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Energy efficient dewatering of far offshore grown green macroalgae *Ulva* sp. biomass with pulsed electric fields and mechanical press

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ABSTRACT

Offshore macroalgae biomass production is a promising, yet challenging, pathway to provide feedstock for biorefineries. In this work, a device and a process for dewatering offshore grown biomass of the green macroalgae *Ulva* sp. using high-voltage pulsed electric fields (PEF) was developed. *Ulva* sp. was cultivated attached to fish cages 15 km offshore. Increasing the applied voltage from 250 V to 500 V and invested PEF energy from 9.3 ± 0.4 J g^{-1} FW to 54.6 ± 0.2Jg $^{-1}$ FW increased the extracted water from 0.033 ± 0.006 g Water g^{-1} FW to 0.150 ± 0.031 g Water g^{-1} FW. The energy consumption to achieve similar moisture content with air convection drying was lower by 78.73 ± 10.41 (JgFW $^{-1}$) for 250 V and 339.31 ± 48.01 (JgFW $^{-1}$) for 500 V, pulse duration 50 µs, pulse number 50, pulse repetition frequency 3 Hz. PEF leads to biomass compression of 8.45 ± 1.72% for 250 V protocol and 25.66 ± 2.53% for 500 V protocol. In addition, PEF leads to the reduction of water diffusivity of 18–19% in the treated biomass, reducing air drying kinetics.

1. Introduction

Global population growth in the era of changing climate will increase the demand for food, chemicals, and fuels. A possible, sustainable direction for addressing this challenge is the production of macroalgae biomass offshore- Seagriculture (Roesijadi et al., 2010; Wargacki et al., 2012; Yun et al., 2015). Seagriculure could provide a sustainable feedstock for biorefineries, for the production of food, chemicals, and fuels without competition with food crops for arable land or potable water. For example, using a metabolism and growth rate model of the green marine macroalga *Ulva*, coupled with essential inputs from climatological oceanographic data, we showed that offshore cultivation of macroalgae has the potential to provide some of the basic products required for human society in the coming decades (Lehahn et al., 2016). For example, this includes displacing 20% of the used fossil fuels in the transportation sector and providing for 100% of the predicted demand for ethanol, acetone, and butanol, and 5–24% of the demand for proteins (Lehahn et al., 2016).

Nevertheless, offshore production and processing of the biomass, in the high-energy environment is challenging. Thus, to date macroalgae still present only a tiny percent of the global biomass supply of ~$17{\cdot}10^{6}$ fresh weight (FW) ton of macroalgae in comparison to $16{\cdot}10^{11}$ tons of terrestrial crops, grasses and forests (Pimentel, 2012; Pimentel and Pimentel, 2008; Roesijadi et al., 2010). The current approaches for offshore seaweed cultivation include near farm concepts for kelp cultivation (Bird, 1987), tidal flat farms, floating cultivation (Bird, 1987), ring cultivation (Bak et al., 2018; Buck and Buchholz, 2004), wind-farm integrated systems ("Marine biomass from offshore wind parks. http://www.submariner-project.eu/index.php?option=com_content&view=-article&id=159:marine-biomass-from-offshore-wind-parks&catid=62:regionalactivitiesdenmark&Itemid=402," n.d.) and intensified with mixing and aeration cages. Yet, the most recent analysis shows that there are no efficient harvesting and storage methods for large scale seaweed production offshore (Nilsen, 2018). Studies on the agricultural processing systems (French, 1960) and seaweed biorefinery energy efficiency analysis (Golberg et al., 2014) show that feedstock transportation costs limit the distance of the cultivation site to the processing facility. Analyzing the energy expenses required for transportation of seaweeds from remote farms, we found that the distance of the large-sale offshore cultivation site to the processing facility is limited to 114–689 km when lower water content allowed for longer transportation distances. Thus, technologies for rapid offshore

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dehydration are needed (Golberg et al., 2014; Nilsen, 2018). However, the technology for rapid seaweed biomass dehydration is not yet available.

The goal of this work is to develop a device and a process for dewatering offshore grown macroalgal biomass using high-voltage pulsed electric fields (PEF) coupled to the mechanical pressing. PEF is an emerging, non-thermal method used in the food industry for food disinfection, enzyme activity modification and molecules extraction from the biomass. The PEF process uses high-voltage short-duration electric fields for modifying biological membrane permeability. Application of PEF as an intensification method of the extraction has been widely used in the processing of fruits and vegetables (Bodenes, 2017). In the recent work it was shown that PEF can be used for the selective extraction of proteins from *Ulva* sp. (Polikovsky et al., 2019, 2016) biomass. In addition, it was shown that PEF can be used for seaweed biomass deashing (Robin et al., 2018). Extraction of water-soluble proteins and carbohydrates from macroalgae using PEF was also demonstrated by other authors (Postma et al., 2017). Although used for dewatering of cassettes in the sugar beets processing (Almohammed et al., 2015; Sack et al., 2010) and energy-efficient dehydration of green biomass (Sack et al., 2009; Vorobiev and Lebovka, 2008) to the best of our knowledge PEF coupled to mechanical pressing has not been used for seaweed processing. As PEF technology is scalable (Golberg et al., 2016; Sack et al., 2010), it could address the needs to seaweed dehydration in offshore farming.

This work specifically examined the application of PEF for dewatering of *Ulva* sp. biomass, which is a potential feedstock for marine biorefineries (Glasson et al., 2017; Postma et al., 2017). PEF coupled with mechanical pressing can partially dewater and compress *Ulva* sp. biomass, providing an essential tool for the development of offshore Seagriculture, particularly in offshore farms as dragging wet seaweed biomass to the shore for processing to energy and food does not make energetic basic and leads to biomass quality loss.

2. Materials and methods

2.1. Biomass inoculum production

The model seaweed used in this study belongs to the genus *Ulva* sp., green seaweeds of worldwide distribution commonly found within the Israeli Mediterranean Sea intertidal zone. 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* (Krupnik et al., 2018), and are referred as *Ulva* spp. in this study.

2.2. Ulva spp. Offshore cultivation coupled with the open-sea fish farm

Offshore cultivation was conducted by attaching the cages with *Ulva* spp. biomass to the commercial fish cages farm installed 15 km west of Ashdod port, Israel. The detailed description of the single point mooring submergible technology appears in refs (Drimer, 2019; Milich and Drimer, 2019). The depth of farm installation (seabed depth): 73–82 m. Type of soil- dirt. Streams: 70% of the time North, 30% of the time South. The farm has a single point mooring and that it the way it always rotates to the direction of the stream. The farm can be submerged to the depth of 42 meters (top part of the cage). During the seaweed cultivation period, the farm produced gilt-head (sea) bream (*Sparus aurata*). From the 8 cages, cages #1 and #8 were empty (Fig. 1), cage#2 contained 400,000 fish till 1 June and 200,000 fish till 25 August, consuming 110 ton of feed from March to August. Cages #3 and #4 contained 430,000 fish and consumed 180 ton and 152 ton during the cultivation period, Cage#5 contained 510,000 fish and consumed 30 ton of feed during the seaweed cultivation period, Cage#7 had 500,000 fish from June 20 and consumed 9.7 ton of feed till August 25. The fish composition was 45% protein and 18–22% lipid for cages #2,3,4,5 and 50% protein and 18–22% lipid for cages #6,7. A 2 cm layer of *Ulva* spp. thalli were placed between two layers of polypropylene tubular nets (TENAX, Gallo Plastik, Italy) to allow for full illumination and prevent grazing. The seaweed cages were attached to the fish cages at 6 locations at 0.5 m, 5 m, and 10 m depths. The initial load of the biomass was done by an inoculum grown in the laboratory photobioreactor as described below. At the following points the initial load was done with the biomass from stocks grown in the cages offshore.

2.3. Ulva spp. Biomass maintenance in the photobioreactor.

Ulva spp. biomass grown offshore was kept under controlled conditions using 40 L macroalgae photobioreactors (MPBR) incorporated in a building's south wall under daylight conditions in a system described in ref (Chemodanov et al., 2017b). Nutrients were supplied by adding ammonium nitrate (NH_4NO_3) and phosphoric acid (H_3PO_4), (Haifa Chemicals Ltd, IS) to maintain 6.4 g m^{-3} of total nitrogen and 0.97 g m^{-3} of total phosphorus in the seawater. The sole CO_2 source was bubbled air. Other conditions such as pH (8.2), salinity, and airflow rate (2–4 L min^{-1}) were maintained steady in all the reactors. The surface water was removed from the harvested biomass with a standard protocol by centrifuging the algal biomass in an electric centrifuge (Spin Dryer CE-88 (6.0 kg) 2800RPM Stainless Steel Housing, Beswin, China) until all surface water was removed (< 1 mL separated).

2.4. Pulsed electric field coupled to mechanical press for seaweed biomass electroporation and dewatering

A custom made pulsed electric field generator was developed for seaweed biomass PEF treatment. The generator provides at a maximum voltage of 1000 V and current of 120 A at the 5 Ohm load. The maximum pulse duration, the number of pulses and pulse frequencies are limited by the permissible heating of the IGBT transistors. In our system, described below, for 5 Ohm load and 1 Hz pulse repetition rate the maximum pulse duration is 100 μs. The functional circuit diagram of the developed pulsed generator is shown in Fig. 3. The main functional nodes of the system include: 1) energy storage capacitor (ESC) with a capacity of 50 μF for voltage 1.25 kV; 2) high-voltage source of charge of energy storage capacitors (CCM1KW (Spellman, NY)); 3) parallel-connected high-voltage switches for pulsed discharge of ESCs (IXYN120N120C3 (IXYS, CA) with parameters of 1200 volts, 120 A; 3) driver of high-voltage switch with electrical circuits of control of transistors gates and own power supply (Gate Driver Optocoupler FOD3184 (Fairchild, CA)); 4) high-power current-limiting resistors (RR02- 3 OHM-2 W); 5) circuit node for manual control of high-voltage switch and high-voltage power supply in testing mode; 6) microcontroller for controlling the process of PEF treatment, calculating the current at the treated biomass, and transferring the results of calculations to the computer for writing to the experiment file; 7) low-voltage power supply for control circuits and fans of the device. The device is connected by a USB interface to a computer for input the experiment parameters in the microcontroller, displaying the current state of the process and record the received data in the experiment file. Currents were measured using a PicoScope 4224 Oscilloscope with a Pico Current Clamp (60A AC/DC), (Pico Technologies Inc., UK). The voltage was measured with PicoScope TA044 70 MHz 7000 V differential oscilloscope probe 100:1/1000:1. Currents and voltages were analyzed with Pico Scope 6 software (Pico Technologies Inc., UK).

The gravitational press-electrode device (diameter 2.5 cm), for the separation of solid and liquid phases during electroporation, is shown in Fig. 3b. A load weighing up to 10 kg can be placed on the load-receiving platform connected to the sliding electrode to create the necessary inter-electrode pressure on the biomass (Fig. 3b). A displacement sensor (optoNCDT, Micro-Epsilon, NC), Fig. 3b is used to monitor the volume change of the biomass during electroporation. For continuous liquid extraction, narrow slit-like openings were made in the lateral part of the

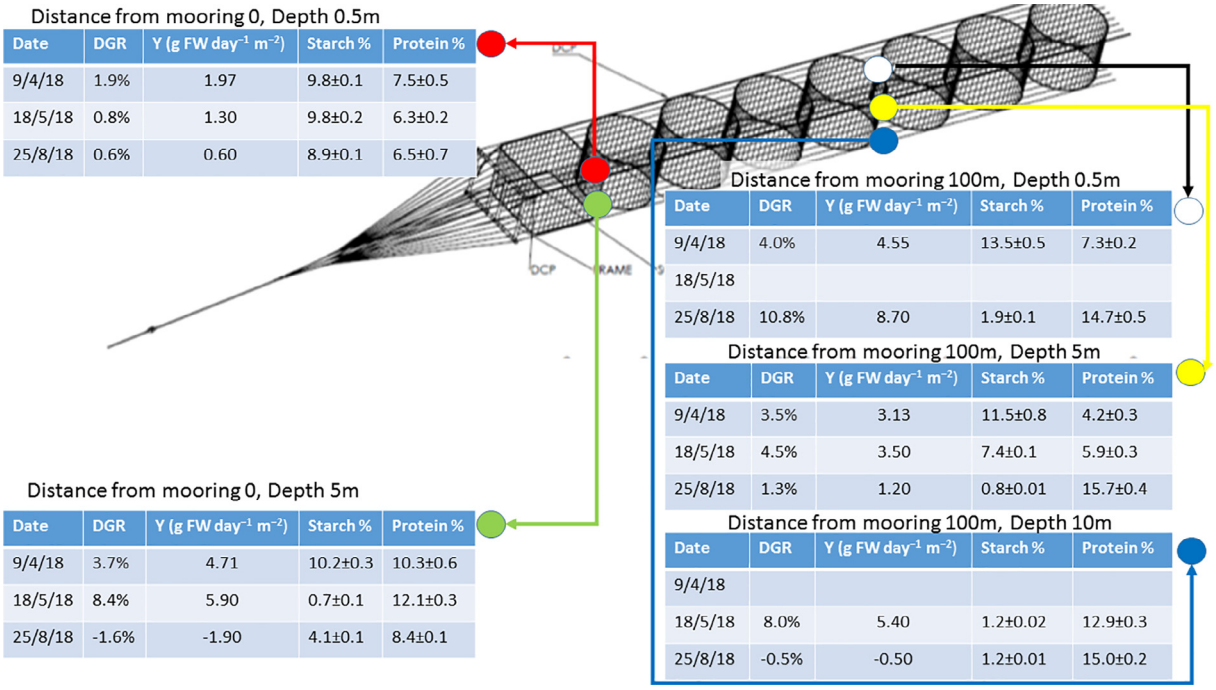

Fig. 1. *Ulva* spp. cultivation far offshore attached to the fish farm. Fish farm design, details appear in refs (Drimer, 2019; Milich and Drimer, 2019). Positions of seaweed cages in relation to fish cages are shown. *Ulva* biomass growth and biochemical composition are shown.

electroporation cell. The extracted liquid is collected and discharge through a groove at the base of the cell (Fig. 3b).

For PEF treatment, 1 g of the centrifuged to constant fresh weight (FW) biomass was loaded in the chamber and PEF were applied. The used protocols were 250 V and 500 V, pulse duration 50 µs, pulse number 50, pulse repetition frequency 3 Hz. The currents and the distance between the electrodes were measured continuously as described above. Three repetitions were conducted for each of the experimental settings.

The *Ulva* biomass volume compression (%) after PEF treatment was calculated with Eq. (1)

$$\%compression = 100\% \bullet \frac{U_0 - U_{PEF}}{U_0} \quad (1)$$

where U_0 (mm^3) is the initial volume of the biomass in the electroporation chamber before PEF and UPEF (mm^3) is the volume of the biomass in the chamber after PEF. For controls, the biomass was in the chamber for 1 min under the same pressure as the PEF treated biomass.

The energy used (J_{in} (Joule) for Ulva biomass treatment with PEF was calculated with Eq. (2)

$$J_{in} = \sum_{i=1}^{i=N} V \bullet I_i \bullet t_p \quad (2)$$

where V (V) is the applied voltage, I_i (A) is the measured current for each pulse, t_p(µs) is the pulse duration and N is the total number of pulses.

The energy saved on evaporation (J_s) by Ulva biomass dewatering with PEF coupled to mechanical press in comparison with evaporation was calculated with Eq. (3)

$$J_s = c_p^{water} \Delta M \Delta T + L_{vap} \Delta M - J_{in} \quad (3)$$

where c_p (4.2 J g^{-1}) is the specific heat capacity of water, ΔM (g) is the mass of removed by PEF and press water, ΔT (°C) is the temperature difference between room temperature and evaporation temperature (25–100 °C in our example), L_{vap} (2300 J g^{-1}) is the latent heat of water vaporization.

2.5. Biomass drying

Moisture and DW content was determined by drying the sample at 105 °C for 12.5 min (till all moisture was removed), using moisture analyzer (BM-50-5, Biobase Biodustry (Shandong) Co. Ltd., China). Records for total weight (g), solid content (%) and moisture content (%) were taken every 30 sec.

Assuming that the tissue is isotropic with respect to water transport, water diffusivity in seaweeds can be described with the Fick's second law of diffusion (Eq. (4)):

$$\frac{dw}{dt} = \nabla D \nabla w \quad (4)$$

where D ($m^2\ s^{-1}$) is the diffusivity of water in the sample, t (s) is drying time, and w is the dimensionless moister content calculated as in Eq. (5)

$$w = \frac{M(t) - M_e}{M_0 - M_e} \quad (5)$$

where $M(t)$ is the moisture content at drying time t(s), M_e is the moisture content at equilibrium and M_o is the initial moisture content.

Under the assumption of the equal distribution, negligible external resistance, constant diffusivity, and negligible shrinkage through the drying process, the solution for the seaweed slab is given by Eq. (6) (Crank, 1975) validated in the food drying field (Kechaou and Maalej, 2000; Zogzas and Maroulis, 1996) and used as a first approximation in multiple studies on PEF assisted drying (Adedeji et al., 2008; Amami et al., 2008; Lebovka et al., 2007):

$$w(D_{eff}, t, l) = \frac{8}{\pi^2} \sum_{i=0}^{\infty} \frac{1}{(2i+1)^2} \exp\left(-\frac{(2i+1)^2 \pi^2 D_{eff} t}{4l^2}\right) \quad (6)$$

where l (m) is the half-thickness of the infinite slab and D_{eff} is the empirical parameter that characterizes the drying rate (Lebovka et al., 2007).

2.6. Biochemical characterization of the biomass

2.6.1. Determination of starch concentrations

Starch concentrations were measured by using a K-TSTA-100A total starch assay kit (Megazyme, Ireland) as described before (Prabhu et al., 2019a). Briefly, dry biomass (at 40 °C) powder was taken (10 mg sample (n = 3)) in 2 ml tubes and washed twice in 500 µl, 80% (v/v) ethanol to remove any glucose present. Two hundred microliters of 2 M potassium hydroxide (KOH) were then added and the tubes were shaken horizontally for 30 min at 37 °C and 150 rpm. The mixture was further incubated at 100 °C for 1 min to completely dissolve the starch. Tubes were short spun for 1 min at 23 °C and sodium acetate buffer (800 µl, 1.2 M, pH 3.8) was added. Immediately, 10 µl α-amylase, 10 µl amyloglucosidase were added and mixed using a vortex mixer. The mixture was shaken for 2 h at 50 °C and 150 rpm. The tubes were then centrifuged at 1800*g* for 10 min (Eppendorf centrifuge 5424, Hamburg). The glucose released was measured at 510 nm, by reacting 0.01 ml supernatant with 0.3 ml glucose oxidase-peroxidase (GODPOD) enzyme mixture for 20 min. Starch concentration, as a percentage of the DM, was calculated with the molar mass conversion from glucose to anyhydroglucose (the starch monomer unit) of 0.9.

2.6.2. Determination of protein concentration

Total protein content in the *Ulva* spp. biomass was analyzed using a modified version of the Lowry method (Lowry et al., 1951). Biomass dried at 40 °C was accurately weighed (~15 mg) in 2 ml tubes and filled to one-third with beads (zirconia, 2 mm, Sarstedt) and 1.5 ml of 2 M sodium hydroxide (NaOH) solution. The tubes were run through 3 sessions of bead beating of 60 sec each, in a bead beater (Biospec (Ok, USA) with intermittent cooling for 10 minutes at 23 °C. The tubes were then centrifuged at 14,000 rpm for 20 min. The supernatants from all the tubes were appropriately diluted with ultrapure water. Diluted samples were analyzed by adding 100 µL in a well of a 96-well plate. Biuret reagent was prepared by mixing 0.5 ml of 1% cupric sulfate with 0.5 ml of 2% sodium potassium tartrate, followed by the addition of 50 ml of 2 % sodium carbonate in 0.1 N NaOH. Two hundred microliters of biuret reagent were added to each well and mixed thoroughly using a micropipette. The mixture was then equilibrated at 23 °C for 10 minutes prior to the addition of 20 µL per well of 1.0 N Folin & Ciocalteu's reagent. Samples were mixed immediately by repeated pipetting following each addition. The color was allowed to develop for 30 minutes at 23 °C, following which, absorbance was measured at 750 nm using a spectrophotometer (Infinite 200 Pro, TECAN, Switzerland). A Standard curve was produced using bovine serum albumin (BSA) at different concentrations (0–500 µg/mL). As a blank, water was used in place of the sample. Analyses were done in triplicate and the results were expressed as BSA equivalent in mg/L.

2.6.3. Elemental CHNS analysis

CHNS Elemental analysis was performed using Flash 2000 Organic elemental analyzer (Thermo Scientific). Sample, 2–3 mg was weighed along with 8–10 mg of vanadium in tin crucible. Combustion Temperature was 950 °C, and carrier gas was helium (99.999%, flow rate of 140 ml min^{-1}) with addition of O_2 at 250 ml min^{-1} for 5 sec. Cyctine, BBOT, Sulphanilamide, Methionine were used as standards.

2.7. Statistical analysis

Statistical analysis was performed using R-studio, fitdistrplus, ggplot2 and dplyr packages (RStudio: an Integrated development environment for R (Version 1.1.383) [Windows]. Boston, MA) and Matlab (MathWorks, MA).

2.7.1. Comparison of drying kinetics of PEF dewatered seaweed

The comparison between the drying kinetics of seaweed biomass treated with different protocols was performed in two steps. In the first step, we performed a point-wise comparison by a two-tailed Student T-Test method of the dimensionless moisture content at each time point during drying. In the second stage, we combined all the drying time point-based statistics into a single p-value according to Fisher's combined probability test (Fisher, 1932) (Eq. (7)):

$$\chi^2 = -2\sum_{t=1}^{T} ln[p_t] \tag{7}$$

where t is the time point during drying for each comparison sub-set ($T = 26$ time points during drying: 30–13 min with intervals of 30 s) and p is the 2-tail p-value statistics of the test. The resulting score is of χ^2 distribution with $2T = 16$ degrees of freedom.

2.7.2. Estimation of the diffusion coefficient

The diffusion coefficient D_{eff} was estimated separately for each experiment as an arithmetic average of three different numerical approximation approaches (Eq. (8), which minimize (i) the Mean Square Error (MSE, Eq. (9)); (ii) the Mean Absolute Error (MAE, Eq. (10)); and (iii) the Mean Relative Error (MRE, Eq. (11)) between the measured and the predicted dimensionless moisture content, *w,* across the experimental

replicate time points

$$Total_{D_{eff}} = \frac{1}{3}(MSE_{D_{eff}} + MAE_{D_{eff}} + MRE_{D_{eff}}) \tag{8}$$

where:

$$MSE_{D_{eff}} = \underset{D_{eff}}{argmin}\left\{\frac{1}{T}\sum_{t=0}^{T}(w_t^{measured} - w_t^{predicted}(D_{eff}, l, t))^2\right\} \tag{9}$$

$$MAE_{D_{eff}} = \underset{D_{eff}}{argmin}\left\{\frac{1}{T}\sum_{t=0}^{T}|w_t^{measured} - w_t^{predicted}(D_{eff}, l, t)|\right\} \tag{10}$$

$$MRE_{D_{eff}} = \underset{D_{eff}}{argmin}\left\{\frac{1}{T}\sum_{t=0}^{T-1}\left|\frac{w_t^{measured} - w_t^{predicted}(D_{eff}, l, t)}{w_t^{measured}}\right|\right\} \tag{11}$$

where t is the measurement time point id; $T = 26$ is the total number of measurements and time, t is the measurement time in seconds. The $w_t^{measured}$ refers to the measured, normalized dimensionless moisture content, which is always equal to 1.00 at the $t[0] = 0$ sec and equal to 0.00 at the $t[T] = 12.5$ min. The $w_t^{predicted}(D_{eff}, h, t)$ refers to the predicted dimensionless moister content calculated with Eq. (6) using the predicted value of D_{eff}, measurement t and l, which is the half-thickness of the infinite slab in meters.

A single-number estimation of the diffusion coefficient D_{eff} for each experimental setup (control, 250 V, 500 V) was performed in a two-step manner. First, a median value of moisture content was calculated per each time-point in the experimental setup. Then, total diffusion coefficient was calculated as in Eq. (8) based on these median values.

3. Results and discussion

3.1. Far offshore grown biomass growth rates and yields

Our experimental design allowed us to verify the impact of the season (Date), the cultivation depth (Depth) and the seaweed cage location in relation to the single point mooring of the fish farm (Distance) on Daily Growth Rate (DGR, in %), Yield (gFW m^{-2} d^{-1}) and chemical composition, such as starch content, protein content and elementary composition (CHNS), Fig. 1.

Importantly, we achieve positive DGR (mean 3.47%, median 3.5%, standard error (SE) 0.99%) and Yield (mean 2.82, median 3.13, SE 0.79 gFW m^{-2} d^{-1} were both positive for the whole cultivation period (Fig. 1). Individually, Date and Depth did not have significant impact of the DGR and yields. In addition, we also found that in this specific

experiment the Distance from mooring did not impact the DGR and Yield alone or in combination with Date and Depth. This could be because of the continuous rotation of the farm, which could cause to local mix of nutrients. However, we found that cultivation Date and Depth combined affected significantly the DGR ($p = 0.02$) and yields ($p = 0.01$) of Ulva biomass. Date and Depth predicate the essential for biomass parameters: light and temperature.

The highest DGR (10.8%) and yield (8.7 gFW m^{-2} d^{-1}) were measured on 25 August at 10 m depth at 100 m distance from the mooring point and the lowest DGR (−1.6%) and yield (−1.9 gFW m^{-2} d^{-1}) were measured on 25 August at 5 m depth at 0 m from the mooring point, probably because of sporulation of biomass washout. These results, which show a net positive growth in April-August in the far offshore, are completely different from our previous near-shore data, obtained for two years of *Ulva* cultivation in the shallow sea waters near Tel Aviv, Israel, where no growth was observed in June, July, and August (Chemodanov et al., 2019, 2017a). In this study, we show that moving the cultivation 15 km from the shore could enable year-round production of the biomass once the technology for large-scale cultivation in these waters is available. Additional previous studies on Ulva cultivation near stationary, not rotating like in our case, fish cages located 3–4 km offshore in August 2012 reported up to 17% specific daily growth rates in downstream from fish cages (Korzen et al., 2015a). Additional work in the same location in September-December 2013 reported up to 13% specific daily growth rates downstream the stationary fish cages (Korzen et al., 2015b). In both these studies, no growth was observed upstream the cages. Future detailed studies should address the fundamental differences in conditions between near-shore and far offshore production and the impact of fish farms design and dynamics on seaweed productivity.

3.2. Offshore grown biomass starch, protein, and elemental composition

The starch content of the biomass varied from 0.7% to 13.5% (mean 6.05%, median 7.5%, SE 0.64%) during the entire cultivation period. The lowest value was measured on 18 May at 5 m depth at Distance 0 m and the highest value was measured on 9 April at 0.5 m depth at a Distance of 100 m from a mooring. We found that cultivation Depth ($p = 2.02{\cdot}10^{-5}$), Date ($p = 2.89{\cdot}10^{-6}$) and combination of Date and Distance ($p = 1.71{\cdot}10^{-5}$) and Date, Depth and Distance ($p = 0.002$) affected the *Ulva* starch content (Fig. 2a–c). The starch content on 9 April (was higher in comparison to 18 May and 25 August (Fig. 2a). Also, starch content on 0.5 depth was higher than on 10 m depth (Fig. 2a, c). The results of starch content dependence with date correspond to our previous work on Ulva starch content in the laboratory and near-shore data, where we showed that starch decreases in the July-August and is the highest in December (Prabhu et al., 2019b).

The protein content of the biomass varied from 4.2% to 15. 7% (mean 9.56%, median 8.56%, SE 0.57%) (Figs. 1, 2d). The lowest value was measured on 9 April at 5 m depth at Distance 100 and the highest value was measured on 25 August at 5 m and 10 m depth at Distance 100 m from a mooring. Date ($p = 9.41{\cdot}10^{-5}$), Depth ($p = 1.34{\cdot}10^{-4}$), Distance ($p = 0.04$) and combination of Date/Depth ($p = 0.02$), Date/Distance ($p = 1.71{\cdot}10^{-10}$) Date/Distance ($p = 0.03$) affected significantly the protein content of the biomass (Fig. 2d–f). The trend shows that large distance from the mooring, deeper cultivation in August lead to the higher protein content of the biomass. These results of protein content could be partially explained by a higher flux of nutrients released from the cages farther from the mooring points, local streams and longer day time (in August in comparison to April/May).

Analysis of elementary composition showed that the total carbon content (%N) of the biomass was in the 19–37% range (mean 26.61%, median 25.96%, SE 1.15%). The total nitrogen (%N) was in the 19–37% range (mean 2.03%, median 1.75%, SE 0.18%). The total hydrogen (%H) was in the 3.76–6.45% range (mean 4.89%, median 4.92%, SE 0.18%) and the total sulfur (%S) was in the 2.25–7.01% range (mean 4.21%, median 3.99%, SE 0.38%). We found that Date slightly affected the %S ($p = 0.04$); however, we did not observe any significant impact of Date, Distance, Depth or their combinations on the total carbon, nitrogen and hydrogen content suggesting that they were affected by other factors. Alternatively, a large sampling size is needed to determine the impact of the tested factors.

These results, however, only provide the first indication on the impact of the multiple environmental conditions on the Ulva biomass in the far-offshore environment growth and chemical content. The conditions far-offshore are dynamic and are very complex for measurement and interpretations.

3.3. Ulva spp. biomass compression and dewatering with pulsed electric fields coupled with a mechanical press

The *Ulva* spp. biomass was dewatered with a combination of electroporation coupled with mechanical pressing. The topology of the developed pulsed electric field circuit is shown in Fig. 3a. The digital image of the PEF generator is shown in Fig. 3b. The press electrode device is shown in Fig. 4c. The detailed design of the electroporation chamber is shown in Fig. 3d. The measured currents and calculated field strengths for the first and last pulses and invested total energy for each experiment are shown in Table 1. The shapes of voltage and current curves for the first and last pulses are shown in Fig. 3e. One of the currently unsolved issues with PEF application on the seaweed biomass with our device is the appearance of uncontrolled current spikes at the beginning and end of the pulse (highlighted in Fig. 3e). These spikes are most probably the result of the accumulated whole circuit inductivity and capacitance, which depends on the multiple setup parameters including cables length and contact points. These spikes lead to biomass exposure to high current but for a very short time during the pulse. Such abnormal current spikes could affect the biomass electroporation threshold (HO et al., 1995).

Previous works on green terrestrial biomass showed the PEF with pressing leads to biomass compression (Yu et al., 2016), important for biomass transportation from the cultivation sites to biorefinery (Zoulalian, 2010). This volume reduction is an extremely important feature for the seaweed biomass grown offshore. As in the nearest future, we expect that the major processing will take place on land, the transportation volumes of vessels will limit the distance of the farm from the processing facility (Lehahn et al., 2016). Here we show that pressing alone led to the biomass volume change of 301 ± 50 mm^3 (Table 2), which is 3.66 ± 0.46% compression (Eq. (1)). The applied 250 V protocol led to the 647 ± 207 mm^3 volume change (8.45 ± 1.72% compression) and 500 V protocol led to 1981 ± 321 mm^3 (25.66 ± 2.53% compression) compression of the biomass from the initial volume (Table 2, Eq. (1)).

Increasing the applied voltage from 250 V to 500 V and J_{in} from 9.3 ± 0.4 J g^{-1} FW to 54.6 ± 0.2 J g^{-1} FW increased the extracted water from 0.033 ± 0.006 g Water g^{-1} FW to 0.150 ± 0.031 g Water g^{-1}FW (Table 2). This also led to the saved on evaporation energy: J_s of 78.73 ± 10.41 (J gFW^{-1}) for 250 V protocol and J_s of 339.31 ± 48.01 (J gFW^{-1}) for 500 V protocol (Table 2, Fig. 4a). Pressing alone lead to only 0.01 gWater g^{-1}FW Ulva extraction and saved 26.15 (J gFW^{-1}), Table 2, Fig. 4a. Previous work on PEF dehydration showed that PEF could increase the dry matter of the sugar beets cassettes after pressing from 35% to 40% (Almohammed et al., 2015; Sack et al., 2010). However, in comparison to seaweed, the cassettes have small initial water content. In addition, PEF has also been used for dehydration of green terrestrial biomass as described in (Sack et al., 2009; Vorobiev and Lebovka, 2008).

3.4. PEF dehydration impact on water diffusion in air-convention drying

We also determined the kinetics of water removal (Fig. 4b) and effective diffusivity coefficient D_{eff} in the control and PEF treated Ulva

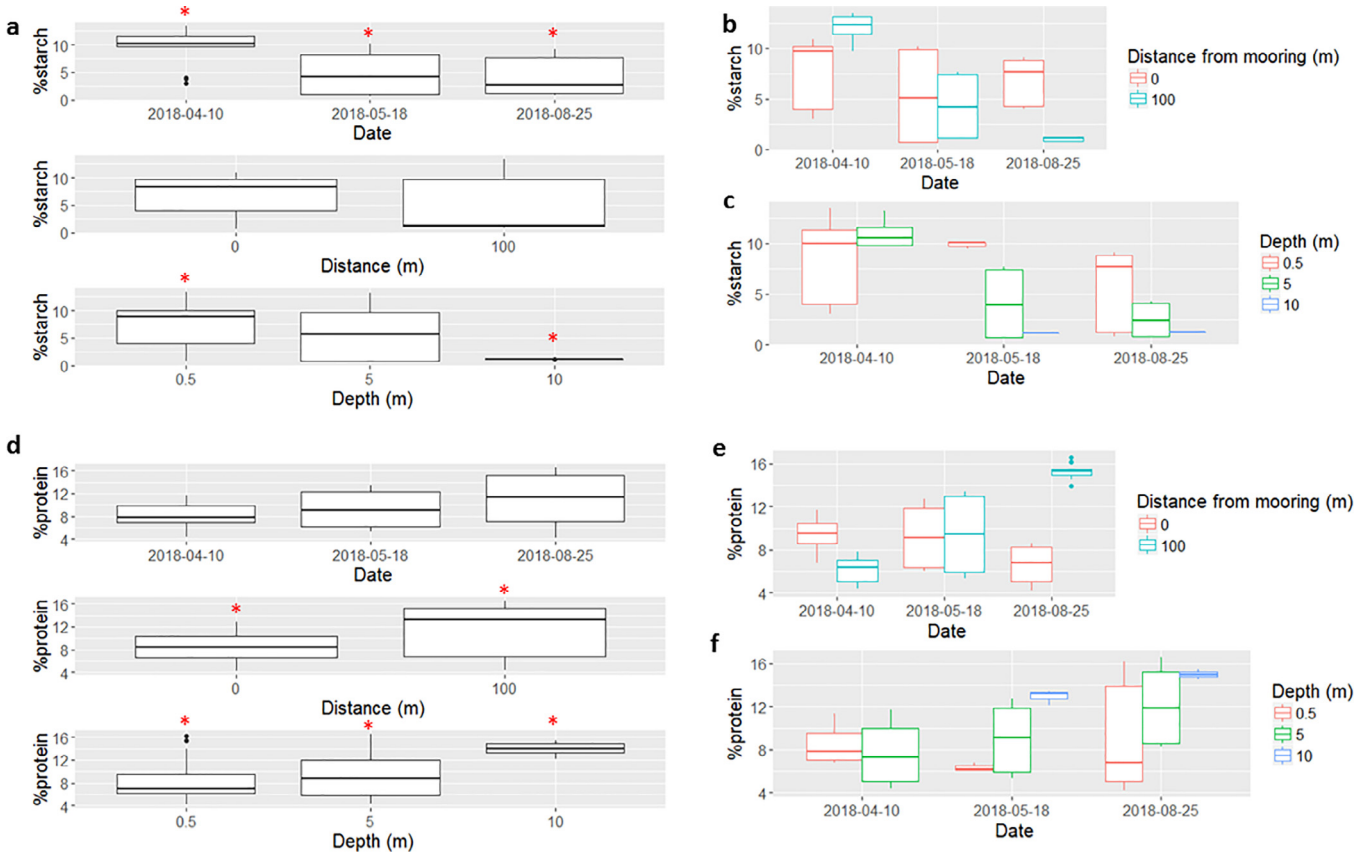

Fig. 2. The impact of Date, Depth and Distance from mooring on the starch (a–c) and protein (d–f) content.

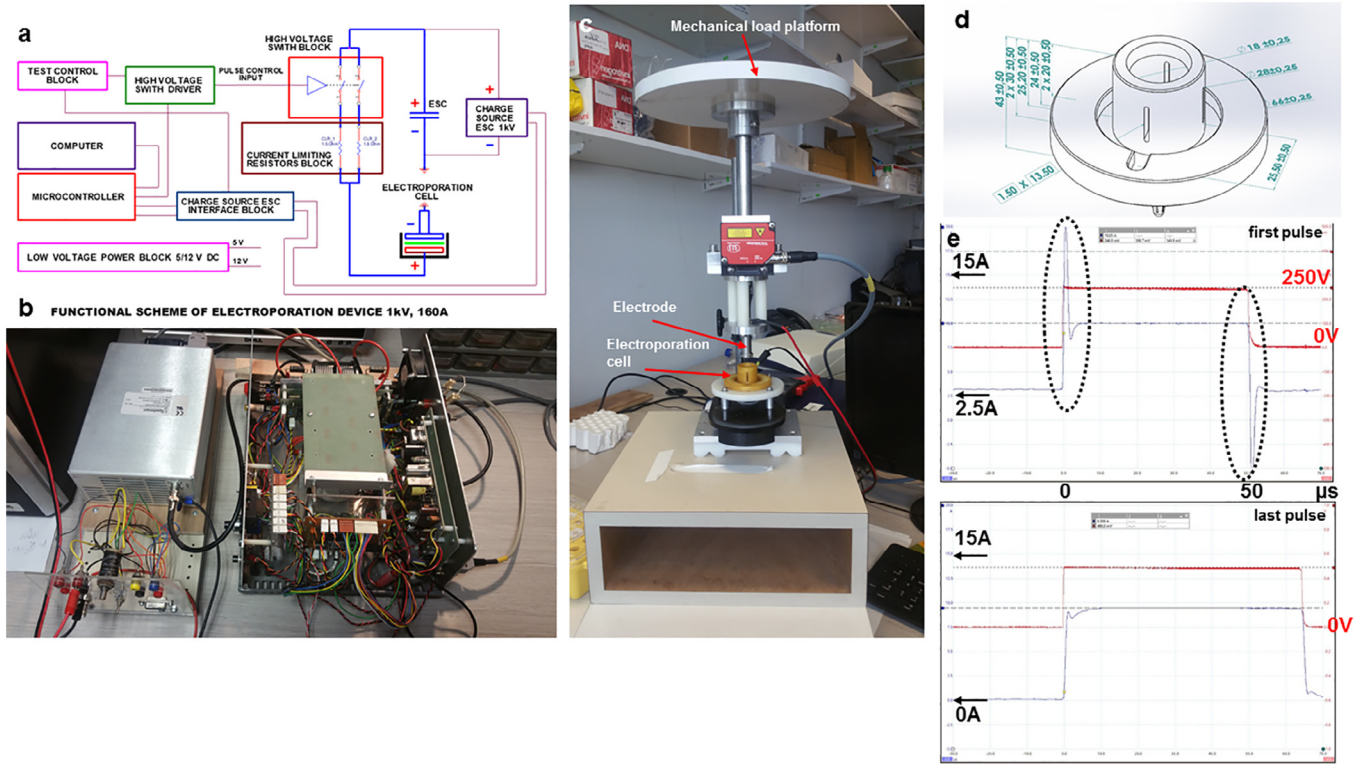

Fig. 3. Pulsed electric field (PEF) setup for Ulva biomass dewatering. a. Schematic design of the high voltage PEF generator. b. Digital image of the assembled PEF generator. c. Sliding electrodes with a coupled mechanical load. d. The design of electroporation chamber. e. Characteristic voltage (red) and current (black) measurements during the first and the last pulses.

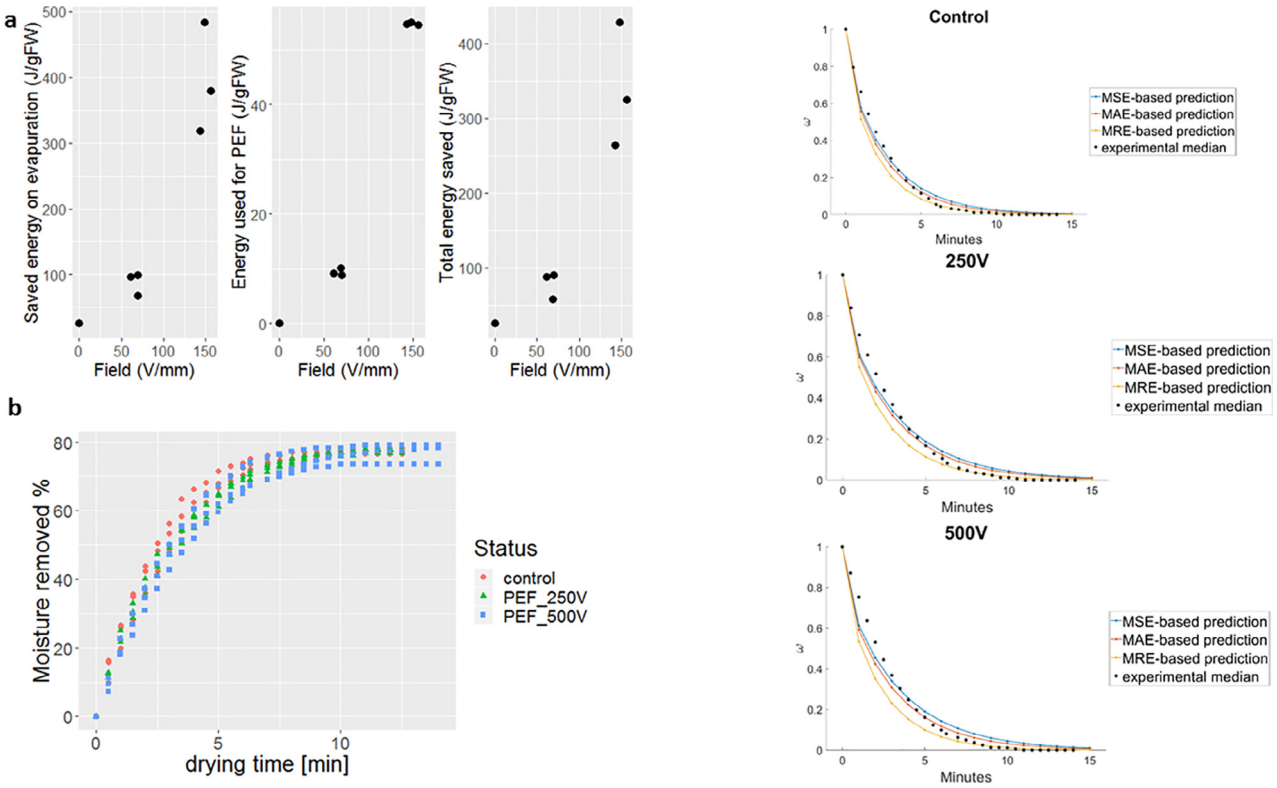

Fig. 4. Pulsed electric field dewatering of Ulva biomass. a. Energy saved with PEF dewatering process. b. *Ulva* biomass moisture loss during drying. c. Predicted and modeled Ulva drying curves after various PEF pretreatments.

biomass samples. The experimental data for w appears in Fig. 4c (dots). Using numerical approximation with three different error-estimating approaches (Eqs. (8)–(11)) we determined the D_{eff} (Table 3) for both control and PEF samples (Fig. 4c solid lines). The predicted vs measured data for w is shown in Fig. 4c. These results show that PEF treatment decreased the effective diffusivity coefficient of the seaweed biomass by 18–19% (depending on the error model), explaining the observed experimentally reduced drying kinetics (Fig. 4b). Although the kinetics of water removal by air convection (Fig. 4b, Table 3) show that after PEF treatment, the diffusion coefficient of water reduced ($p < 0.0093$ for Control vs 250 V and $p < 0.0179$ for Control vs 500 V), we did not observe any significant differences between post-PEF diffusion coefficient between the used treatments ($p < 0.97$ for both treatments). This reduction of the water diffusivity after PEF could be explained by the biomass compression and achieved a saturation already at 250 V treatment (Table 2). The limitation of this diffusion estimation study is that the D_{eff} was established based on the classical diffusion models under equal distribution, negligible external resistance, constant diffusivity, and negligible shrinkage through the drying process. These models have limitation in the description of the experimental drying data (Hamdami et al., 2004) and validation and testing this assumption is an important future step for this process scale-up.

4. Conclusions

A long-term positive growth and productivity was achived when *Ulva* spp. was grown offshore in the proximity of fish cages. Substantial amounts of starch and proteins can be extracted from the produced biomass. Larger distance from the mooring, deeper cultivation in August lead to the higher protein content. Electroporation coupled to mechanical press allowed for rapid seaweed biomass dewatering and compression. This dewatering, which could be done offshore leads to potential reduced costs of evaporation energy. In addition, electroporation led to the reduction of water diffusivity of 18–19% in the treated seaweed biomass and reduced drying kinetics by air convection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

Table 1
Ulva sp. biomass dewatering with PEF. Applied PEF parameters.

Experiment number	Voltage (V)	Pulse duration (μs)	Number of pulses	Pulse Repetition Frequency (Hz)	Total energy input (Joule)	E (Vmm^{-1}) First/Last pulse	Current (A) First/Last pulse
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	250	50	100	3	8.76	65.27/73.96	5.5/8.5
5	250	50	100	3	10.02	66.31/71.84	6.5/9.5
6	250	50	100	3	9.13	59.10/62.81	6.3/8.3
7	500	50	100	3	54.63	126.26/159.74	17.8/60
8	500	50	100	3	55.01	125.63/170.65	17.5/60
9	500	50	100	3	54.39	128.87/183.15	17.1/60

Table 2
Ulva sp. biomass compression and energy saved on evaporation after PEF coupled with mechanical pressing.

Experiment number	Voltage (V)	d1* (mm)	d2* (mm)	J_{in} (J gFW^{-1})	E (Vmm^{-1}) First/Last pulse**	Biomass volume change after PEF (mm^3)	Water removed (g)***	J_s (J gFW^{-1})
1	0	3.33	3.18	0	0	343	0.01	26.15
2	0	3.43	3.33	0	0	246	0.01	26.15
3	0	3.35	3.23	0	0	316	0.01	26.15
4	250	3.83	3.38	8.76	65.27/73.96	883	0.038	90.60
5	250	3.77	3.48	10.02	66.31/71.84	569	0.026	57.97
6	250	4.23	3.98	9.13	59.10/62.81	490	0.037	87.62
7	500	3.96	3.13	54.63	126.26/159.74	1628	0.122	264.39
8	500	3.98	2.93	55.01	125.63/170.65	2060	0.188	428.77
9	500	3.88	2.73	54.39	128.87/183.15	2256	0.145	324.78

* d1 (mm) is the initial distance between the two electrodes inside the electroporation chamber. d2 (mm) is the final distance between the two electrodes inside the electroporation chamber.

** Values of electric field strength (Vmm-1) during the first and the last pulses.

*** Mass of water removed (g) from a seaweed sample by a combination of PEF and mechanical pressure.

Table 3
Water effective diffusivity from Ulva sp. tissue under convective air drying. Pairwise group-based comparison of Control vs PEF250v and of Control vs PEF500V showed a significant difference between the two groups with the combined p-value of 0.0093 and of 0.0179 respectively. We did not find any significant difference between PEF250V and PEF500V groups.

Method	$D_{eff\_control}$ (m^2 s^{-1})	Error_Control (w^2)	D_{eff_PEF250} (m^2 s^{-1})	Error_PEF (w^2)	D_{eff_PEF500} (m^2 s^{-1})	Error_PEF500 (w^2)
Mean Square Error (MSE)	$2.98{\cdot}10^{-8}$	0.0013	$2.14{\cdot}10^{-8}$	0.0020	$2.18{\cdot}10^{-8}$	0.0030
Mean Absolute Error (MAE)	$3.25{\cdot}10^{-8}$	0.0264	$2.31{\cdot}10^{-8}$	0.0346	$2.43{\cdot}10^{-8}$	0.0392
Mean Relative Error (MRE)	$3.89{\cdot}10^{-8}$	0.2124	$2.88{\cdot}10^{-8}$	0.2796	3.1510^{-8}	=0.3102
Total	$3.37{\cdot}10^{-8}$		$2.44{\cdot}10^{-8}$		$2.59{\cdot}10^{-8}$	

influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.122229.

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