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Net primary productivity, biofuel production and CO_2 emissions reduction potential of *Ulva* sp. (Chlorophyta) biomass in a coastal area of the Eastern Mediterranean

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ABSTRACT

Offshore grown macroalgae biomass could provide a sustainable feedstock for biorefineries. However, tools to assess its potential for producing biofuels, food and chemicals are limited. In this work, we determined the net annual primary productivity (NPP) for *Ulva* sp. (Chlorophyta), using a single layer cultivation in a shallow, coastal site in Israel. We also evaluated the implied potential bioethanol production under literature based conversion rates. Overall, the daily growth rate of *Ulva* sp. was 4.5 ± 1.1%, corresponding to an annual average productivity of 5.8 ± 1.5 g_{DW} m^{-2} day^{-1}. In comparison, laboratory experiments showed that under nutrients saturation conditions *Ulva* sp. daily growth rate achieved 33 ± 6%. The average NPP of *Ulva* sp. offshore was 838 ± 201 g C m^{-2} $year^{-1}$, which is higher than the global average of 290 g C m^{-2} $year^{-1}$ NPP estimated for terrestrial biomass in the Middle East. These results position *Ulva* sp. at the high end of potential crops for bioenergy under the prevailing conditions of the Eastern Mediterranean Sea. We found that with 90% confidence, with the respect to the conversion distribution, the annual ethanol production from *Ulva* sp. biomass, grown in a layer reactor is 229.5 g ethanol m^{-2} $year^{-1}$.This translates to an energy density of 5.74 MJ m^{-2} $year^{-1}$ and power density of 0.18 W m^{-2}. Growth intensification, to the rates observed at the laboratory conditions, with currently reported conversion yields, could increase, with 90% confidence, the annual ethanol production density of *Ulva* sp. to 1735 g ethanol m^{-2} $year^{-1}$, which translates to an energy density of 43.5 MJ m^{-2} $year^{-1}$ and a power density 1.36 W m^{-2}. Based on the measured NPP, we estimated the size of offshore area allocation required to provide biomass for bioethanol sufficient to replace 5–100% of oil used in transportation in Israel. We also performed a sensitivity analysis on the biomass productivity, national CO_2 emissions reduction, ethanol potential, feedstock costs and sizes of the required allocated areas.

1. Introduction

Growing population, increasing quality of life and longevity imposes new pressures on all industrial sectors involved in the production of food, chemicals and fuels for humans, including the use of land, drinking water, fossil fuels and natural resources. An expanding body of evidence, nonetheless, has demonstrated that marine macroalgae can provide a sustainable source of biomass for food, feeds, fuel and chemicals generation [1–6]. Macroalgae, which contain very little lignin and do not compete with food crops for arable land or potable water, have stimulated renewed interest as additional future sustainable food and transportation fuel feedstock [1–11,7,8]. Still, the models and tools developed for terrestrial biomass analysis are not yet available for macroalgae. Moreover, the application of advanced genomic tools to characterize various macroalgae strains only happened in recent years [9]. Additional significant efforts are required in order to establish macroalgae breeding programs and to develop strains with specific properties for food, chemicals and fuel applications [10]. In addition to fundamental biological aspects macroalgae based biorefinery engineering and engineering economics is also

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not yet developed. Although multiple techno-economical and policy factors affect the viability of biorefineries [11,12], several parameters such as local net primary productivity (NPP, defined in units of as g DW m^{-2} day^{-1}, a measurement of the conversion efficiency of solar energy into potentially useful chemical energy), and species specific conversion efficiencies are critical for any of these assessments. Moreover, major gaps in local data pertaining to these parameters obstruct the development of reliable estimates of macroalgae crops potential for biorefineries at the local/national levels.

Previous studies estimated an average, global NPP of macroalgae at 1–3 kg C m^{-2} $year^{-1}$ [2,13,14]; yet, these numbers should be locally established for each cultivation point and for each potential seaweed crop. In addition, previous studies used oceanography based computational tools to estimate the potential of green macroalgae as provide food, chemicals and biofuels feedstock on the global level [15].

The goal of this work is to establish a measurement based methodology to assess macroalgae potential as a crop for biorefinery applications. Here we report on the NPP of the green macroalga *Ulva* sp. in the coastal areas of Israel. We also used a statistical approach, incorporating seasonal changes in growth rates, to estimate the potential local transportation bioethanol production from *Ulva* sp. Based on these data, we modeled the required offshore allocations that can provide, with high confidence, any given fraction of the national needs for transportation fuels.

2. Materials and methods

2.1. Macroalgae biomass inoculum

The model seaweed used in this study belong to the genus *Ulva* (the taxonomic status of the species under investigation), a green marine macroalga of worldwide distribution found in the intertidal and shallow waters within the Israeli Mediterranean Sea shores. For the current study, specimens were taken from stocks cultivated in an outdoor seaweed collection at Israel Oceanographic & Limnological Research, Haifa, Israel (IOLR), in 40 L fibreglass tanks supplied with running seawater, aeration and weekly additions of 1 mM NH_4Cl and 0.1 mM NaH_2PO_4. With each nutrient application, the water exchange was stopped for 24 h to allow for nutrients absorption.

2.2. Cultivation location choice

Biomass was cultivated in a shallow nearby area in the sea close to an electricity power station in Tel Aviv, Israel (Fig. 1a). The considerations for choosing the field site included easy access from the beach, restricted access to the general public or small sport navigation vessels, no warm water outputs from the power plant and a solid breakwater system for easy work. The experimental site also underwent intensive ecological restoration in recent years. The location choice allows for continuous weekly monitoring without interference from the general city activities.

2.3. Thin flat reactor design for NPP measurements

To estimate the biomass growth potential of *Ulva* sp. for biorefineries we designed the thin flat cultivation reactor. In nature, *Ulva* generally grows attached to a substrate (usually rocks) yet it may also be found growing in a floating stage within the water column. To investigate the possibility of offshore *Ulva* sp. growth for biorefineries, we designed a flat cultivation reactor where a thin, 2 cm layer of thalli were placed between two layers of nets (TENAX Tubular nets for Mussel Breeding &Packaging Shellfish Polypropylene, mesh configuration – rhomboidal, 32 G 223 neutral. 74 N 140 green, Gallo Plastik, Italy. The cage (0.15 m × 0.3 m, total illuminated area 0.045 m^2) was built from Polyethylene (PE) (D = 32 mm) and high-density polyethylene (HDPE), (D = 16 mm) pipes and a TENAX (Gallo Plastik, Italy) net (Fig. 1b) to allow for full illumination and prevent grazing of algae by fish. The three flat cultivation reactors used in this study were connected to the rope and located 5–20 m from the shore (Fig. 1a). Here measurements of biomass were taken each week to estimate growth rates with a total number of 28 experiments.

Daily growth rate (DGR%) was calculated as in Eq. (1) following Refs. [16,17].

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

where N (d) is the number of days between measurements, m_{out} is the wet weight (WW) (g) measured at the end of each growth period, and m_{in} is the WW (g) of the inoculum.

2.4. Laboratory macroalgae photobioreactor

A closed custom made macroalgae photobioreactor (MPBR) system with 6 spherical reactors of 1 L was developed to grow macroalgae under controlled conditions. The MPBR maintained uniform conditions in each one of the 6 reactors in terms of flow rate, pH, temperature, salinity, and NH_4^+ and PO_4^{2-} nutrients concentration. The system consisted of a 130 L central tank from which the water was continuously recirculated through the 6 reactors with a pump. The flow rate of recirculated water in the system was set to 850 ml min^{-1} with a rotameter (FS, Emproco Israel). Temperature was controlled at the major tank with a 300 W heating body and a thermostat (JEBO 2010, China). Each reactor was equipped with a matching light emitting diode (LED) system (60 W PAR, Flora Photonica, Israel) enabling to control the illumination parameters for each spherical reactor. The LED light included 6 colors in the PAR wavelengths (380, 430, 460, 630, 660, 740 nm). Each LED was connected to a signal generator and a power supply (MCH-303A, 30V/3A, Lion electronics Israel). To avoid the co-lateral effect of light treatments, cardboards were placed between the test tubes.

Ulva sp. thalli from the same type as the one used for offshore cultivation were grown in artificial seawater prepared from dried Red Sea salts (Red Sea Inc. Israel) with distilled water. The salinity was adjusted to 3.5% and the pH was set at 8.2 in all experiment. NH_4Cl and NaH_2PO_4 (Haifa Chemicals, Israel) were used to adjust the levels of nitrogen (N) to 6.36 ppm and phosphorus (P) to 0.97 ppm. Irradiance was set on 1000 μM photons m^{-2} s^{-1} and the temperature set at 23 °C with a thermostat. These parameters were chosen based on previous studies of light, nutrients and temperature effects on *Ulva* sp. growth [18–20]. To compensate for potential biological differences between thalli, we cut 4 large thalli to 6 equal parts and cultivated them in 6 different reactors. Initial wet weight of each inoculum was 0.6 ± 0.01 g per reactor. Light: Dark cycle was 9 h:15 h. The thalli were harvested for the experiments after 3 days.

2.5. Macroalgae biomass growth rate and energy conversion offshore

To measure the growth rates and solar energy conversion to macroalgae biomass, we inoculated each of the cultivation reactors with 40 g WW of *Ulva* (888.9 g m^{-2}), using the inoculum density optimization suggested in [3]. Biomass measurements were taken every 7–11 days and the daily growth rates (DGR) were determined as described in Eq. (1). On one hand, increasing the frequency of biomass measurements could have led to excessive water loss of the thalli and to stress. More extended period of cul-

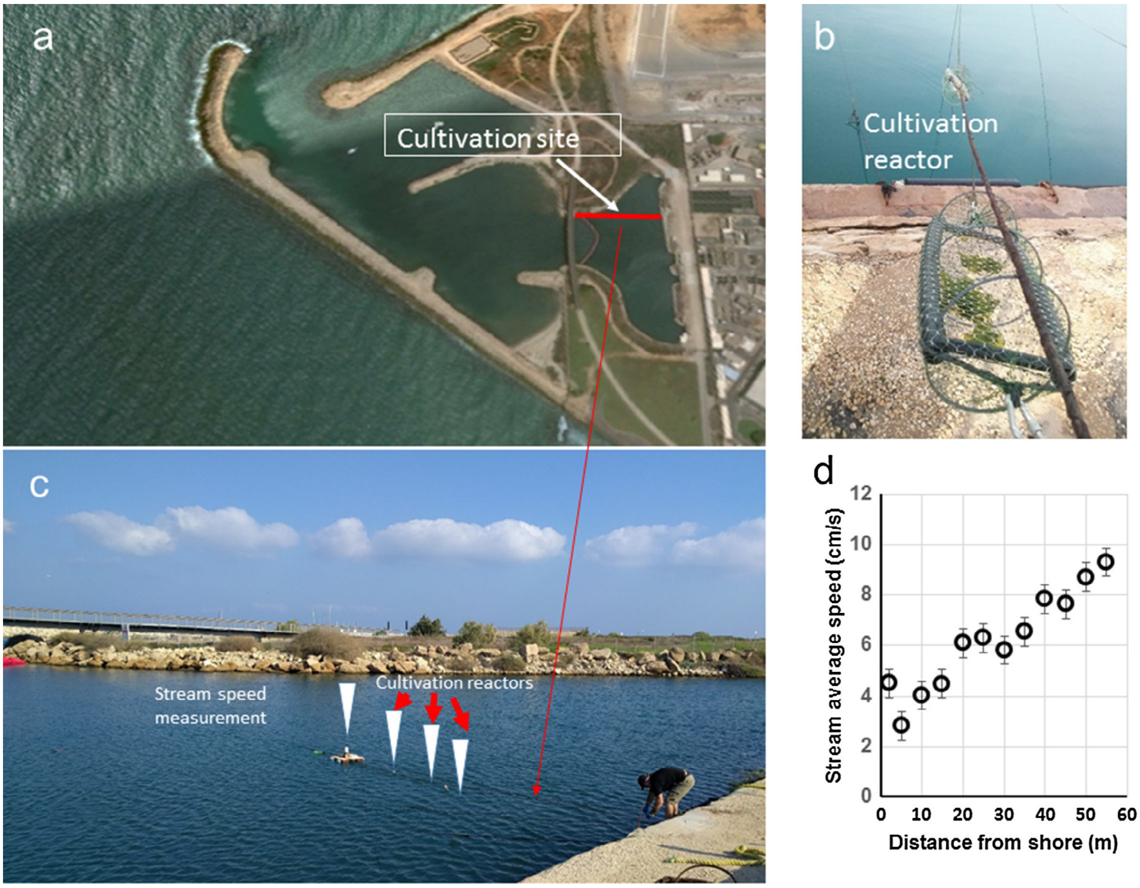

Fig. 1. Experimental setup for NPP measurements. (a) Macroalgae cultivation site at Reading power station in Tel Aviv, Israel; (b) Flat thin cultivation reactor with a signal cultivation depth and double net design. (c) Positions of cultivation reactors during one year measurements; (d) Water current speed profile at the cultivated area, measured at the same depths as the Flat thin cultivation reactor (N = 10 for each point).

tivation, on the other hand, could miss actual growth due to the natural fluctuations in the environmental conditions. The same culture of macroalgae was kept in the thin cultivation reactor and m_{in} was adjusted to 40 g either by removing the extra biomass or by adding additional biomass from the onshore inoculum.

The average Net Primary Productivity (NPP) in units of g C m^{-2} year^{-1} for the specific initial biomass density and the specific thin flat cultivation reactor geometry used in this study was calculated as appears in Eq. (2):

$$\langle NPP \rangle = \frac{1}{A}\frac{DW}{WW}\cdot\frac{C}{DW}\sum_{k=1}^{n}(m_{k\_out} - m_{k_{in}}) \qquad (2)$$

where DW is the dry weight (g), WW is the wet weight (g), C is the carbon fraction in the DW biomass, m_{k_out} (g) is the WW of the biomass as measured at measurement point k, and m_{k_in} (g) is WW of the initial biomass, n is the total number of measurement points, A (m^2) is the area of the cage. The average from three separate flat cultivation reactors is reported.

2.6. Solar irradiance

Solar irradiation for the cultivation period was extracted from the Israel Meteorological Services (IMS: http://www.ims.gov.il/IMS/CLIMATE/LongTermRadiation/) for the Beit Dagan Israel measurement station. The daily global solar irradiance (kJ m^{-2}) was calculated as the irradiance from 5 am to 7 pm on each day of the cultivation experiment. The IMS data base provides information of the accumulated global irradiance with 1 h resolution.

2.7. Temperature

Daily sea surface water temperature for Tel Aviv was extracted from http://seatemperature.net/current/israel/tel-aviv-tel-aviv-israel-sea-temperature.

2.8. Water current measurements

The flow at the cultivation site was measured using acoustic Doppler method with 3-axis (3D) Argonaut-ADV's (SonTeck, CA). The flow was measured along the cultivation rope by installing the device on the connected to the rope raft, ensuring the flow was measured at the same depth as the cages were installed (Fig. 1c). We used Argonaut-ADV firmware version 11.9 for data analysis. At least 10 measurements were taken at each point.

2.9. Bioethanol potential estimation of Ulva sp. biomass

Ulva biomass composition changes with seasons and environmental stimuli [21–23]. Other sources of variance for bioethanol production from the same biomass are due to the variations in the fermentation process: hydrolysis conditions, microorganisms used, product separation efficiencies and other factors [24–26]. Therefore, we based a first approximation of the annual bioethanol potential production from *Ulva* biomass on a careful recent literature review of *Ulva* fermentation. We screened twenty one papers that reported measurements of *Ulva* fermentation to bioethanol from algae grown/collected at different locations and seasons, representing a distribution of possible chemical compositions of the growth environment [27–47].

Based on the literature derived fermentation metadata, we constructed a probability density function that describes the *Ulva* biomass to bioethanol conversion efficiency. This conversion efficiency distribution, denoted CED, is based, at this first stage, on uniformity assumptions with respect to the conditions reported in the literature – each paper gets an equal weight. The CED takes values in [$g_{ethanol}$ g^{-1} DW_{Ulva}]:

$$CED\left[\frac{g_{ethanol}}{gDW_{Ulva}}\right] = \frac{m_{ethanol}[g_{ethanol}]}{m_{UlvaDW}[gDW_{Ulva}]} \tag{3}$$

For a growing period between two consequent measurements $d = 1 \ldots n$, where n is the number of measurements taken during the year, let $EPR(d)$ denote the random variable that describes the Ethanol Production Rate during the growing period between the two points. As the biomass yield (DGR) varies during the year, to obtain the distribution of daily *EPR*(d), for each measurement period, in units of g Ethanol m^{-2} d^{-1}, we multiply the fixed distribution of the conversion rate CED by the growth rate DGR measured:

$$EPR(d)[g_{ethanol}m^{-2}d^{-1}] = DGR[g\ WW_{Ulva}\ m^{-2}d^{-1}] \cdot \frac{DW_{Ulva}}{WW_{Ulva}} \cdot CED\left[\frac{g_{ethanol}}{g\ DW_{Ulva}}\right] \tag{4}$$

The annual production of ethanol is the sum of production yields at the measured periods: $d = 1 \ldots n$. Therefore, to obtain the distribution of the annual ethanol production rate (AEPR) we need to compute the distribution of the random variable defined in Eq. (5):

$$AEPR = \sum_{d=1}^{n} EPR(d) \tag{5}$$

The random variable AEPR assumes units of g Ethanol m^{-2} $year^{-1}$. Assuming independence of conversion rates in any two periods, the distribution of AEPR can be obtained by repeatedly convolving the distributions of its summands *EPR*(d). This convolution was calculated using a modular custom developed script in Matlab (MathWorks, ver 2016b, MA).

2.10. Fuel properties characterization of Ulva sp. biomass by combustion

Twenty gram (DW) of biomass, harvested in April 2016, dried at 40 °C to constant weight, were 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 Israel Electric company.

2.11. Target biomass cost estimation

To estimate distribution of the maximum justifiable cost of for the biomass for bioethanol we used the following Eq. (6):

$$C_{DW\_biomass}[\$ \cdot \text{ton}^{-1}] = P_{ethanol}[\$ \cdot L^{-1}] \cdot CED \cdot f \tag{6}$$

where $C_{DW\_biomass}$ ($/ton) is the maximum cost of the biomass ex-processing facility, $P_{ethanol}$ ($/L) is the current price for the ethanol futures on the market, CED is the conversion efficiency of macroalgae biomass to bioethanol as defined in Eq. (3) (with appropriate unit conversion), and f is the fraction of biomass cost in the total cost of bioethanol production.

2.12. Statistical analysis

Statistical analysis was performed with Excel (ver. 13, Microsoft, WA) Data analysis package, Matlab (ver. 2016b, MathWorks, MA) and R software (ver.2015, RStudio Inc., Boston, MA). All experiments and controls were done at least in triplicates unless stated differently. All experiments and controls were done at least in triplicates unless otherwise stated. Standard deviation (±STDEV) is shown in error bars. For average annual DGR standard error of the mean is reported (±SE).

3. Results and discussion

3.1. Ulva sp. net primary productivity

The growth of *Ulva* sp. in flat cages at the coastal waters cultivation site was followed and monitored for 12 months, from January 2016 to January 2017. The measured DGRs are shown in Fig. 2b. Positive growth was observed from January to June and from October to December, however, from July to September the biomass deteriorated resulting in no growth or even biomass losses (Fig. 2a). The highest DGRs (75–87%) were observed in March–April. The annual average maximum daily productivity was 67.9 g_{WW} m^{-2} day^{-1} (equivalent to 10.2 g_{DW} m^{-2} day^{-1}), with a maximum observation of 35.49 g_{DW} m^{-2} day^{-1}; average minimum daily productivity was 7.6 g_{WW} m^{-2} day^{-1} (~1.1 g_{DW} m^{-2} day^{-1}). The grand total average productivity (14 January 2016–12 January 2017 was 38.8 g_{WW} m^{-2} day^{-1} (~5.8 g_{DW} m^{-2} day^{-1}) or 14,167 g_{WW} m^{-2} $year^{-1}$, at an average DGR of 4.5 ± 1.1% for the entire measurement year.

To estimate the NPP potential of *Ulva* sp. we conducted a series of laboratory experiments where the environmental conditions were fixed. Laboratory experiments showed that under nutrients and light saturation conditions *Ulva* sp. daily growth rate (DGR) was 33 ± 6%. These results are significant as they show the biological potential of *Ulva* sp. NPP in constant stable environments. These results show that higher NPP *Ulva* sp. can be reached if the conditions of cultivation are optimized offshore with new technologies.

The annual plot of the surface seawater temperature near the experimental site is shown in Fig. 2c, and the annual global irradiance is shown in Fig. 2d. The high solar radiation of July–September was not converted into biomass as there was no growth detected during this period, perhaps contributed by the high water temperatures (~29 °C). Previous studies have reported 25 °C as the optimal temperature for high growth in various *Ulva* species [18,19], with growth limitation observed at higher temperatures [20]. The local currents, induced by the local power station cooling pumps, varies from 3 to 6 cm s^{-1} at the cultivation area (Fig. 1d).

The previous study of seaweed offshore cultivation in Israel was conducted on *Ulva* (cultivated from September 2013 to October 2013) attached in ropes downstream of intensive fish cages, and reported an average DGR of 12%, compared to <0.5% for controls grown in the open sea, distant from the fish cages [40].

Assuming 0.15 Dry weight/Wet weight ratio and 37% carbon content [48], the average NPP calculated for *Ulva* in this current study was 838 ± 201 g C m^{-2} $year^{-1}$. These results position *Ulva's* grown in the coastal Tel Aviv area at production levels higher than other biofuel crops, cultivated in other locations, such as switchgrass (624 g C m^{-2} $year^{-1}$), corn (713 g C m^{-2} $year^{-1}$), wheat (378 g C m^{-2} $year^{-1}$) and rice (631 g C m^{-2} $year^{-1}$) (Table 1), but less than *Miscanthus* (1546 g C m^{-2} $year^{-1}$) and sugar cane (1721 g C m^{-2} $year^{-1}$) (Table 1). However, the cultivation of the above mentioned terrestrial crops for eventual biofuel production presents a very limited opportunity as land and irrigation water

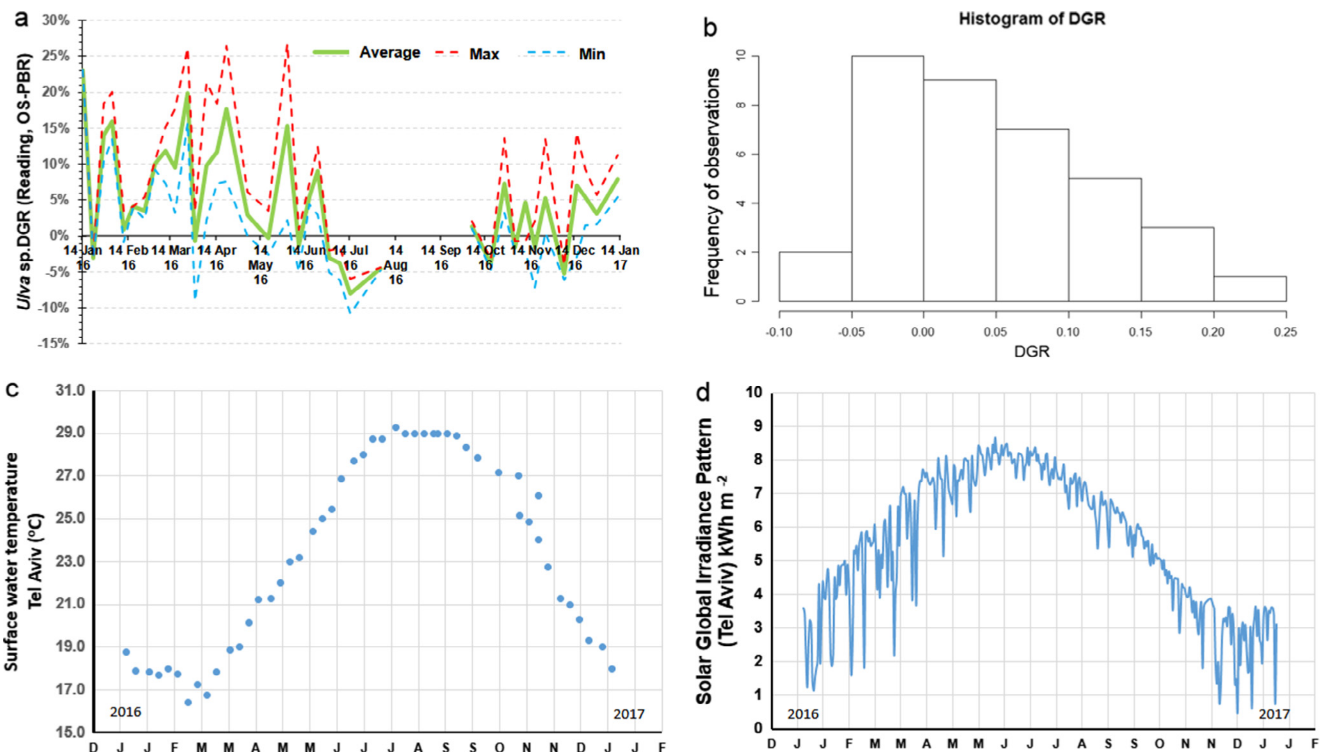

Fig. 2. Annual growth rates and environmental conditions at the cultivation site. (a) Measured annual daily growth rate (%DGR) of *Ulva* biomass at Reading (N = 3 for each point). (b) Histogram of DGR observed during the year; (c) Annual profile of surface water temperature (°C) in Tel Aviv; (d) Annual global irradiance (kW h m^{-2}) at Tel Aviv.

Table 1
Net primary productivity (NPP) of terrestrial crops biomass, and that of marine macroalgae.

Biofuel crops	NPP (g C m^{-2} $year^{-1}$)	Reference
Switchgrass	622	[61]
	624	[62]
Miscanthus	1546	[61]
	1489	[62]
Rice	631	[63]
Corn	408	[63]
	713	[62]
Wheat	378	[63]
	320	[62]
Sugar cane	1721	[63]
Food crops	613	[63]
Middle East (C_4, perennial, leguminous and woody)	290	[49]
Marine macroalgae		
Laminaria-Ascophyllum (Nova Scotia)	1900	[64]
Macrocystis (Kerguelenn archipelago)	2000	[65]
Laminaria (South-West England)	1225	[66]
Macrocystis (California)	400–820	[67]
Codium fragile (Long Island)	696–4700	[68]
Ulva sp. (Ria Formosa Lagoon (estimation))	190	[69]
Ulva compressa (Minicoy Atoll)	1460	[70]
Ulva rigida (Venice lagoon)	358	[71]
	646	[72]
Ulva sp. Reading Power Station, Tel Aviv (grown in a single layer photobioreactor)	838	This study

are very scarce in a region such as Israel. Indeed, the combined NPP of C4, perennial, leguminous and woody biomass in the Middle East was shown to be 290 g C m^{-2} $year^{-1}$ [49].

In comparison with other reports on *Ulva* biomass NPP, we show results higher than *Ulva* NPP monitored in Ria Formosa (190 g C m^{-2} $year^{-1}$) or Venice (max 646 g C m^{-2} $year^{-1}$) lagoons, but lower than Minicoy Atoll (1460 g C m^{-2} $year^{-1}$), Table 1. In addition to environmental conditions, the differences between the previous studies on *Ulva* and our results can also be due to technical sources of variation, such as incubation techniques, environmental differences, age, thallus part, reproductive state, external morphology, crowding, macrohabitat, microhabitat, desiccation, and physical injury, discussed in [50]. To address part of these technical issues, we cultivated all the biomass at a single layer, all at the same depth (~7 cm) inside the special OS-PBR design. Therefore, all thalli got exactly the same amount of light during the cultivation and these experiments appeared to be repeatable.

Although the total annual NPP of *Ulva* biomass in the coastal area of Tel Aviv, Israel, shows promising cumulative numbers, our results are far below the maximum NPP reported for other macroalgae species such as *Laminaria-Ascophyllum* in Nova Scotia, Canada (1900 g C m^{-2} $year^{-1}$), *Laminaria* sp. in South-West England (1225 g C m^{-2} $year^{-1}$), *Macrocystis* sp. in Kerguelen Archipelago, (2000 g C m^{-2} $year^{-1}$), *Macrocystis* sp. in California, USA (max 820 g C m^{-2} $year^{-1}$), *Codium fragile* in Long Island, USA (max 4200 g C m^{-2} $year^{-1}$), Table 1. However, these peak productivities are reported for completely different geographical areas with high surface water nutrient concentrations that do not exist in the Eastern Mediterranean.

Our results further show that *Ulva* follows a complex pattern of growth, first upon initiation of the experiment a rapid spiked with high growth rate followed by the significant fall as close as the following week (Fig. 2a). These biomass fluctuations can be explained mostly by sporulation likely induced by stress resulting from the implantation of fresh biomass in a seemingly different environment. As depicted in Fig. 3,

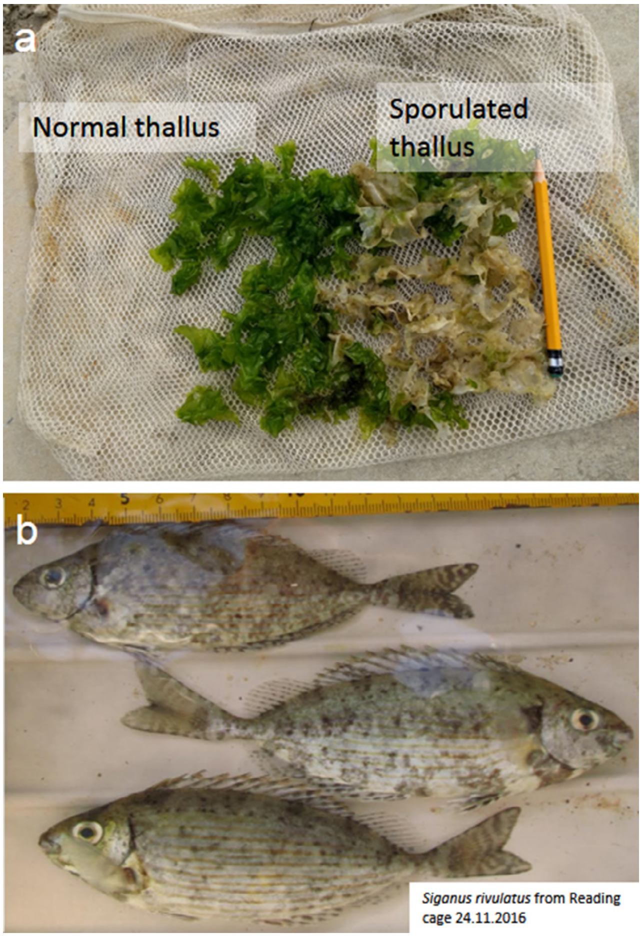

Fig. 3. Biomass losses. (a) Example of rapid *Ulva* sp. thallus sporulation. (b) Grazing fish as observed in the cultivation cages without double net protection.

lighter area represents the thallus parts with released spores. Degradation and disappearance of the biomass will follow this step, induced by various environmental conditions and specific algae stage of life cycle [51–54]. The timing of these biomass fluctuations are still poorly understood and, in contrast to laboratory conditions [55,56], at this point are difficult to control in the offshore open environment. Future studies should test the impact of the initial inoculum weight and sampling frequency on the %DGR.

In addition to the sporulation, additional loss of the biomass could be from grazing: Fig. 3b shows the digital images of *Siganus rivulatus*, species known to include *Ulva* in its diet in the Eastern Mediterranean [57], from the cultivation cage. To avoid grazing by fish, a double net structure of the OS-PBR was used. Attempts to cultivate with a single net led to very high biomass losses most probably because of grazing and crustaceans [58–60].

3.2. The fuel properties of dried Ulva sp. biomass determined by direct combustion

The remaining moisture (RM%) of the dried biomass was 7.07%, and contained 36.02% ash, 1.09% sulfur and 8.53% volatile compounds. The energetic low heating value (LHV) of the dried biomass as fuel was 2697 kcal kg^{-1} (11.29 MJ kg_{DW}^{-1}). Hence, with an average production of 5.8 g_{DW} m^{-2} day^{-1}, *Ulva* biomass can produce 23.9 MJ m^{-2} $year^{-1}$ (0.75 W m^{-2} for year round operation) for direct combustion.

3.3. An estimation of the Ulva biomass potential as a feedstock for transportation biofuels, a case study of Israel, in the Eastern Mediterranean

The conversion efficiency distribution (CED), a function that describes the probability distribution of biomass conversion into ethanol, appears in Fig. 4a. The corresponding annual ethanol production rate (AEPR) and its cumulative distribution function are shown in Fig. 4b and c. The mean AEPR of *Ulva* sp. cultivated at Reading site is 229.5 g Ethanol m^{-2} $year^{-1}$ (which, assuming 25 MJ $kg_{ethanol}$, translates to an energy density of 5.74 MJ m^{-2} $year^{-1}$ and power density of 0.18 W m^{-2}). In comparison, corn bioethanol energy density is 7.2–8.9 MJ m^{-2}, corn stover 3.7 MJ m^{-2}, *Miscanthus* 16.6 MJ m^{-2}, switchgrass 4.8 MJ m^{-2}, and sugar cane 16.1 MJ m^{-2} [73]. However, none of these crops fit the generally arid cultivation areas in Israel or in the East Mediterranean.

Intensification of the growth to the rates observed at the laboratory conditions with the currently reported conversion yields could increase the annual ethanol production efficiency of *Ulva* to 1735 g Ethanol m^{-2} $year^{-1}$, which translates to an energy density of 43.5 MJ m^{-2} $year^{-1}$ and a power density 1.36 W m^{-2}. All of these at 90% confidence with respect to the CED derived from literature.

Israel Government resolutions No. 1354 and No. 2790 support the transition to alternative to petroleum sources, with the goal of reducing the weight of petroleum-based fuels as an energy source for transportation at a rate of approximately 30% by 2020, and by approximately 60% by 2025. According to the Central Bureau of Statistics, the total consumption of transportation fuels in Israel in 2014 was 2797.20 ton, which is ~4,195,800 ton of ethanol for the same energy content. In Fig. 4d we show the total annual requirements for bioethanol to replace different fractions of transportation energy needs that are currently fulfilled by oil. Next, based on our field results of biomass cultivation and computational simulation of the fermentation process, we calculated the required marine area needed for allocation needed for different fractions of bioethanol to come from the offshore cultivated biomass (Fig. 4e). In addition, we calculated the percentage of Israel Exclusive Economic Zone (EEZ, ~26,000 km^2) which these offshore farms should occupy (Fig. 4f, Table 2).

Our models show that, without intensification, to supply 5% of the Israel transportation energy requirement (with 90% confidence) there is a need to cultivate 914 km^2 (4% of EEZ); 10% with 90% confidence would require 1828 km^2 (7% of EEZ); 20% with 90% confidence will require 3656 km^2 (14% of EEZ); 50% with 90% confidence will require 9141 km^2 (35% of EEZ); 60% (as required in the long term by Israeli Government resolutions No.1354 and No. 2790) with 90% confidence will require 10,969 km^2 (42% of EEZ(; and complete replacement of energy for transportation by bioethanol derived from algae will required 18,282 km^2 with 90% confidence (70% of the Israel EEZ).

Area allocation is one the most critical part of the industrial offshore biomass programs development with multiple other stake holders involved [74–76]. We did the sensitivity analysis on the required offshore areas allocation (Fig. 5, Table 3). The required area allocations change with the confidence levels of the conversion processes. To replace 60% of the oil used for transportation fuels with macroalgae derived bioethanol with 99% confidence, 13,285 km^2 will be needed; with 95% confidence, 11,693 km^2 will be needed; with 90% confidence, 10,965 km^2 will be needed; with 80% confidence, 10,180 km^2 will be needed; and with 70% confidence, 9660 km^2 will be needed (Fig. 5a, Table 3). Required areas to displace 5% and 10% of oil for transportation appear in Fig. 5b and Table 3, and 20%, 50% and 100% appear in Fig. 5a and Table 3. In comparison, the total area under agriculture in Israel as for 2015 was 4700 km^2 [77].

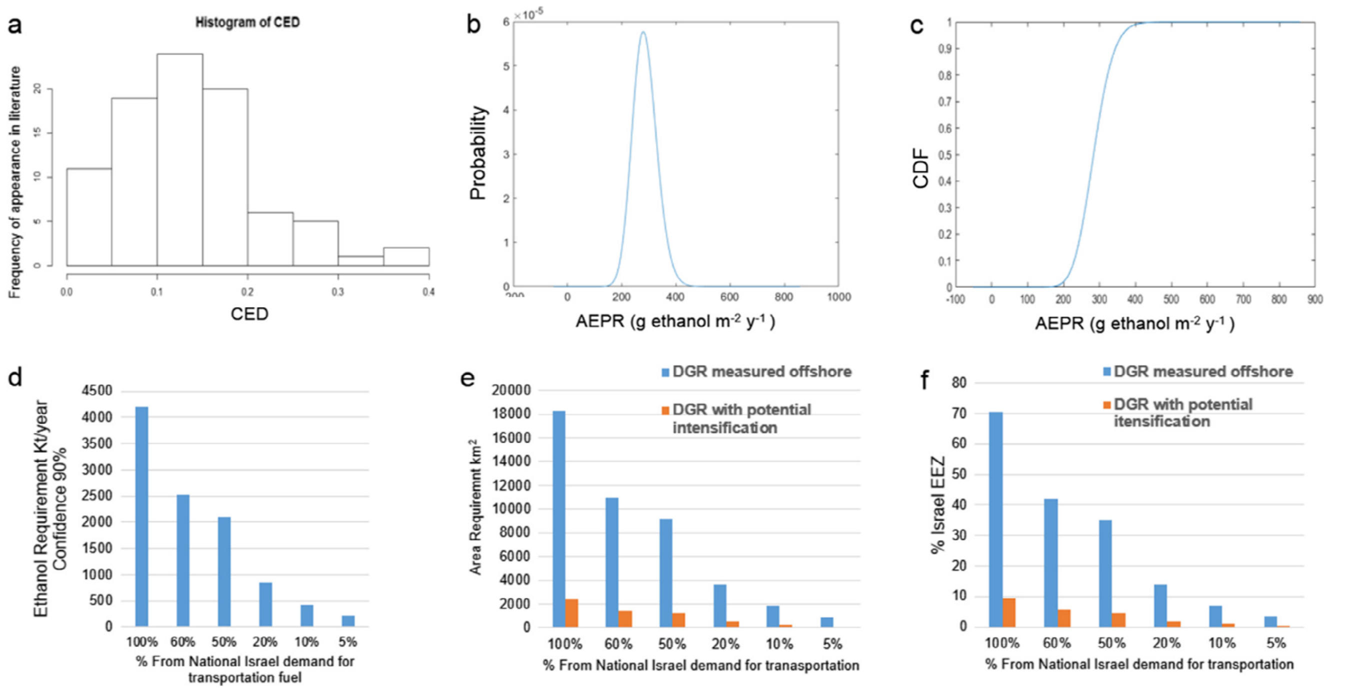

Fig. 4. Bioethanol production estimation from the offshore-cultivated biomass. (a) Conversion efficiency distribution (CED) based on *meta*-data analysis of conversion literature. (b) Annual ethanol production rate (AEPR) probability density function. (c) Annual ethanol production rate (AEPR) cumulative density function; (d) Ethanol requirements (Kton/year) to supply a fraction of Israel transportation fuels. Comparison between areas required for biomass grown in an in a single layer photobioreactor with no intensification as observed in the offshore experiment with biomass grown under nutrients saturation as observed in laboratory experiments (e and f). (e) Offshore area allocation (km^2) requirement to displace oil in transportation in Israel. (f) Fraction of Israel Exclusive Economic Zone (EEZ) required to displace a fraction of oil in transportation in Israel.

Table 2
Bioethanol and Offshore area allocation requirements to replace oil for transportation fuel needs in Israel from macroalgae grown in a single layer photobioreactor with no intensification with 90% confidence.

% From National Israel demand for transportation fuels	Requirement for bioethanol (kton)	Area required to provide biofuel with 90% confidence km^2	% Israel EEZ[a]
100%	4195	18,282	70
60%	2517	10,969	42
50%	2097	9141	35
20%	839	3656	14
10%	419	1828	7
5%	209	914	4

[a] EEZ - exclusive economic zone.

All reported scenario show that significant marine areas should be allocated for biomass production. Allocation of such areas in the coastal or commercial sea areas is problematic because of the numerous other usages such as recreation and transports. Removing the production significantly offshore will be needed. Yet, the environmental conditions in the open sea are different from those in the coastal area. There is a rapid drop in nutrients (NO_3^{1-} and PO_4^{2-}) concentration offshore in Israel [78–80] and the surface water nutrient concentration will not be sufficient for large-scale biomass production.

Importantly, our laboratory experiments show that under nutrient saturation conditions, DGR of 33 ± 6% can be achieved. Under the same initial density, these growing rates, if achieved offshore, could reduce the required areas allocation by 87%. Our modeling results show that with a DGR of 33 ± 6% it would be possible to supply 100% of transportation fuels in Israel by allocating 2418 km^2 (9.2% of EEZ in comparison to 70% with no intensification), 60% would require 1451 km^2 (5.5% of EEZ vs 42% without intensification); 50% would require 1209 km^2 (4.6% of EEZ vs 35% without intensification); 20% would require 484 km^2 (1.85% of EEZ vs 42% without intensification); 10% would require 242 km^2 (0.9% of EEZ vs 7% without intensification) and 5% would require 121 km^2 (0.46% of EEZ vs 4% without intensification). These results clearly demonstrate the need to develop technology for intensification of macroalgae offshore growth. Nonetheless, energy and environmental implications of growth intensification offshore are still unknown.

One possible solution for small scale cultivation could be afforded by integrated multi-trophic aquaculture, which has already been shown in *Ulva* cultivation downstream from offshore installed fish cages [40]. This approach can be useful in the near-future for both increasing the sustainability of offshore fish farms and for developing and testing offshore macroalgae cultivation technologies. However, large-scale cultivation, required for biofuel production, will entail dedicated area with dedicated nutrients supply. One possible large scale fertilization approach can be artificial upwelling. Energetic, environmental and scale up aspects of this approach have been discussed in recent reviews [81,82]. Additional aspects such as energetic cost of transportation and environmental impacts of large scale offshore cultivation have been discussed in [1,15,83,84]. Further monitoring of NPP for annual variation and the development of intensified cultivation techniques [85] and of more efficient carbon utilization of macroalgae derived biomass in the fermented products can decrease the sea area footprint of the offshore biomass used to respond to transportation fuel needs.

3.4. Offshore biomass biofuels potential impact on CO_2 emission reduction on the national level in Israel

Data in Table 3 show that the allocation of areas for offshore biomass cultivation will reduce the fossil fuel derived CO_2 emissions in Israel by 827–16,554 Gg per year (1.5–25%) depending

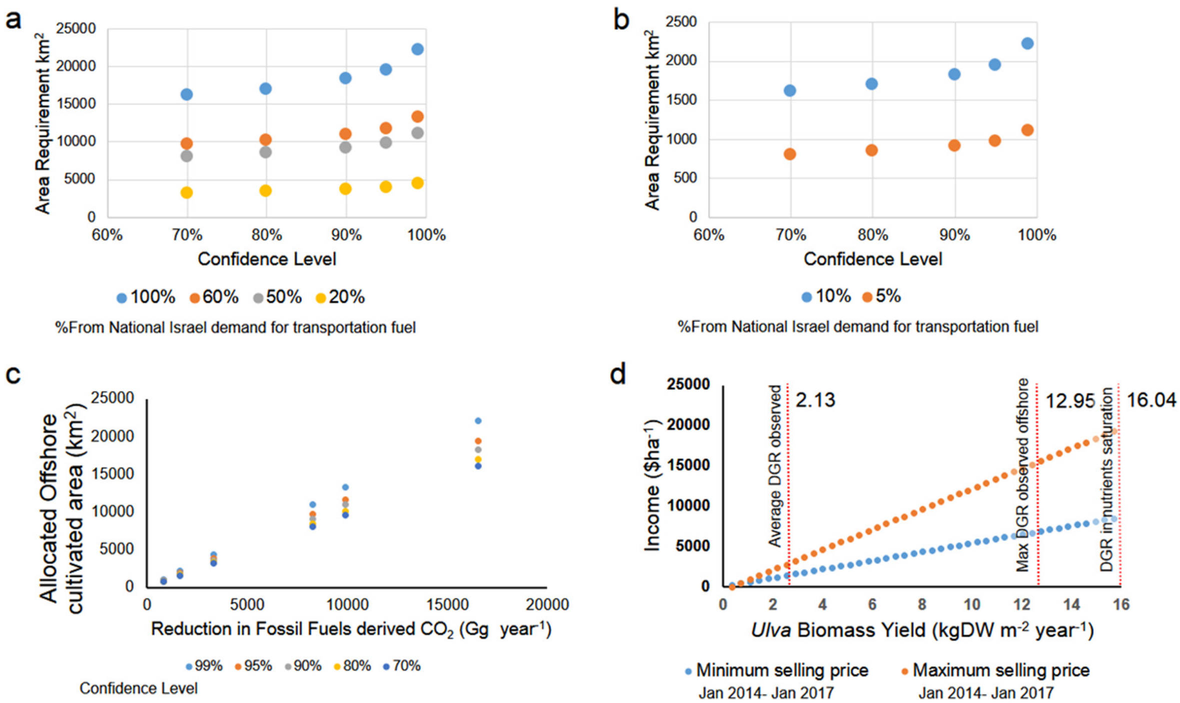

Fig. 5. Allocated offshore areas sensitivity analysis for *Ulva* sp. biomass cultivated in a single layer photobioreactor with no intensification. (a) To supply biomass for bioethanol production to displace 100%, 60%, 50% and 20% of oil used in the transportation sector in Israel. (b) To supply biomass for bioethanol production to displace 5% and 10% of oil used in the transportation sector in Israel. (c) Allocated for offshore cultivation area required to produce biomass for bioethanol to reduce new CO_2 emissions from the fossil fuels on national levels in Israel. (d) Estimated annual income from the production of bioethanol derived from offshore cultivated macroalgae.

Table 3
Sensitivity analysis of the required offshore area allocation for macroalgae production in a single layer photobioreactor with no intensification for bioethanol.

% From National Israel demand for transportation fuels	Potential reduction in fossil fuels CO_2 emission (Gg) (%National total in 2013)[a]	Requirement for bioethanol (kton)	Area requirements for confidence of AEPR				
			99%	95%	90%	80%	70%
AEPR (g Ethanol m^{-2} $year^{-1}$)			189.5	215.3	229.5	247.3	260.6
100%	16,554 (25%)	4195	22,141	19,488	18,282	16,966	16,101
60%	9932 (15%)	2517	13,285	11,693	10,969	10,180	9660
50%	8277 (12%)	2097	11,071	9744	9141	84,83	8050
20%	3310 (4.9%)	839	4428	3898	3656	33,93	3220
10%	1655 (2.5%)	419	2214	1949	1828	1,97	1610
5%	827 (1.5%)	209	1107	974	914	848	805

[a] Israeli Central Bureau of Statistics. Retrieved from: http://unfccc.int/essential_background/library/items/3599.php?rec=j&priref=7667#beg.

on the size of the allocated area. According to the Israel Ministry of Environmental Protection, the national Israel GHG emission reduction target for 2030 is 26% from the emissions in 2005 (total 64,334 Gg CO_2). This reduction is equal to 16,726 Gg CO_2 emission reduction.

Our experimental data and simulations show that offshore cultivated biomass for transportation bioethanol could contribute to the national targets of fossil fuel derived CO_2 emissions. Reduction of 26% from the emission of CO_2 in 2005 by the production of transportation bioethanol from the offshore cultivated macroalgae (with 90% confidence) would require allocation of 18,475 km^2 (or 71% of national EEZ). The sensitivity analysis for the amount of CO_2 (Gg) from fossil fuels that can be reduced by allocating offshore areas for macroalgae biomass cultivation for bioethanol production appears in Fig. 5c.

3.5. Preliminary economic analysis and costs requirements for offshore derived biomass for bioethanol

A critical part of the biofuel resource assessment is economics. The problem is that large offshore farms for the biomass for biofuel production do not yet exist. The information about the investments in the pilot scale systems is also scarce and species specific [86,87]. With the market price of \$0.39 per liter (January 2017) to \$0.91 per liter (March 2014), production of bioethanol form *Ulva* is costal area of Israel (229.5 g Ethanol m^{-2} $year^{-1}$ with 90% confidence) will lead to the income of \$0.11 m^{-2} $year^{-1}$ (\$1150 ha^{-1} $year^{-1}$) to \$0.26 m^{-2} $year^{-1}$ (\$2615 ha^{-1} $year^{-1}$). These market prices also sets the top limit for the biomass costs and farm investment. With the average productivity of ($\sim$5.8 g_{DW} m^{-2} day^{-1} or 2125 g_{DW} m^{-2} $year^{-1}$), the maximum cost of the biomass and its processing to maintain the breakeven is $\sim$\$54–\$123 ton^{-1}. Previous studies on the engineering economic of lignocellulosics bioethanol refineries showed that cost of raw material (*f* in Eq. (5)) is $\sim$30% of the final bioethanol cost [87,88]. Therefore, the maximum cost of the biomass should be at \$16–\$37 ton^{-1} (for AEPR 229.5 g Ethanol m^{-2} $year^{-1}$), which is at the low end of the current costs of lignocellulosic biomass (\$30–\$100 ton^{-1} ex-biorefinery) [86,87]. The sensitivity analysis of the required costs for the biomass and potential incomes from the offshore cultivated areas, with the reported until now conversion efficiencies, appear in Table 4. The prices for macroalgae ex-farm in Asia, world largest producing region, are at \$230–770 ton^{-1} [89,90].

Table 4
Sensitivity analysis of the income and required biomass costs with the reported *Ulva* biomass to bioethanol conversion efficiencies.

Confidence of AEPR	99%	95%	90%	80%	70%
AEPR (g Ethanol m^{-2} $year^{-1}$)	189.5	215.3	229.5	247.3	260.6
Low income ($0.34 lit^{-1} selling price) $ ha^{-1}	960	1091	1163	1253	1321
High income ($0.91 lit^{-1} selling price) $ ha^{-1}	2183	2480	2643	2848	3002
$C_{DW\_biomass}$ $ ton^{-1} (Low)	14	15	16	18	19
$C_{DW\_biomass}$ $ ton^{-1} (High)	31	35	37	40	42

The sensitivity analysis for potential income as a function of observed yeals in this study appear in Fig. 5d. Increasing the yields from the average observed (5.8 g_{DW} m^{-2} day^{-1}) to the maximum observed in the offshore cultivation system (35.49 g_{DW} m^{-2} day^{-1}) could increase the income from bioethanol sales to $6883–$16,060 ha^{-1}. Intensification to the DGR observed at the laboratory system would lead to 43.95 g_{DW} m^{-2} day^{-1} (with 133 g_{DW} m^{-2} initial cultivation density), potentially increasing the income from bioethanol sales to $8625–$20,190 ha^{-1}). Development of the technology for growth intensification offshore [85], as has been shown on-shore [3] is a major challenge for the future of this new branch of bioenergy production.

3.6. Biorefinery potential of offshore cultivation macroalgae

Because of the current low prices for fuel energy, biofuels would generate the lowest income per biomass conversion to stay economically viable. Producing low cost biomass would require complex automation and tremendous scale up. To enable scale up and technology development offshore biomass farms should provide additional sources of income. The approach of producing several co-products from the same biomass, with differences market prices, is known as biorefinery. Although discussed frequently in literature, de facto actual experimental reports of macroalgae biorefinery are rare. In a recent paper on *Ulva* biorefinery, the experimental approach to co-produce mineral rich liquid extract (MRLE), lipid, ulvan, and cellulose was reported [91]. This work suggests that one ton of fresh *Ulva* biomass could give approximately 37 kg of MRLE, 3.8 kg of lipid, 34.6 kg of ulvan and 14.0 kg of cellulose (5.85 kg ethanol if fermented) on a DW basis. An additional recent study on *Ulva* biorefinery has shown the co-production of protein rich extract that can be used as animal feed and production of acetone, butanol, ethanol and 1,2-propanediol by clostridial fermentation from hydrolysates [92]. The scalability of these processes and their economic viability are still to be determined.

3.7. Social-economic potential of offshore macroalgae biorefinery for Eastern Mediterranean countries

Development of offshore macroalgae biorefinery in Israel with its high labor costs provides new directions for the workforce development. First, offshore biomass production could provide an income for fisherman, as their income is under pressure due to governmental regulations and global overfishing in the Mediterranean Sea [93]. The development of offshore biorefinery could also develop a new research-industrial sector for the growing number of life-sciences graduate students (~22% of all PhD students, according to the Central Bureau of Statistics in Israel) in the region.

4. Conclusions

In coastal areas with scarce arable land and freshwater for irrigation, marine offshore production of biomass can provide a solution to facilitate transition from fossil fuel to a sustainable bioeconomy. We measured the NPP of *Ulva* sp. biomass grown in the coastal area of Israel and estimated its potential to provide for bioethanol for the transportation sector in Israel. Our results show that *Ulva* sp. NPP, 838 g C m^{-2} $year^{-1}$, falls within the high range of other biofuel crops. Yet, the area of sea needed for the cultivation of the biomass to provide 60% of Israel transportation needs (as of 2014) is between 9660 and 13,285 km^2. This area represents 62–85% of the Israel EEZ. Substitution of 10% of oil for transportation sector by bioethanol derived from offshore grown macroalgae will require 1610–2214 km^2, or 6–9% of EEZ. Reduction of 26% from the emission of CO_2 in 2005 by the production of transportation bioethanol from the offshore cultivated macroalgae (with 90% confidence) would require the allocation of 18,475 km^2 with growing rates achieved in this study with no intensification offshore. Importantly, cultivation intensification to the growth rates observed in laboratory could reduce the required areas allocations by 87%. Development of additional technologies, such as artificial upwelling for offshore fertilization or deep-water bioreactor for natural fertilization and artificial lighting, is required for a large-scale offshore biomass production. Future technologies should provide the biomass at $14–$42 ton^{-1} to enable economic viability of the offshore bioenergy project.

Contributions

AC design the offshore reactor. AC and AG performed the offshore growth rates measurements. OH, AC, AL and AG designed the closed bioreactor, OH built the reactor and did the measurements. AI grew the initial biomass inoculum for all experiments. ZY and AG developed the ethanol conversion model. All authors contributed to design of the research, the discussion of the data and the writing of the paper.

Conflict of interest

The authors declare no competing financial interests.

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Corrigendum

Corrigendum to "Net primary productivity, biofuel production and CO_2 emissions reduction potential of *Ulva* sp. (Chlorophyta) biomass in a coastal area of the Eastern Mediterranean" [Energy Convers. Manage. 148 (2017) 1497–1507]

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The authors regret about the mistake we found in the sentence (page 1500, section 3.1) "The highest DGRs (75–87%) were observed in March–April". The actual highest DGRs were observed in March–April (17.6–19.9%).

The authors would like to apologise for any inconvenience caused.

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