess the economics of building and creating biorefineries and the exact product mix appropriate to various conditions. This work has quantified a combination of derivative products from the green macroalga *Ulva*, and established that it might lead to a substantial level of revenue per kg of dry seaweeds. The annual average daily growth rate for *Ulva* sp. is 6.58 g DM day^{-1} m^{-2} (Prabhu et al. 2019). With this growth rate, the total biomass produced will be 5.76 t ha^{-1} year^{-1}. With our calculated TR of US\$1.56 to US\$3.93 kg^{-1} (Table S2), the total revenue generated will be US\$ 8917.03 to US\$ 22,464.06 ha^{-1} year^{-1}. The major demand driving factors that are expected to boost the market include the availability of raw materials at a reduced cost, increasing consumer awareness towards and subsequent demand for bio-based products and government initiatives to promote green products among others. However, much more research is required to assess the commercial viability of the technology. One needs to improve the assessment of revenues and understand how it depends on location, scale and government policies. For example, if carbon pricing is introduced, the by-products engaged in applications that reduce greenhouse gases (biofuel) are becoming much more valuable. Commercial viability depends on the cost of harvesting and processing the feedstock, and it may change over time as a result of learning.

Conclusion

In this work, the lab-scale extraction of six different products (salt, starch, lipid, ulvan, protein, and cellulose) from the same initial *U. ohnoi* biomass in an integrated manner was demonstrated. Extractable yields obtained for all the products in this biorefinery approach amount 90.31% of the DM used. The estimated revenue is US\$1.56 to US\$3.93 kg^{-1} DW *Ulva* when sold fractionated to ingredients. In a sustainable biorefinery, maximum biomass conversion and "zero residual waste" is important for sustainable economic development and market growth. Green seaweeds, although its properties are unpredictable but can be defeated, have properties to contribute to sustainable bioeconomy. This work suggests a new marine biorefinery design for the extraction of value-added products from green seaweeds. Such efficient and sustainable use of biomass resources will form the foundation of a future bio-based blue economy in coastal communities and helping them to become more sustainable.

Funding All authors received financial support from the US-Israel Binational Agricultural Research and Development Fund, Israel Ministry of Energy, and Israel Ministry of Science and Technology (grant number 57514).

Compliance with ethical standards

Competing interest The authors declare that they have no competing interests.

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