In the Field

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Feasibility study of *Ulva* sp. (Chlorophyta) intensive cultivation in a coastal area of the Eastern Mediterranean Sea

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Abstract: Increasing biomass production yields is a critical challenge for macroalgae biorefineries. The continuous tumbling and mixing of free-floating algae through water or airflow has been shown to increase the productivity of algae in land-based cultivation systems. This approach has not been tested thoroughly in offshore cultivation. We report, here, a field feasibility study on the increase in green macroalga *Ulva* sp. growth rates in offshore cages, achieved by the combined effect of tumbling and mixing of the algae using influxes of water and air. The experimental system was tested in a shallow coastal area in central Israel, in the eastern Mediterranean Sea. A maximum daily growth rate of 19.2%, areal productivity of 33.72 g dry weight (DW) day^{-1} m^{-2}, and volumetric yields of 37.78 g DW day^{-1} m^{-3}, together with 38.47 ± 0.01% ash and 5.28% protein content on a dry matter basis were achieved in the cages with intensified cultivation in the first week of May 2017. Our study shows that cultivation with tumbling and mixing of biomass with air, and water exchange with the environment is a feasible method to increase *Ulva* sp. biomass productivity offshore. © 2019 Society of Chemical Industry and John Wiley & Sons, Ltd

Supporting information may be found in the online version of this article.

Keywords: macroalgae; seaweed; intensive offshore production; offshore aeration; offshore mixing

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†AC designed the offshore reactor. AC and AG performed the offshore growth-rate measurements. AI grew the initial biomass inoculum for all experiments. GJ and DI performed the combustion experiments. AR performed biochemical analysis of biomass composition. AG conceived the project. AC, AL, and AG designed the study. All of the authors contributed to the design of the research, the discussion of the data, and the writing of the paper.

Introduction

Agriculture is the primary method to produce biomass for food, biochemicals, and biofuels, but the European Biorefinery Joint Strategic Research Roadmap for 2020 indicates that 'a key issue for biomass production in Europe is land availability.'[1] Countries that have the problem of limited arable land for energy crop cultivation can find marine macroalgae farming a useful alternative that can provide a sustainable feedstock biomass for downstream processing in biorefineries.[2–5] For instance, a methodology for macroalgae biorefinery design for rural areas in developing countries has been developed.[6,7] A key challenge in the field of macroalgae biorefinery remains the sustainable production of the biomass.[8–10]

Offshore cultivation of macroalgae is one sustainable strategy to produce bioenergy and bioproducts without using arable land and scarce freshwater resources.[11,12] On a global scale, most offshore seaweed biomass is produced in Indonesia, the Philippines, China, India, and Tanzania, and is currently under investigation in US and EU biomass programs.[13,14] The concepts of offshore marine biomass cultivation include farms for kelp growth,[15] tidal flat farms, floating seaweed cultivation settings,[15,16] ring cultivation systems,[17] and, most recently, wind-farm integrated systems[18] and underwater ropes.[19]

Following the success of on-land photobioreactors in providing high biomass yields when the major cultivation parameters of temperature, light, mixing, and nutrients were controlled,[20,21] theoretically possible intensified offshore cultivation methods were proposed.[22,23] However, to the best of our knowledge, the intensification methods that control key parameters offshore, have not been demonstrated in the field.

The goal of this work was to perform a feasibility study of intensified macroalgae cultivation offshore. In this study, an intensification method was applied to a ~2 m^3 cage, deployed in a shallow area in Tel Aviv, Israel, close to a power plant, with tumbling and mixing of biomass with air and water supplied by an airlift pump from a deeper layer. The green seaweed *Ulva* sp. was chosen as the model species as it is very common on the shores of Israel and displayed high biomass productivity in extensive cultivation offshore in Israeli waters.[24,25] Furthermore, the production of proteins and starch,[26–28] and biomass fermentation to acetone, ethanol, butanol, and polyhydroxyalkanoates from several *Ulva* species has already been demonstrated.[29–35]

Tumbling with air previously intensified *Ulva* sp. growth at low nutrient levels in on-land reactors.[36] It causes movement of the algae in the reactor, reducing shading limitations, and increasing exposure to light and available dissolved nutrients, thus enhancing photosynthesis and productivity.[37] Tumbling with air may also prevent the development of competitive macroalgal grazers and epiphytes, such as diatoms.[38] Our results show that growth rates of *Ulva* sp. can be intensified by a combination of tumbling, air mixing, and external water supply.

Materials and methods

Cultivation site

The *Ulva* sp. cultivation site was located in a shallow coastal area in the proximity of an electric power plant in Tel Aviv (32° 07′ 00″ N 34° 49′ 00″ E), Israel (Fig. 1(a)). The reasons for choosing this particular experimental site included easy access from the shore and a breakwater for additional protection, power supply availability, restricted access for the general public and water sports activities, and no effects of warm water outflux from the power plant. Altogether, the location allowed for continuous monitoring of the biomass cultivation site conditions.

Macroalgae biomass inoculum

The model seaweed used in this study belongs to the genus *Ulva* sp., a green marine macroalga distributed worldwide and found in the intertidal and shallow waters within the Israeli Mediterranean Sea shores. The exact taxonomic status of the *Ulva* sp. used in this study suggests a mix of two morphological and genetically similar types, *Ulva rigida* and *Ulva fasciata*.[39] Specimens were taken from stocks cultivated in an outdoor seaweed collection at Israel Oceanographic and Limnological Research, Haifa, Israel (IOLR), in 40 L fiberglass tanks supplied with running seawater, tumbling with air, and weekly additions of 1 mmol L^{-1} NH_4Cl and 0.1 mmol L^{-1} NaH_2PO_4. With each nutrient application, the water exchange was stopped for 24 h to allow for nutrient uptake.

Offshore cultivation in cages with tumbling, air mixing and water exchange

Although, in nature, *Ulva* grows primarily attached to hard substrates, it is frequently found growing in a floating stage within the water column. Cultivation of free-floating algal biomass provides an opportunity to use water volumes for cultivation instead of large areas used for attached biomass cultivation,[22] thus reducing the area used for cultivation.[24]

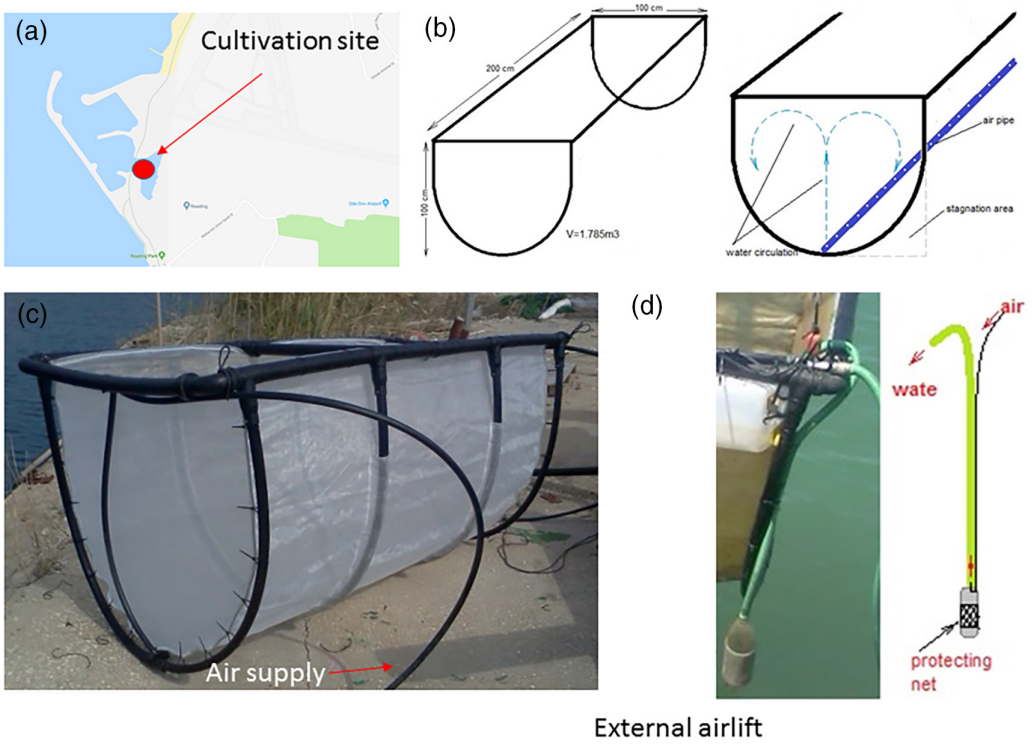

Figure 1. (a) Cultivation site. (b) Schematic design of the reactor with intensification with tumbling, mixing and water exchange. (c) Digital image of the reactor for intensified cultivation. (d) External airlifts for water exchange enhancement.

To test the potential of tumbling, air mixing, and external water exchange on the intensification of the *Ulva* sp. biomass growth, we designed a floating cage equipped with air-flow outlets at the bottom for constant aeration (Fig. 1(b)). The U-shape carcass (working volume 1.785 m^3, total illuminated area 2 m^2, Fig. 1(c)) was built from high-density polyethylene pipes (Ø = 50 and 35 mm) and a Ginigar anti-insect net (25 mesh, Fig. 1(c)), which effectively prevented fish grazing. Air was supplied to the bottom of the cage through a polyethylene pipe (Ø = 20 mm) at 40–45 L min^{-1} LPM / reactor or 20–22.5 LPM m^{-3} of water, depending on the load density of the biomass (ranging from 1 kg m^{-3} at the beginning of the cultivation to 4.5 kg m^{-3} at harvesting). Additional water was pumped into the cage from 1 m depth using four airlifts made from an HDPE single wall corrugated pipe (Ø = 20 mm) and 7/4 PVC pipes (Fig. 1(d)). The airlift pumped 11.03 m^3 of water per day, which equals 618.2% day^{-1} water exchange in the cage.

The system was installed ~30 m from the shore (Fig. 3(a,b)). The average streamflow at that point was measured and found to be in the range of 6–8 cm s^{-1}.[24] Air was supplied from 6 a.m. to 6 p.m. through a central bottom pipe through 2 mm holes (Fig. 3(c)). For harvesting, the reactor was removed from the water using a series of pulleys, hung up to remove excess water, and drained by gravitation (Fig. 3(d)).

Two experiments were performed. The first experiment started on April 20, 2017 and ended on May 29, 2017. The second experiment started on June 15, 2017, and ended on July 12, 2017. Sampling was also done every 2 weeks, so that the yield was harvested, and 2 kg of algae were loaded to the reactor every 2 weeks. Daily growth rate (DGR%) was calculated as in Eqn (1):[40,41]

$$DGR = \frac{1}{N} \cdot \frac{m_{out} - m_{in}}{m_{in}} \cdot 100\% \quad (1)$$

where N (d) is the number of days between measurements, m_{out} is the dry weight (DW) measured in g at the end of each growth period, and m_{in} is the DW (g) of the inoculum. We used a standard protocol for surface water removal by centrifuging the algal biomass in an electric centrifuge (portable washer spin dryer CE-88 (6.0 kg) 2800 rpm, stainless steel housing, Ningbo Beswin Electric Co., Ltd., Ningbo, China) until all surface water was removed (< 1 mL separated). Drying was done at 40 °C until constant weight (< 5% change in consequent measurements). Dry matter was determined by drying in 105 °C for 3 h.

Extensive cultivation

For extensive cultivation, a 2 cm layer of thalli was placed between two layers of nets (TENAX Tubular nets for Mussel Breeding and Packaging; Shellfish Polypropylene, mesh configuration – rhomboidal, 32 G, 223 neutral., 74 N 140 green, Gallo Plastik, Italy) in the cage that was neither tumbled nor mixed, which had free water exchange with the surrounding sea. The cage (0.15 m × 0.3 m, total illuminated area 0.045 m^2) was built from polyethylene (D = 32 mm), high-density polyethylene (HDPE) (D = 16 mm) pipes, and a TENAX (Gallo Plastik, Italy) net (Fig. 2(a)) to allow for full illumination and to prevent grazing of the algae by fish. The cages were connected to the rope and located ~30 m from the shore, at a distance of ~10 m from the aerated cage (Fig. 3(b)). Unlike the aerated cage, the biomass was held at a depth of ~ 10 cm in a single layer with no aeration supplied. Fresh weight (FW) of 20 g of *Ulva* was loaded to each cage every 2 weeks.

Determination of the effect of tumbling with air on *Ulva* sp. growth rate in the controlled on-land cultivation system

To understand better the effect of tumbling with air on the *Ulva* sp. biomass growth rate in a controlled environment, the biomass was cultivated in polyethylene terephthalate (PET) plastic bottles (1.5 L) with modified caps, to allow for water exchange and air supply (Fig. 2(c)). Five grams FW of *Ulva* were loaded per bottle. Artificial seawater (salinity 3.5%, pH 8.2) was supplied with 21.4 mg L^{-1} of NH_4NO_3 and 4 mg L^{-1} of H_3PO_4 (Haifa Chemicals Ltd, Haifa, Israel). Air was supplied at 0.36 LPM per bottle from 6 a.m. to 6 p.m. The water with nutrients was changed daily. The total cultivation time was 7 days per experiment. Two separate experiments were conducted. The first experiment was conducted from June 12, 2018 to June 19, 2018 (three replicates for tumbled with air and mixing and three replicates for non-tumbled and not-mixed bottles). The second experiment was conducted from June 19, 2018 to June 26, 2018 (six replicates for aerated and six replicates for non-aerated bottles). Thalli rotation velocity was measured for a single thallus for half and full cycle (Fig. 2(b)) in three bottles with a stopper watch.

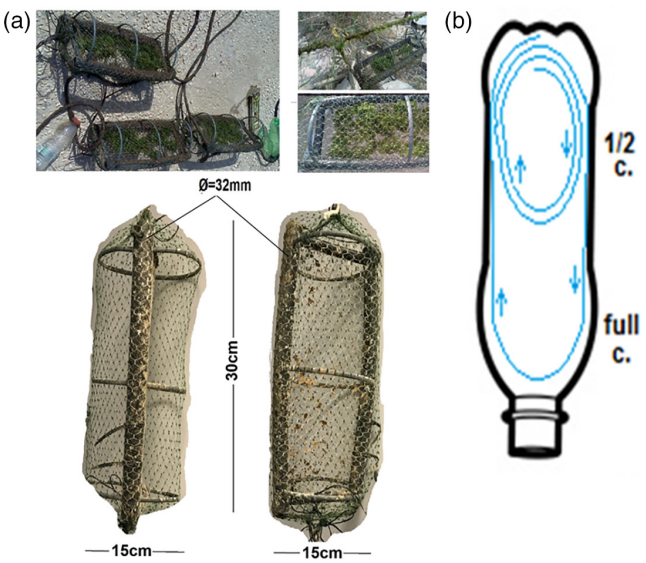

Figure 2. (a) Cages for extensive cultivation. Digital images and geometry. (b) Bottles for cultivation on-land in controlled conditions. The trajectories for measured thalli velocity are schematically shown.

Solar irradiance and temperature

Solar irradiance and temperature were measured every 15 min, using an Onset HOBO Pendant® temperature / light 64K data logger (Onset Inc., Bourne, MA), installed at 40 cm depth inside the aerated cage with the biomass. The additional sensor was installed inside the flat cage that was not aerated at ~10 cm depth. For the on-land system, temperature and irradiance were measured in the water and outside of the bottles with two sensors. Lux values were converted to μmoles m^{-2} s^{-1} by multiplying measured lux values by 0.019, a constant used for sun illumination ({http://www.egc.com/useful_info_lighting.php}).

Nutrients measurement at the cultivation site

To measure nutrients, 50 mL of water was sampled at the cultivation site every 2 weeks with biomass sampling / loading. Nutrients were analyzed less than 1 h after sampling in duplicate. Ammonia, nitrite, nitrate, and phosphate were quantified using a SMART3 colorimeter (LaMotte, Chestertown, MD) with kits and protocols supplied by the manufacturer.

Biomass composition analysis

For ash analysis, the biomass (DW) was ignited in pre-weighed, clean crucibles at 550 °C for 3 h in a muffle furnace (Thermolyne muffle furnace, Thermo Scientific, Waltham, MA). The crucibles were finally removed from the furnace, kept in a desiccator to cool them to room temperature, and weighed. The analysis was done in triplicate. Protein content was determined according to AOAC 981.10 with an automatic Kjeldahl system for total protein quantification. A protein calculation factor of 5 was used.[42] The analysis

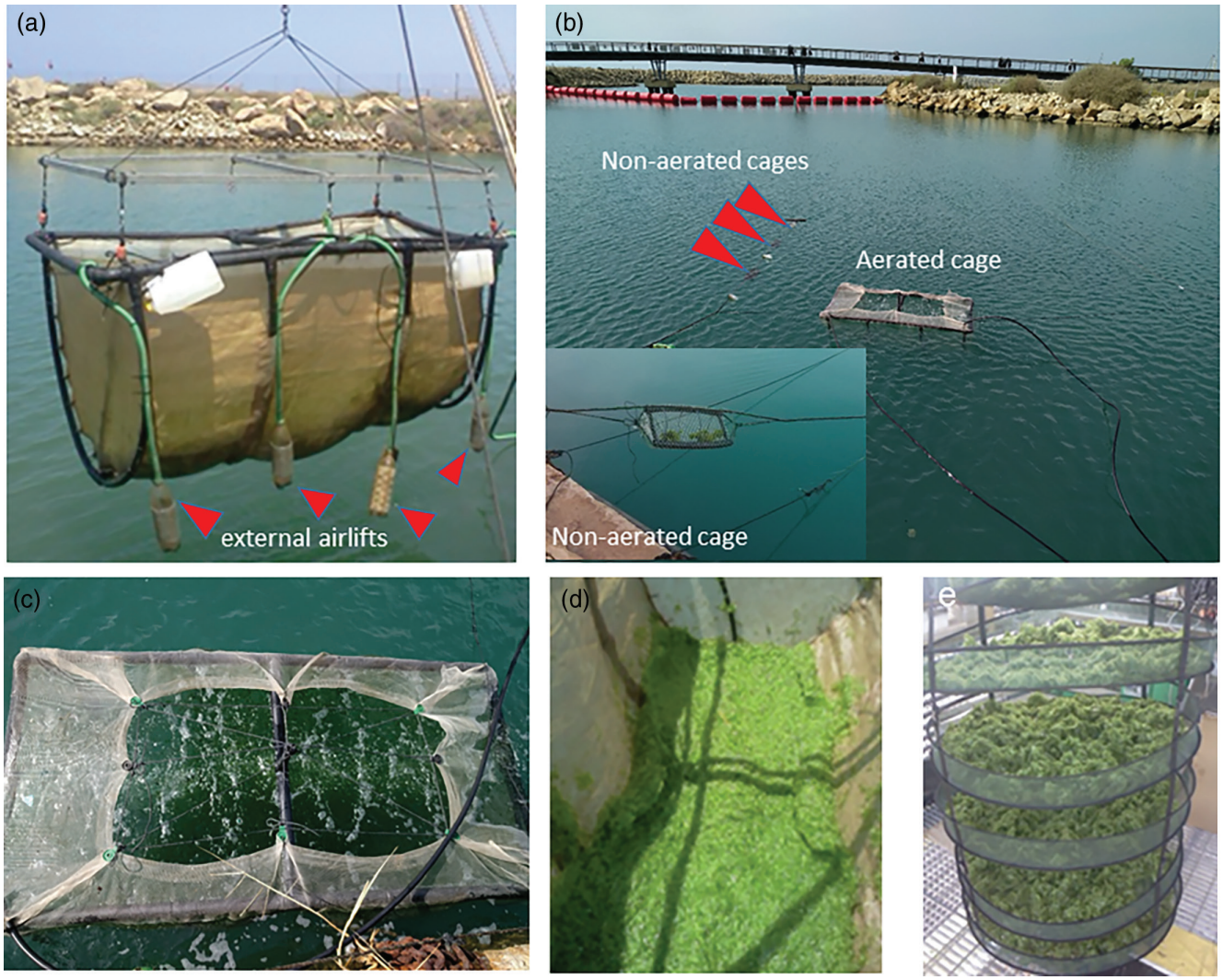

Figure 3. (a) Digital image of the cultivation reactor with external airlifts. (b) Deployment of the reactor with algae to the cultivation site. (c) Tumbling with air and mixing of *Ulva* sp. biomass in the reactor. (d) Harvested *Ulva* biomass after water removal with gravitation. (e) Solar dried *Ulva* biomass.

was conducted by a certified food chemistry company (AminoLab, Rehovot, Israel). For caloric value analysis, 20 g (DW) of biomass, harvested on May 3 and 17, 2017, dried at 40 °C to constant weight, was analyzed for energy content according to ASTM D5865 – 13 (Standard Test Method for Gross Calorific Value of Coal and Coke) by a certified laboratory of the Israel Electric Corporation. Element analysis, CHNS, was done using a Thermo Scientific carbon, hydrogen, nitrogen, sulfur (CHNS) Analyzer (Flash2000) at Technion, Israel Institute of Technology chemical characterization and surface chemistry unit.

For monosaccharide quantification the biomass was hydrolyzed as optimized by Jiang *et al.*[43] (2% sulfuric acid, 1:20 solid to solvent ratio, 30 min, 121 °C) in 10 mL Nalgene™ Oak Ridge High-Speed PPCO centrifuge tubes (Thermo Fisher Scientific, Waltham, MA) in an autoclave (Tuttnauer 2540MLV, Breda, Netherlands). The monosaccharide content in the hydrolysates was quantified by high-pressure anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) using a Dionex ICS-5000 platform (Dionex, Thermo Fischer Scientific) with an analytical column (Aminopack 10) and its corresponding guard column. An electrochemical detector with an AgCl reference electrode was used for detection. The analysis was performed using an isocratic flow of 4.8 mM KOH for 20 min. The column was then washed with 100 mmol L^{-1} KOH between each run and re-equilibrated with 4.8 mmol L^{-1} KOH prior to injection. The column temperature was kept at 30 °C, and the flow rate was set to 0.25 mL min^{-1}. Calibration curves

were produced for each sugar with internal standards. In this work we quantified rhamnose, arabinose, galactose, glucose, xylose, glucuronic acid, mannitol, fucose, and mannose. The glucuronic acid and uronic acid derivative content were monitored using a program that involved three eluents (NaOH, ultrapure water, and sodium acetate) – see supporting information, Table S1. Two additional to glucuronic peaks were observed in all samples that were assumed as aldobiouronic acid and iduronic acid as stated in[44] and reported here as uronic acid derivatives. Each algal sample was hydrolyzed in duplicate before analysis. Each of the hydrolysates was analyzed in duplicate using high-pressure ion chromatography (HPIC). All data are reported as the weight fraction of the specific monosaccharide biomass (μg of monosaccharide mg^{-1} DW biomass).

Statistical analysis

Statistical analysis was performed with the Excel (ver. 13, Microsoft, WA) data analysis package and R software (version 2015, RStudio Inc., Boston, MA, USA). Standard deviation (±STDEV) is shown in error bars. For group comparison, a one-tail Student-t analysis was performed.

Results and discussion

Environmental parameters during the sea cultivation period

The illumination and temperature profile in the aerated reactor at 40 cm depth is shown in Fig. 4. Importantly, the temperature in the cages increased from ~24 to 32 °C during the cultivation period. Comparison between an average temperature in the cages that were mixed and the cages that were not mixed with aeration showed that, until July, an aerated cage was at least 2 °C cooler than a non-aerated cage (see supporting information, Fig. S1). This is important, as the temperature at these levels (close to 30 °C) slows *Ulva* sp. growth.[45,46] Measured nutrient levels are shown in Table 1. Large fluctuations in nutrients levels (NH_3 0.09–2.16 ppm, NO_3^- 0.44–2.11 ppm, NO_2 0.13–1.53, and PO_4^{-3} 0.05–0.99 ppm) were observed.

Growth rates and area productivity

The biomass was weighed every 2 weeks and the yield was harvested. In the first and second experiments, the highest growth rates (19.2% and 4.1%) were measured after

Table 1. Nutrients levels measured at the cultivation site. Data shown is an average of a duplicate measurement.

Measured date	NH_3 (ppm)	NO_3^- (ppm)	NO_2 (ppm)	PO_4^{-3} (ppm)
20 April 17	0.61	0.44	0.20	0.06
3 May 17	0.64	0.84	0.07	0.10
9 May 17	2.16	0.88	0.69	0.99
29 May 17	0.04	0.57	0.66	0.11
15 June 17	0.62	1.32	0.13	0.05
28 June 17	0.09	2.11	0.69	0.09
12 July 17	0.94	1.36	1.52	0.08

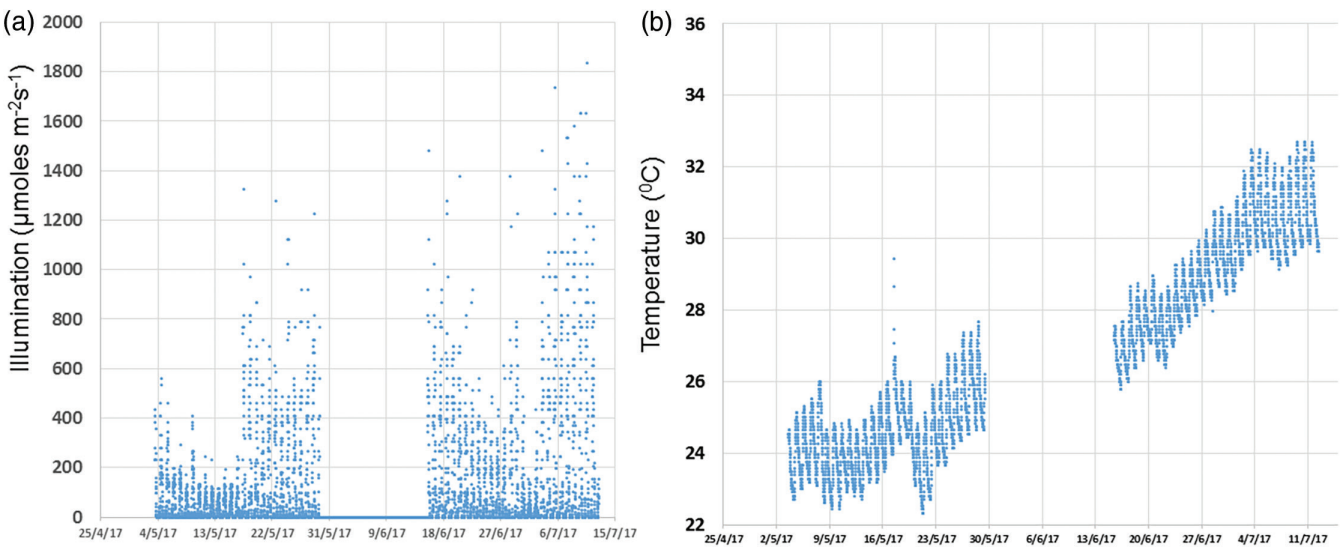

Figure 4. Illumination (a) and Temperature (b) profile inside the cultivation reactor. Information was recorded with 15 min resolution continuously.

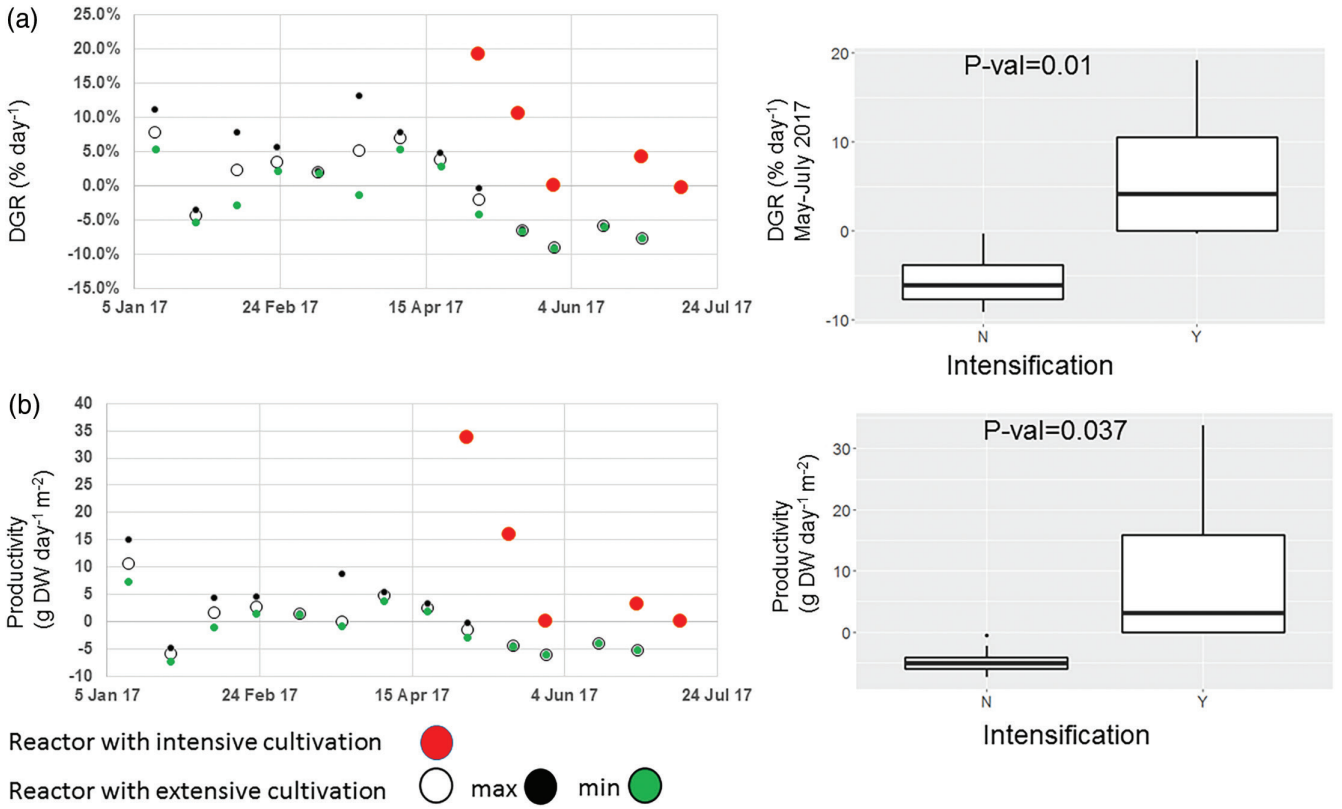

Figure 5. Daily growth rates (a) and productivity (b) of *Ulva* in the intensified and extensive cultivation systems in the sea (n = 3). Minimum, maximum and average measurements are shown.

the first 2 weeks of cultivation. This could be the result of accumulated nutrients during inoculation for both groups. Higher levels of NH_3 and NO_3^- were also observed on May 9, 2017, June 15, 2017, and June 28, 2017, and these could support the growth. Higher growth rates were observed at lower temperatures (10.6–19.2% DGR was observed when the temperatures were at 24–26 °C) and lower growth rates were observed at higher temperatures (26–32 °C), an effect that has been reported before for *Ulva* sp.[45,47] Interestingly, the growth rates were higher in the intensified growth reactors than in the extensive growth reactors in the same season (Fig. 5(a)). Consequently, the tumbling of macroalgae with air, mixing, and external water supply to the cages in the sea led to the highest yield observed in the entire season (Fig. 5(b) and Table 2). The highest yield was observed after 13 days of cultivation, on May 3, 2017 and it was 33.72 g DW day^{-1} m^{-2} (6.74 g C day^{-1} m^{-2}, 0.33 g N day^{-1} m^{-2}), or 37.79 g DW day^{-1} m^{-3}. In comparison, the highest yield in the extensive cultivation from January 2017 to July 2017 was 15 g DW day^{-1} m^{-2} in January 2017. There was no growth from May to July in the cages with extensive cultivation (Fig. 5(b)), probably due to high temperature (see supporting information, Fig. S1). It is important to note that cultivation in the cages that were tumbled with air, with mixing but without external water supply from outside the cage (Fig. 1(d)), resulted in biomass loss when growth was observed in the extensive system from January to May 2017. This implies that tumbling, air mixing, and external water flux, which provides nutrients and colder water, play a combined role in growth-rate intensification. It is difficult to establish the extent to which each component contributes to the growth-rate intensification due to complex interactions between the components, such as the reduction of photoinhibition, the enhanced supply of nutrients, the enhanced gas exchange and hydrodynamic stimulus, just to mention a few. Furthermore, air and biomass movement in the reactor might also prevent the development of damaging viruses or bacteria, as was shown for some on-land systems.[48] The water supply, using airlift pumps from deeper layers, will also typically reduce the temperature in the reactor, working against the temperature inhibition factor.

To shed light on some of the coupled interactions mentioned above, we performed a series of experiments in the on-land system where the only changed parameter between cultivation reactors was aeration.

Table 2. *Ulva* sp. biomass growth rates and yields. Two separate experiments were performed (highlighted in white and light grey) in the cage with intensification, achived with with tumbling, air mixing and external water exchange. FW- fresh weight, DW- dry weight.

Date	Density (FW) in the cage with intensification		Yield in the cage with itensification		Growth Rate	
	g FW m^{-2}	g FW m^{-3}	gDW day^{-1} m^{-2}	gDW day^{-1} m^{-3}	DGR in cage with intensification % day^{-1}	DGR in extensive cages % day^{-1}
20 April 17	1,174[a]	1,315[a]				
3 May 17	4,096	4,590	33.72	37.79	19.2%	–4.2%
17 May 17	2,482	2,781	15.88	17.79	10.6%	–6.7%
29 May 17	1,000	1,120	0	0	0.0%	–9.1%
15 June 17	1,165[a]	1,306[a]				
28 June 17	1,786	2,001	3.11	3.47	4.1%	–5.9%
12 July 17	954	1,069			–0.3%	Not measured

[a]Stays for initial density, the rest is density at harvesting.

The experiments in the controlled environment showed that under an air temperature between 21.9 °C (at night) and 40.53 °C (during the day), and water temperature between 21.6 °C (at night) and 37.4 °C (during the day), and maximum solar illumination intensity (outside the reactors) of 942 µmoles m^{-2} s^{-1}, mixing by aeration (5.8–8.6 rpm) increased the DGR from 7.6 ± 2.6% to 29.9 ± 2.9% ($P < 2.8 \cdot 10^{-5}$). These results in the controlled land-based system showed that tumbling with air and mixing increased the growth rates of *Ulva* sp. biomass (see supporting information, Fig. S2) when nutrients were available in excess, corroborating the view that a combination of tumbling with air, mixing, and nutrient supply is needed for the intensification of growth, when other parameters, such as illumination and temperature, are equal.

Previous studies on the *Ulva* sp. cultivation offshore reported a maximum 17% DGR when the algae were cultivated downstream from fish cages and –15% upstream of the cages.[49] Previous work also compared various methods for *Ulva* sp. growth in the on-land tank, on ropes, and with spray systems. Studies with *Ulva* sp. growth in tanks with tumbling reported on DGR of 10–45%,[50–55] with high values achieved in nitrogen-rich wastewaters such as manure streams.[56] *Ulva* sp. cultivation in the tumbled with air tanks led to high DGR in comparison with spray culture (16.9% versus 11.8%).[57] A study that compared tumbled and not-tumbled *Ulva* sp. growth in tanks reported yields of 12.1 g DW m^{-2} d^{-1} with tumbling regimes of 12 h, versus 4.7 g DW m^{-2} d^{-1} without aeration.[58] Additional research on the production of *Ulva ohnoi* in the intensive land-based system with controlled cultivation conditions reported 20–80 g DW m^{-2} day^{-1}.[59] This study showed 3.1–33.7 g DW m^{-2} day^{-1} in the aerated offshore system (0.15 DW:FW ratio) and a maximum of 15 g DW day^{-1} m^{-2} in the non-aerated cages. The highest DGR of 65% we found in the literature was reported for *Ulva* sp. grown on ropes inside tanks.[60]

Compositional analysis of the cultivated *Ulva* sp. biomass

The ash content of the dry matter from the samples with the highest yields, 33.72 (g DW day^{-1} m^{-2}) and 15.86 (g DW day^{-1} m^{-2}), harvested on May 3, 2017 and May 17, 2017, from cages with intensified cultivation, was 38.47 ± 0.01% and 37.87 ± 0.01%, respectively. This ash composition is at the lower boundary of the *Ulva* ashes reported in Mexico, where 35.8–57.6% ash was reported,[61] but higher than ash content reported for *Ulva* sp. in Spain, which showed 11–29%.[62]

The harvested biomass had significantly lower protein content than biomass grown in laboratory conditions (2.9–6.2% protein in the intensified cages in the sea, 0.53–9.08% in cages with extensive cultivation versus 33% in the lab). Low protein (5.9–17%) has been reported for multiple natural stocks of various *Ulva* species,[63–66] suggesting that precise nitrogen control is required to maintain high protein content.[67]

The elementary composition of the biomass harvested from cages with intensified cultivation varied as follows during the entire cultivation period: C% 19.6–22.5; H% 3.7–4.6, N% 0.65–1.4, and S% 3.54–6.74 (Table 3). It was not significantly different from the composition of the biomass cultivated in extensive cages (Table 4). These results indicate the potential of *Ulva* sp. to capture carbon and nitrogen, two important climate-change factors, for the mitigation of which *Ulva*

Table 3. Protein content and elementary composition of harvested from a tumbled with air cage *Ulva* biomass. or extensive cultivation (white). Protein was measured according to AOAC 981.10 with a multiplication factor of 5. CHNS shows the average of at least two technical repeats.

Harvesting date	Cultivation days	Protein content (%)	C (%)	H (%)	N (%)	S (%)
03 May 2017	13	5.28	19.9	4.6	1.05	6.74
17 May 2017	27	2.96	21.3	4.4	0.65	6.79
29 May 2017	39	4.64	21.3	4.1	0.98	3.54
28 June 2017	13	4.8	22.5	4.6	1.06	5.52
12 July 2017	27	6.24	19.6	3.7	1.40	3.15

Table 4. Protein content and elementary composition of harvested from an extensive cultivation. Protein was measured according to AOAC 981.10 with a multiplication factor of 5. CHNS shows the average of at least two technical repeats.

Harvesting date	Cultivation days	Protein content (%)	C (%)	H (%)	N (%)	S (%)
12 January 2017	14	8.81	17.59	3.94	1.76	4.79
26 January 2017	14	9.08	22.99	4.13	1.82	1.73
13 February 2017	17	8.55	17.65	3.84	1.71	4.81
28 February 2017	15	8.60	18.92	3.68	1.72	4.04
23 March 2017	14	7.07	21.81	4.21	1.41	4.93
06 April 2017	14	5.06	15.59	3.22	1.01	3.53
20 April 2017	16	3.83	20.81	4.24	0.77	4.71
03 May 2017	13	0.53	5.73	2.27	0.11	3.18
17 May 2017	14	2.62	22.81	4.41	0.52	5.84
18 May 2017	14	4.32	24.16	4.44	0.86	3.30
26 May 2017	10	6.07	19.59	4.09	1.21	5.28
29 May 2017	10	3.88	22.40	4.08	0.78	3.64
17 July 2017	14	4.71	21.89	3.93	0.94	3.55

Table 5. Monosaccharides content of the *Ulva* sp. biomass cultivated with intensification achieved with tumbling with air, mixing and external water supply (gray) or extensive cultivation (white).

Harvesting date	Cultivation days	Fructose (mg g^{-1} DW)	Galactose (mg g^{-1} DW)	Glucose (mg g^{-1} DW)	Rhamnose (mg g^{-1} DW)	Uronic acid derivatives (mg g^{-1} DW)	Xylose (mg g^{-1} DW)
03 May 2017	13	2.49 ± 0.15	0.76 ± 0.25	21.76 ± 7.84	15.70 ± 5.32	19.58 ± 9.57	13.64 ± 3.55
17 May 2017	27	0.96 ± 0.13	1.02 ± 0.55	29.42 ± 0.08	15.85 ± 0.74	15.23 ± 0.44	13.71 ± 0.34
29 May 2017	39	0.52 ± 0.03	1.80 ± 0.01	18.58 ± 0.17	9.61 ± 0.17	6.93 ± 0.70	12.47 ± 0.44
28 June 2017	13	3.33 ± 2.65	2.26 ± 0.70	39.16 ± 2.57	21.91 ± 2.57	18.05 ± 0.67	16.72 ± 2.31
12 July 2017	27	1.95 ± 0.11	3.80 ± 0.85	18.43 ± 2.59	12.47 ± 2.59	5.59 ± 0.04	9.08 ± 1.56

sp. has been investigated[47] using offshore cultivation at 6.74 g C m^{-2} d^{-1} (25 g CO_2 m^{-2} d^{-1}) and 0.34 g N m^{-2} d^{-1}, at maximum growth rate achieved in this study (assuming 0.15 DW:FW ratio). According to the Israel Ministry of Environmental Protection, the Israel's national GHG emission reduction target for 2030 is 2.7 ton CO_2 per capita (26% from the emissions in 2005).[68] Therefore, ~0.108 km^2 of the sea area should be allocated per capita, if *Ulva* sp. with intensified cultivation is used to achieve these goals.

Analysis of carbohydrate content (Tables 5 and 6) showed that glucose was the major carbohydrate, followed by rhamnose, xylose, and uronic acid derivatives. This is consistent with our previous data on the same species harvested from the sea and cultivated in the on-shore reactor integrated into the building.[69] Longer offshore cultivation in the cages with intensified cultivation (39 versus 18 days) led to lower fructose, glucose, rhamnose, uronic acid derivatives, and xylose content (Table 4). This result is consistent with our previous finding that the content of these sugars in acid hydrolysates was higher when cultivated in the controlled photobioreactor in comparison with the same biomass harvested from the wild stocks from the

Table 6. Monosaccharides content of the *Ulva* sp. biomass cultivated under extensive cultivation.

	Cultivation days	Fructose (mg g^{-1} DW)	Galactose (mg g^{-1} DW)	Glucose (mg g^{-1} DW)	Rhamnose (mg g^{-1} DW)	Uronic acid derivatives (mg g^{-1} DW)	Xylose (mg g^{-1} DW)
12 January 2017	14	0.35 ± 0.29	1.6 ± 0.42	6.67 ± 8.22	16.43 ± 4.15	67.43 ± 25.64	7.68 ± 1.89
26 January 2017	14	0.35 ± 0.04	0.31 ± 0.03	15.58 ± 1.26	7.1 ± 0.54	21.33 ± 3.84	4.97 ± 0.18
13 February 2017	17	0.44 ± 0.09	0.5 ± 0.4	7.73 ± 4.64	6 ± 3.76	29.24 ± 13.65	3.4 ± 1.58
28 February 2017	15	0.36 ± 0.07	0.24 ± 0.51	5.9 ± 6.2	11.39 ± 2.87	39.72 ± 1.42	4.96 ± 0.06
23 March 2017	14	1.01 ± 0.95	0.94 ± 0.01	23.79 ± 1.17	13.13 ± 0.03	49.77 ± 2.63	6.37 ± 0.02
06 April 2017	14	0.46 ± 0.03	0.3 ± 0.19	9.39 ± 0.93	5.42 ± 0.5	16.01 ± 0.09	3.77 ± 0.17
20 April 2017	16	0.58 ± 0.2	1.16 ± 0.04	48.02 ± 2.85	23.58 ± 0.88	71.44 ± 0.68	10.85 ± 0.11
03 May 2017	13	0.3 ± 0.1	0.09 ± 0.04	12.84 ± 5.41	3.68 ± 1.22	13.19 ± 5.67	1.93 ± 0.63
17 May 2017	14	1.05 ± 0.17	1.12 ± 0.23	32.27 ± 5.53	23.59 ± 5.22	48.52 ± 9.15	15.61 ± 2.32
18 May 2017	14	0.55 ± 0.04	1.66 ± 0.22	29.91 ± 4.12	32.2 ± 3.05	64.39 ± 2.59	16.92 ± 0.52
26 May 2017	10	0.23 ± 0.03	0.91 ± 0.01	8.07 ± 0.91	12.03 ± 0.91	29.88 ± 0.86	5.49 ± 0.09
29 May 2017	10	0.34 ± 0.03	1.01 ± 0.02	13.12 ± 1.33	7.93 ± 0.36	20.22 ± 1.43	8.91 ± 0.2

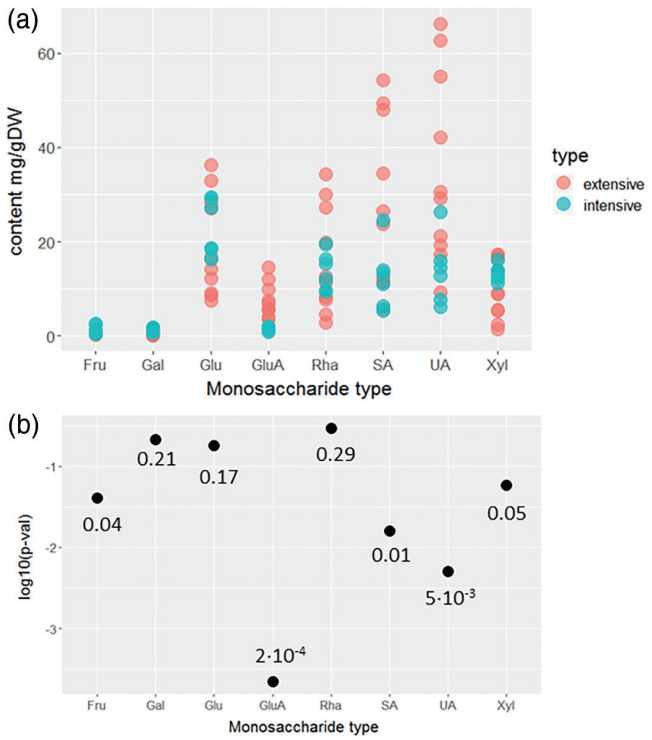

Figure 6. Monosaccharides content in the acid hydrolysate of the biomass harvested from cages with intensified and extensive cultivation in May 2017. (a) Concentration, (b) *P* value for comparison (Student-t) of the content between the two cultivation methods. n = 6 for intensified cultivation and n = 10 for extensive cultivation. Gal – galactose, Glu-glucose, GluA-glucuronic acid, SA-other sugar acids, Rha – rhamnose, Xyl – xylose, Fru – fructose, UA – uronic acid.

sea.[69] As the total carbon content of the biomass did not change with cultivation time (Table 3), we suggest that, under offshore cultivation, the carbon is stored in fibers such as cellulose, which is not hydrolyzed by our protocol.

Comparison of the monosaccharide content of the matched biomass harvested on May 3, 2017, May 17, 2017, and May 29, 2017 (see supporting information, Fig. S3, Tables 5 and 6) shows that intensification of cultivation led to the significant ($P < 0.5$) increase in fructose content, and significant decrease ($P < 0.5$) in glucuronic, uronic, and other sugar acids (Fig. 6).

Energy content of *Ulva* sp. biomass cultivated with intensification

The energetic high heating value (HHV) of the dried biomass as fuel was 8.46 MJ kg_{DW}^{-1} (remained moisture (RM%) 11.21%) for the harvest on 3 May and 9.13 MJ kg_{DW}^{-1} (remained moisture (RM%) 13.79%) for the harvest on May 17. Hence, at the observed maximum, *Ulva* sp. biomass can produce 2 MJ m^{-2} per day or produce a maximum power density of 23 W m^{-2} for direct combustion.

Conclusions

We tested the feasibility of the offshore cultivation of *Ulva* sp. biomass in an intensified offshore reactor, with tumbling and mixing with air and external water supply. This intensification method allowed for the production of *Ulva* sp. biomass during the period from May to July in 2017 while there was no growth in extensive offshore systems. Tumbling with air, and mixing, increased the growth rate

of *Ulva* sp. in controlled, land-based systems in comparison with the same systems without tumbling and mixing. Multiple coupled mechanisms can lead to these changes, including a reduction in photoinhibition, enhanced nutrient flux, the water, enhanced gas exchange, and hydrodynamic stimulus. Air and biomass movement in the reactor might also prevent the development of axenic zones, and the development of detrimental levels of viruses or bacteria or excretion of growth inhibiting photosynthesis byproducts. Water supply using airlift pumps from deeper layers also reduces the temperature of the reactor. Our findings open new directions for the design of offshore cultivation systems that will produce usable biomass without arable land and fresh water.

Competing interests

The authors declare no competing financial interests.

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Alexander Golberg

Alexander Golberg, PhD, is a senior lecturer at the Porter School of Environmental and Earth Sciences. His major research interests are in the development of new technologies for the benefit of the environment and human health. He is the recipient of the Green Talent Award 2012 from the Federal German government for the development of small-scale marine biorefineries, and the Robert B. Lindberg Award from the American Burn Association in 2015 for the development of electroporation and pulsed electric-field technologies for burn wound healing. He graduated from Technion, Department of Biotechnology and Food Engineering and received a PhD in bioengineering from the Hebrew University of Jerusalem.

