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Design of marine macroalgae photobioreactor integrated into building to support seagriculture for biorefinery and bioeconomy

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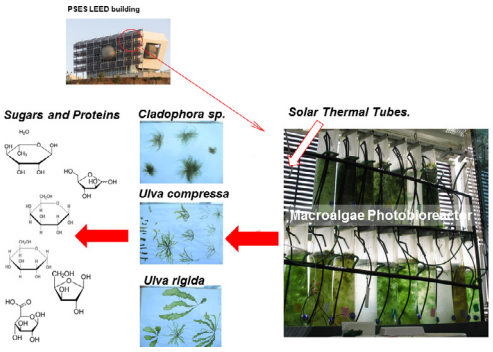
HIGHLIGHTS

- Indoors macroalgae photobioreactor (MPBR) was developed.
- MPBR integrated in LEED building.
- Macroalgae ash, energy density, carbohydrates and protein content was measured.
- Macroalgae accumulated energy was calculated.

GRAPHICAL ABSTRACT



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ABSTRACT

Seagriculture, which can provide offshore grown macroalgae biomass would play a significant role in bioeconomy. Nevertheless, seagriculture development has been hindered by the lack of laboratory photobioreactors that enable fundamental and pilot scale macroalgae research. In this work, a macroalgae photobioreactor (MPBR) was developed and integrated into the building. The MPBR operation was demonstrated for 6 months with cultivation of *Cladophora* sp., *Ulva compressa* and *Ulva rigida* green macroalgae species isolated from 3 sites at the Eastern Mediterranean coast. The growth rate, protein, ash, specific energy density, rhamnose, xylose, arabinose, glucose, galactose and glucuronic acid content of the cultivated species were quantified. The maximum accumulated energy rates were 0.033 Wh L^{-1} d^{-1} for *Cladophora* sp., 0.081 Wh L^{-1} d^{-1} for *U. compressa* and 0.029 Wh L^{-1} d^{-1} for *U. rigida*. This work provides a detailed design of an indoor, urban photobioreactor for cultivation, maintenance and energy balance analysis of macroalgae biomass for biorefinery.

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1. Introduction

Growing population, longevity and desire for a better quality of life put ever-increasing pressure on energy, food and chemicals infrastructures (Sherbinin et al., 2007). Currently, this growth translates worldwide to the increased consumption of fossil fuels and conversion of land for agricultural uses (Hazell and Wood, 2008; Höök and Tang, 2013). However, climate change, environmental pollution and food/energy security questions challenge the capacity of current infrastructures and supply chains to provide for sustainable development (Haberl et al., 2011). An alternative, yet undeveloped, approach to produce biomass for food, chemicals and fuels, is to use seas and oceans (Chen et al., 2015; Roesijadi et al., 2010; Yun et al., 2015). This approach for the offshore biomass production was recently coined seagriculture (http://seagriculture.eu/).

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Seagriculture could play a significant role in the development of bioeconomy, which would lead to the reduction in the use of fossil fuels (Zilberman, 2013). In addition, the expansion of biomass production offshore would slow the rate of land use conversion for agriculture (Lehahn et al., 2016). The basic unit of the seagriculture based bioeconomy is a marine biorefinery. Biorefinery is a system, which includes feedstock cultivation, harvesting, and conversion into products used in different branches of economy (Golberg et al., 2014). The potential of macroalgae feedstock for biorefinery is under intensive investigation on the laboratory and pilot scale (Bikker et al., 2016; van der Wal et al., 2013). However, macroalgae feedstock development has been hindered in comparison with advances in terrestrial crops and microalgae (Wichard et al., 2015).

Unavailability of technologies and research tools to grow and maintain macroalgae in the laboratory conditions slow development of macroalgae feedstock research (Loureiro et al., 2015; Van Hal et al., 2014; Wichard et al., 2015). In comparison, a major scientific tool that advanced multiple applications of microalgae in research and industry is a photobioreactor (PBR) (Singh and Sharma, 2012). Although intensively used in the microalgae research and commercialization (Wang et al., 2012), reports on PBRs for adult macroalgae growth, propagation and research are very rare (Holdt et al., 2014; Mullikin and Rorrer, 1998; Rorrer and Mullikin, 1999). In most studies on macroalgae as a biorefinery feedstock, macroalgae are either harvested from natural stocks, or cultivated in the large outdoor tanks, in the sea or in the small lab tubes.

However, natural stocks harvesting could lead to the inconsistency in genetics and phenotypes between studies and even in the same study. Although cultivation in outdoor tanks and in the open sea (both near shore and offshore) is effective in the concepts of integrated multitrophic aquaculture (Korzen et al., 2015), it prohibits the use of genetically modified macroalgae, required for fundamental studies and biotechnology applications. This limitation is due to the high probabilities of genetically modified biomass runoff to the fragile marine environment. In addition, these outdoor systems are under continuous threat of contamination. Small lab flasks and tubes, which allow for maintenance of clean cultures, can support the work with mutants and allow for precise controlled experiments. However, these systems are usually of a very small scale, and cannot provide optimal cultivation conditions in terms of natural illumination, water and gas exchange, required by macroalgae physiology. Therefore, the results from these controlled studies in tubes can be biased by non-optimal environmental conditions. In a parallel vein, an emerging concept in the recent years is the integration of algae PBRs into buildings (Lakenbrink, 2013; Pagliolico et al., 2017; Pruvost et al., 2016). Although these systems, based on microalgae cultivation, have been proposed for improving the energy balance of buildings by biogas production (Lakenbrink, 2013) and shading (Pagliolico et al., 2017), to the best of our knowledge they never been used for macroalgae and nor was the chemical composition of the cultivated algae reported.

To goal of this work is to address the current limitations of macroalgae research infrastructure by providing a design of a new PBR developed for macroalgae studies in the laboratory conditions and for the small scale biomass production in buildings. The closed macroalgae photobioreactor system (MPBR) allows for species maintenance, research and propagation. The MPBR was incorporated into the Leadership in Energy and Environmental Design (LEED) certified building, with existing solar-thermal absorption chiller air condition (AC) system. The developed MPBR is different from the proposed until now microalgae PBR incorporated into buildings (Lakenbrink, 2013; Pagliolico et al., 2017; Pruvost et al., 2016), as it uses seawater, it is integrated with a thermos-solar powered AC system and it is focused to grow and maintain macroalgae. This work describes the system design and performance for cultivation and maintenance of several local to Eastern Mediterranean species of green macroalgae, which can be potentially used as feedstock for biorefinery. This system provides an example of research infrastructure, required to perform continuous macroalgae research and small scale biomass production for in the closed urban environments.

2. Materials and methods

2.1. Marine macroalgae biomass

Green macroalgae species, *Cladophora* sp., *Ulva compressa* and *Ulva rigida* were collected, from Tel Aviv (Reading), Haifa, Mikhmoret, and Rosh HaNikra areas during spring 2016. These species were chosen to test the developed MPBR as they are known for the fast growth rates (Bruhn et al., 2011) and, thus, could provide rapid feedback on the MPBR. In addition, these species are considered as potential feedstock for biorefinery (Bikker et al., 2016; Ek et al., 1998; Polikovsky et al., 2016).

Collected biomass was transported in the plastic bags filled with the seawater to the laboratory and sorted manually to get clean monocultures. Monocultures were cultivated/maintained in separate MPBRs. Nutrients were supplied by adding ammonium nitrate (NH_4NO_3, Haifa Chemicals Ltd, IS) and phosphoric acid (H_3PO_4, Haifa Chemicals Ltd, IS) to maintain 6.4 g m^{-3} of nitrogen and 0.97 g m^{-3} of phosphorus in the seawater. The sole CO_2 supply was bubbled air. Fresh weight (FW) of the biomass was monitored weekly with analytical scale (Mettler Toledo, PB-S model, Switzerland). Before weighing, all surface water was carefully removed with a hand powered kitchen centrifuge until the removed water weight was less than 1 g.

The daily growth rates, P, expressed in $\%d^{-1}$, were calculated as described in Eq. (1) (Schmidt et al., 2010):

$$P = 100\% \cdot (FW_f - FW_i)/(FW_i \cdot t) \quad (1)$$

where FW_f (g) is the final fresh weight of all the biomass in the reactor, FW_i (g) is the initial fresh weigh in the same reactor, t (d) is the number of days between the measurements.

2.2. Indoor macroalgae photobioreactor (MPBR) to develop macroalgae feedstock for the future offshore marine biorefinery

The major motivation for the development of indoor MPBR was the need to create a research infrastructure develop macroalgae feedstock for the future offshore biorefinery. Development of such a feedstock requires macroalgae cultivation in the relevant physiological conditions in the laboratory where some of the environmental conditions can be controlled and the runoff of species to the marine environment is excluded. To achieve these aims we developed a closed loop, indoor cultivation system with 29 reactors (40.4 L each, with continuously exchanging water) with total operation volume of 1,171.6 L of seawater and with an air bubble mixing. The water was continuously recirculated and filtered. The system allowed for macroalgae biomass acclimation, cultivation and species maintenance under natural sun illumination, with controlled nutrients and mixing intensities levels.

2.3. Vertical polyethylene photobioreactor for macroalgae cultivation

A basic unit of the developed system is a reactor for macroalgae cultivation. In MPBR, the cultivation reactor (Fig. 1a) was welded from 200 μm polyethylene sleeve (Polytiv, Israel, Length 100 m, thickness 200 μm, width 0.4 m) with embedded anti-UV protection. The total volume of each cultivation reactor was 40.4 L. Air bubble mixing was provided from the bottom and water exchange

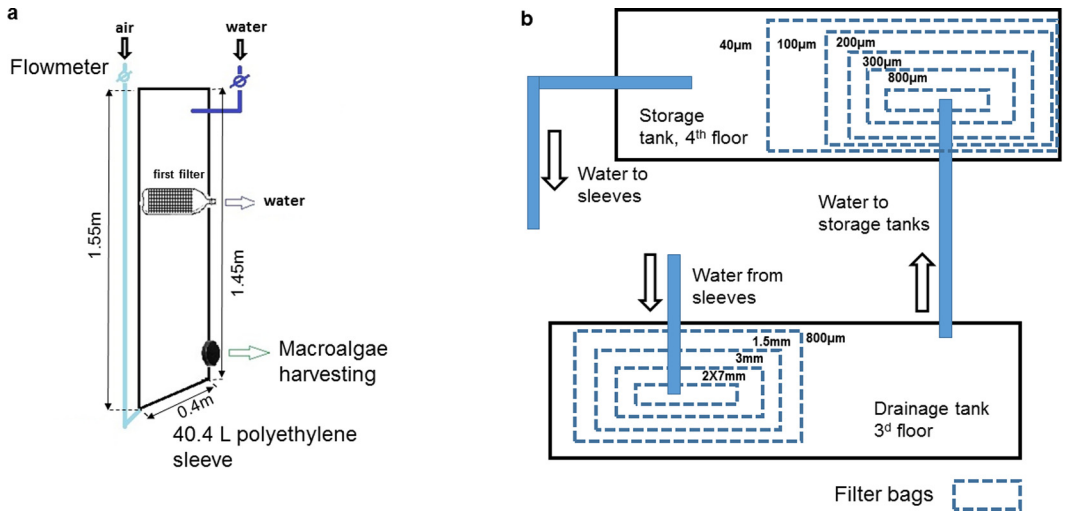

Fig. 1. Schematic representation of a. A single macroalgae photobioreactor (MPBR). b. Recirculated seawater filtration system. Dashed lines show the filter bags.

from the top (Fig. 1a). The rate of aeration and water exchange was controlled manually for each reactor with mechanical valves. The top part of the reactor contained a filtering net (0.5–3 mm, depending on the biomass size) to prevent biomass washing out to the main circulation system. Biomass harvesting was done from the bottom, by opening the cover and filtering the biomass through the aquarium net (Fig. 1a). During the cultivation, continuous water exchange removed the metabolites produced by the algae and associated organisms. For maintenance, every 1–3 weeks, the biomass was removed to a different reactor and the reactor was cleaned mechanically.

2.4. Closed loop seawater system for continuous water exchange

The total operation volume of the system was 6400 L of seawater (salinity 3.9%, pH 8.2). Initial water stock was provided from the Tel Aviv Reading marine area and was partially (1/4–1/3) exchanged every 3 months. Evaporation was compensated by adding deionized water to the system to maintain the salinity at 3.9%.

The total circulating volume was 3400 L. Additional 3000 L were stored separately. 2000 L were stored in two 1000 L plastic storage tanks at the basement. The rest of the water was pumped to the 4th floor of the building using 32 mm pipe and Dm 30 N Pedrollo 1.1 kW pump (Italy). On the 4th floor 1000 L was stored in the plastic storage tank and 3682 L of water were used in the active volume distributed as follows: 1) 2 tanks, with capacity of 1000 L each and 1 tank with a capacity of 500 L (connected) on the 4th floor, 2) 1,171.6 L were in the 29 cultivation reactors with the biomass on the 3th floor; 3) 1500 L of PVC drainage tank, located under the system of reactors on the 3th floor (Fig. 2b). The additional role of the drainage tank was to serve as a safety buffer to absorb all the water from MPBR system in the case of emergency. The water flowed by gravitation from tanks on the 4th floor to the MPBRs (50 mm PVC major pipe and 20 mm distribution pipes (Fig. 2b)), and from them to the drainage tank (25 mm PVC pipe). Once the water in the drainage tank approached a predetermined level, a pump (PQAm70 0.55 kW, Pedrollo, Italy) recirculated the water back to the 4th floor storage tanks. The gravitation driven flow was a key element to save electricity and prolong the pump life.

A critical part of the system was water filtration (Fig. 1b). Filtration was needed to prevent plankton and epiphytes growth and to prevent cross contamination when several species were grown at the same time. As fresh sea water was used for cultivation of several species simultaneously, complete filtration of the all contaminants is very expensive and not practical. Therefore, the following system of filtration meshes was developed. At the exit from the cultivation reactors to the drainage tank, the water passed through a series of 0.5–5 mm filters to remove large pieces of debris and thalli (Fig. 1a). At the entrance to the drainage tank, the water was further filtered up to 800 µm (Fig. 1b). At the entrance to the storage tanks, on the 4th floor, water was further filtered up to 40 µm with membranes, installed at the end of the pipe, (Fig. 1b) and wadding polyester Sintepon (Pentair Aquatic Eco-Systems, Inc., FL). In addition, PVC ribbon with high surface for biofilter (Bio-Fill and BF 250, Pentair Aquatic Eco-Systems, Inc, FL) to prevent contamination was used.

2.5. Solar flux measurements

The variation of solar flux (Photosynthetically active radiation, PAR) between cultivation reactors was measured exactly at noon in August-September 2016 for each cultivation reactor with Li-Cor Spherical Quantum Sensor (LI 193SA, Li-Cor, NE). The measurements were taken at the same point, 15 cm from the top, for each reactor during three cloudless day. The illumination profiles during 24 h outside and inside the single reactor were measured by Onset® HOBO® sensor UA-002-08 (Onset Inc. MA) in August-September 2016.

2.6. Temperature measurements

Minimum and maximum daily temperatures were measured in the system with thermometer (Maxi-mini-thermometer, model: MMG-3, Shanghai QualityWell Industrial Co., Ltd, China) during 6 months of operation. Temperature changes during 24 h inside and outside the reactors were measured by Onset® HOBO® sensor UA-002-08 (Onset Inc, MA) in August-September 2016.

2.7. Air bubble mixing

Air bubble column was used for thalli mixing inside the cultivation reactors. The air system consisted of an air blower (SC201MF 0.4KW Emmecom Srl.,Italy) located at the 4th floor (Fig. 2b) and distribution pipes. The central pipe 50 mm branched to smaller 16 mm, feeding the reactors (Fig. 1a) at 2–4 L min^{-1}. The air flow rate in each reactor was manually regulated with the incorporated in series flow meters (DFA-15, Darhor Technology Co.,Limited,

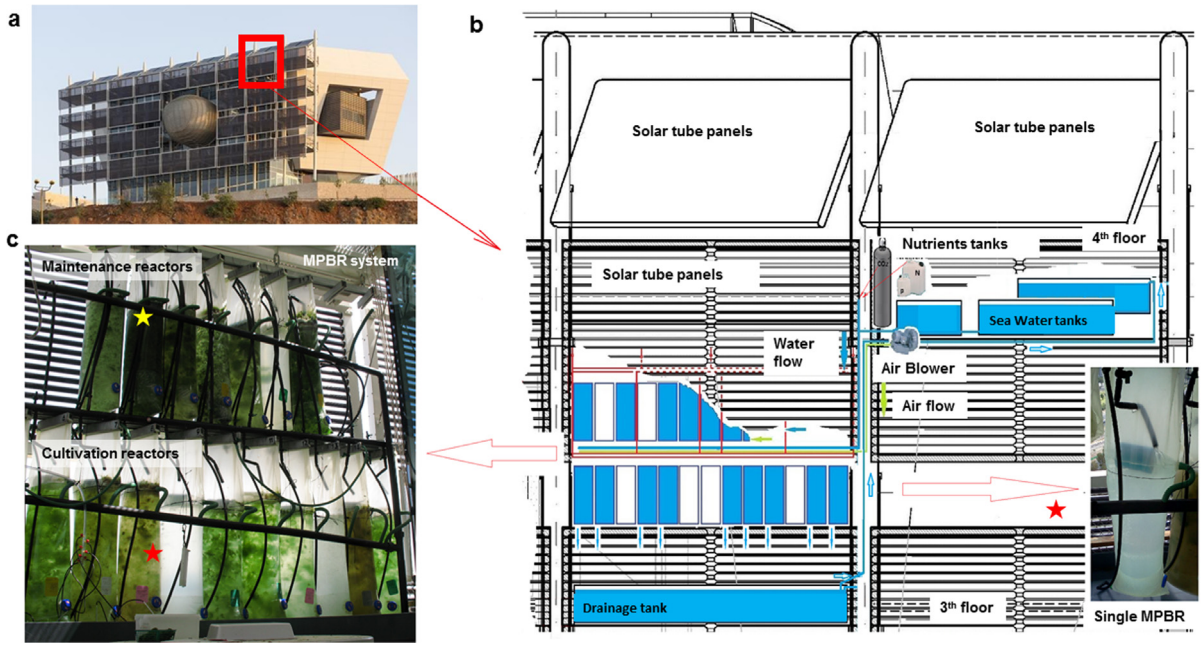

Fig. 2. Closed macroalgae photobioreactor (MPBR) integrated into the LEED building of PSES at Tel Aviv University a. System location inside the eco-wall of the PSES building. b. Detailed plan of the MPBR integrated within solar tubes of the building. Red arrow shows the position of sleeve reactors in the gap between solar tube arrays. Blue lines show water flow; green lines show air flow. c. Digital photography view of the MPBR system. Red arrow indicates the cultivation area, where maximum solar light is available to the reactors. Yellow star indicates the species maintenance reactors where intensive growth is not expected because of the limited by solar tubes illumination.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

China). The flow rates was adjusted for complete circulation of all thalli in the cultivation reactor. The usual flow rates were 2–4 L min^{-1}.

2.8. Protein analysis

Harvested with an aquarium net biomass was dried at 40 °C for 12 h. Five gram of each sample were analyzed according to AOAC 981.10 with an automatic Kjeldahl system for total protein quantification. Protein calculation factor of 6.25 was used. Analysis was done by a certified food chemistry company (AminoLab, Rehovot Israel).

2.9. Carbohydrate and sugar acid analysis

The biomass was dried in an oven at 40 °C until constant weight. The dried biomass was made brittle by liquid nitrogen and then it was grinded into powder manually by mortar & pestle. All chemicals and standards were purchased from Sigma-Aldrich (Israel) if not otherwise mentioned. Thermochemical deconstruction (2% sulfuric acid, 1:20 solid to solvent ratio, 30 min, 121 °C) was conducted in 10 mL centrifuge tubes (Nalgene™ Oak Ridge High-Speed PPCO Centrifuge Tubes (Thermo-Fisher Scientific, CA)) in autoclave (Tuttnauer 2540MLV, Netherlands). For each batch, dried samples of biomass (50 mg) were weighed on analytical balance (Mettler Toledo, Switzerland) Sulfuric acid was added into the tube and the mix was vortexed to make the powder well distributed in acid. The hydrolysates were stored at −20 °C after centrifugation (5 min, 12,000 rpm using a benchtop centrifuge (Epindorf, Germany).

For carbohydrate analysis, the hydrolysates were thawed, an aliquot was taken and diluted 50 times in ultrapure water before being filtered through a 0.22 μm syringe-filter (Millipore, USA) into High Pressure Ion Chromatography (HPIC) vials (Dionex, Thermo Fischer Scientific, MA, USA). Monosaccharide contents in the hydrolyzates were monitored by HPAEC-PAD using a Dionex ICS-5000 platform (Dionex, Thermo Fischer Scientific, MA, USA) with an analytical column (Aminopack 10) and its corresponding guard column. Electrochemical detector with AgCl as reference electrode was used for detection. The analysis was performed using an isocratic flow of 4.8 mM KOH generated by the automatic Eluent Generator (Dionex, Thermo Fischer Scientific, MA, USA) during 20 min. The column was washed with 100 mM KOH between each run and re-equilibrated with 4.8 mM 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 monosaccharide with internal standards.

Glucuronic acid content was quantified with a program that involved three handmade eluents: NaOH, ultrapure water and sodium acetate. Two additional small peaks were observed but not analyzed in the area of glucuronic acid peak in all samples. These were hypothetically identified as aldobiouronic acid and iduronic acid as stated in (Quemener et al., 1997). Total yield was calculate using the following Eq. (2):

$$\%Yield = \frac{10\sum_{i=1}^{6} m_i}{1000 \cdot \%Solid} \quad (2)$$

where m_i (μg) is the mass of carbohydrate i in the sample, %Solid is the solid load of the dried at 105 °C for 24 h biomass for the hydrolysis from the total weight. The summed carbohydrates were rhamnose, xylose, arabinose, glucose, galactose and glucuronic acid. The concentrations of the rest of the released monosaccharides were negligible. Each biomass sample was hydrolyzed in duplicate for the analysis. All hydrolysates were analyzed in duplicates for carbohydrates content.

2.10. Ash content quantification

About 0.5 g (±0.01) of harvested biomass were weighted and then dried at 105 °C using conventional oven for 24 h in pre-weighted clean ceramic crucibles. The crucibles were then cooled down in desiccator, weighted, and ignited at 550 °C for 3 h in a

muffle furnace (Thermolyne muffle furnace, Thermo scientific) and then kept at 105 °C. The crucibles were finally cooled down in desiccator and weighted. The dry weight content (DW) was calculated with Eq. (3):

$$DW = \frac{m_3 - m_2}{m_1 - m_2} \quad (3)$$

where m_1 (g) is the weight of the biomass samples (dried at 40 °C) and the weight of the crucible combined, m_2 (g) is the weight of the ceramic crucible and m_3 is the weight of the biomass samples and the weight of the crucible after drying at 105 °C. The ash content (Ash) was calculated with Eq. (4):

$$Ash\% = \frac{m_4 - m_2}{m_3 - m_2} \cdot 100\% \quad (4)$$

where m_4 (g) is the weight of the biomass samples and the weight of the crucible the after combustion at 550 °C for 3 h.

2.11. Specific energy quantification

Twenty gram (DW) of biomass were analyzed for energy content (specific energy e [MJ kg^{-1}] according to ASTM D5865 – 13 (Standard Test Method for Gross Calorific Value of Coal and Coke) by a certified laboratory of Israel Electric company.

2.12. Energy balance analysis

Solar irradiation for the cultivation period was extracted from the Israel Meteorological Services (http://www.ims.gov.il/IMS/CLIMATE/LongTermRadiation/) for Beit Dagan Israel measurement station. The daily global solar irradiance (kWh m^{-2}) was calculated as the irradiance from 5am to 7pm on each day of the cultivation experiment. The IMS data base provides information of the accumulated global irradiance with 1 h resolution. The total solar (E_{solar}) input to the MPBR was calculated for the total illuminated area of the eco-wall (4.48 m^2).

The maximum PAR to chemical energy conversion efficiency of macroalgal biomass in the MPBR was calculated using Eq. (5)

$$\%\eta_{max} = \frac{\Delta(FW_f - FW_i)_{max} \cdot FW/DW \cdot e \cdot N}{E_{solar} \cdot \Delta t} \cdot 100\% \quad (5)$$

where $\Delta(FW_f - FW_i)_{max}$ (g_{ww}) is the maximum biomass accumulation for each species per reactor; FW/DW is wet: dry weight ratio, measured by biomass drying at 105 °C for 24 h; e (MJ kg^{-1}) is the specific energy determined as described in Section 2.10; N is the number of the cultivation reactors (14), and Δt (d) is the number of days with P_{max}, calculated in Eq. (1).

3. Results and discussion

3.1. Design of MPBR incorporated into the LEED building

The MPBR system was constructed within the south/east corner of the eco-wall of the LEED Platinum certified building of the Porter School of Environmental Studies (PSES) at Tel Aviv University (Fig. 2a). Eco-wall incorporates a series of solar-thermal tubes that provide thermal energy for the building AC system. However, the tubes do not cover completely the south wall (Fig. 2b, red star area); therefore there is still available solar flux that comes to the building and is not used for heating the solar tubes. This location of MPBR behind the solar tubes increased the total energy harvesting of the eco-wall structure. Recent theoretical work analyzed a microalgae PBR incorporated into the building façade for biomass production, energy consumption reduction and thermal regulation of the building (Pruvost et al., 2016).

The MPBR system was divided into two sub-system: cultivation MPBRs and maintenance MPBRs. To further increase the use of solar energy within the building, we decided to locate the system cultivation MPBRs between the gaps between the tubes (Fig. 2b and c red star area). However, it is important to note, that the cultivation MPBRs were not exposed to the maximum solar irradiation, which is attenuated by the solar-thermal tubes. Therefore, we did not expect to maximize the biomass yields in this system in comparison to open sun systems, where reactors are exposed to the open light. The maintenance MPBRs were located behind the solar tubes (Fig. 2c, yellow star area), their goal was to maintain the clean monoculture species stocks alive without intensive growth for all experiments.

3.2. Environmental parameters of the system: light intensity and temperature

As the system was located indoors but in the open air (inside the eco-wall), we did not control the light and temperature during the cultivation time. Depending on specific reactor location, PAR varied between reactors in 238–348 μmole photons m^{-2} s^{-1} (52.12–76.21 W m^{-2}, Fig. 3a). The open sun measurement (outside the solar tubes) at the same location and time showed PAR in 2,035–2065 μmole photons m^{-2} s^{-1} (445.66–452.23 W m^{-2}) range. This order of magnitude intensity lost was caused by shading from the integrated in building thermal-solar tubes. The illumination profile during 24 h outside and inside a single reactor are shown on Fig. 3b. The measurements inside the reactor were taken in a reactor filled only with water and in a reactor with 1.5 gr_{ww} L^{-1} of *U. rigida* biomass. Biomass at this density led to reduction of the illumination inside the reactors two times (Fig. 3b).

The minimum and maximum temperature distribution in the system is shown in Fig. 3c. Day/night temperature differences of 0.5–13 °C in April-first 2 weeks of June and 1–3.5 °C in second half of June-August were observed. The 24 h distribution of temperature inside and outside the single cultivation reactor is shown on Fig. 3d.

3.3. Green macroalgae biorefinery with indoor MPBR

For system validation, we cultivated three green macroalgae species, isolated from different locations in the costs of Israel. The macroalgae were cultivated in the system during spring and summer 2016 at least at 3 repeats per experiment (three cultivation reactors per species) (Fig. 4). On Fig. 4, the minimum (P_{min}), the maximum (P_{max}) and the average (P_{av}) growth rates for each species is reported as measured during the whole cultivation period. On Table 1, the chemical composition and growth rate at the day of chemical composition measurement (P_h) for a representative sample is reported.

Cladophora sp., isolated from Rosh HaNikra sampling site was cultivated for 122 days from 5 May to 26 July 26, 2016. The growth rates changed from P_{min} −3.66% day^{-1} to P_{max} 9.78% day^{-1}, with a P_{av} of 3.47% day^{-1}. The P_{max} was measured for 12 continuous days from 5 May to 17 May 2016. *Cladophora* sp. isolated from Mikhmoret sampling site was cultivated for 122 days from 5 May to 26 July 26, 2016. The growth rates changed from P_{min} 9.32% day^{-1} to P_{max} 95.5% day^{-1}, with a P_{av} 36.63% day^{-1}. The P_{max} was for 12 continuous days from 5 May to 17 May 2016. *Cladophora* sp. isolated from Reading sampling site was cultivated for 38 days from May 30 to July 7. The growth rate was stable at P 95.47% day^{-1} during the entire period of 38 cultivation days from May 30 to July 7, 2016 (Fig. 4a). This is in comparison to P 8–15% day^{-1} reported for *Cladophora* sp. in (de Paula Silva et al., 2013) at optimum pH with enhanced CO_2 or to P of 0.68–0.74 d^{-1} for *Cladophora* sp. at optimum N:P ratio (Liu and Vyverman, 2015).

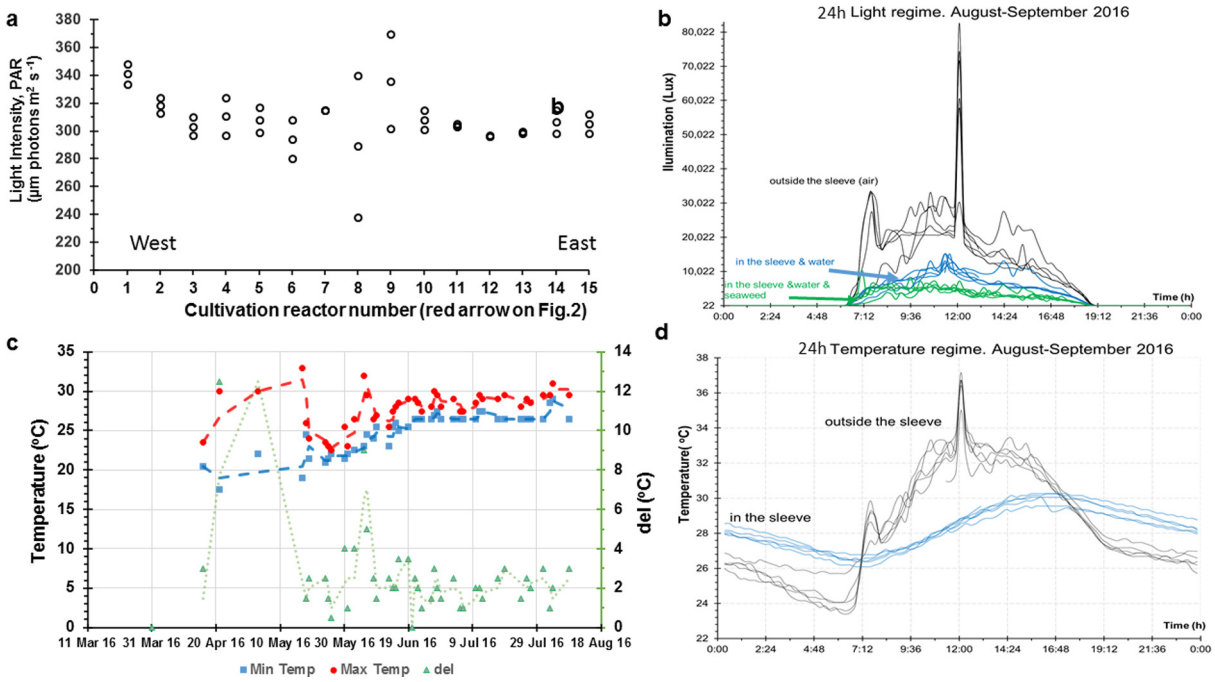

Fig. 3. a. Light distribution between cultivation reactors at noon during August–September 2016 in Israel. Three measurements were done at 12 pm in August–September 2016. b. Illumination profile during 24 h outside the reactor (black line), inside the reactor with sea water (blue line) and inside the reactor with 1.5 gr L^{-1} of *Ulva* biomass (green line) in August-September 2016 are shown. c. Temperature distribution in the system during March-August 2016. d. 24 h distribution of temperature inside and outside the cultivation reactors. Data from 7 consequent measurements in August-September 2016 are shown.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cladophora sp.	Growth rate (%/day)			Time (days)	
Sampling site	P_{min}	P_{max}	P_{av}	**Days with P_{max}**	**Total cultivation days**
Rosh-ha-Nikra	-3.66%	9.78%	3.47%	12	122
Mikhmoret	9.32%	95.5%	36.63%	12	122
Reading	95.47%	95.47%	95.47%	38	38

Ulva compressa	Growth rate (%/day)			Time (days)	
Sampling site	P_{min}	P_{max}	P_{av}	**Days with P_{max}**	**Total cultivation days**
Rosh-ha-Nikra	-2.47%	16.79%	2.89%	18	175
Mikhmoret	1.46%	5.51%	3.65%	27	172
Reading	-82.34%	0.83%	-40.76%	40	143

Ulva rigida	Growth rate (%/day)			Time (days)	
Sampling site	P_{min}	P_{max}	P_{av}	**Days with P_{max}**	**Total cultivation days**
Rosh-ha-Nikra	-3.13%	19.51%	4.61%	16	89
Haifa	-0.88%	8.39%	2.01%	64	175

Fig. 4. MPBR system validation for the cultivation of a. *Cladophora* sp. b. *Ulva compressa*; c. *Ulva rigida*. Thalli morphology is shown on the left panel. Biomass growth rates are shown on in the right.

Carbohydrate profiling was done for *Cladophora* sp. harvested at Mikhmoret site and cultivated in the system (Table 1, Fig. 5). The growth rate at the day of harvest (P_h) was 63.9%. Rhamnose content was 4.37 ± 0.85 µg mg_{DW}^{-1}, arabinose 34.27 ± 3.47 µg mg_{DW}^{-1}, galactose 29.91 ± 3.16 µg mg_{DW}^{-1}, glucose 69.44 ± 6.71 µg mg_{DW}^{-1}, xylose 20.02 ± 2.66 µg mg_{DW}^{-1} and glucuronic acid 1.10 ± 0.09 µg mg_{DW}^{-1}.

Table 1
Macroalgae species grown in the MPBR system approximate composition and energy density content based on DW at 105 °C.

Species (Origin, date of collection in origin)	P_h% (day^{-1}) at harvest from system	Rh* μg mg_{DW}^{-1}	Arb* μg mg_{DW}^{-1}	Gal* μg mg_{DW}^{-1}	Gluc* μg mg_{DW}^{-1}	Xyl* μg mg_{DW}^{-1}	Gluc* acid μg mg_{DW}^{-1}	Ash μg mg_{DW}^{-1}	Protein μg mg_{DW}^{-1}	Specific energyMJ kg^{-1}
Cladophora sp. (Mikhmoret, 16.05.2016)	63.9% (30.5.2016)	4.37 ± 0.85	34.27 ± 3.47	29.91 ± 3.16	69.44 ± 6.71	20.02 ± 2.66	1.10 ± 0.09	267 ± 10	120	15.042
Ulva compressa (Rosh HaNikra, 1.03.2016)	3.5% (14.6.2016)	55.57 ± 4.72	0.33 ± 0.17	10.34 ± 1.52	85.94 ± 5.93	27.26 ± 2.69	20.73 ± 1.87	276 ± 10	290	14.310
Ulva rigida (Haifa, 1.03.2016)	1.4% (20.6.2016)	42.11 ± 1.62	0.52 ± 0.18	4.07 ± 0.54	61.93 ± 2.59	10.29 ± 0.35	18.77 ± 4.98	473 ± 20	330	9.879

* Rh (rhamnose), Arb (arabinose), Gal (galactose), Gluc (glucose), Xyl (xylose), Gluc acid (glucuronic acid).

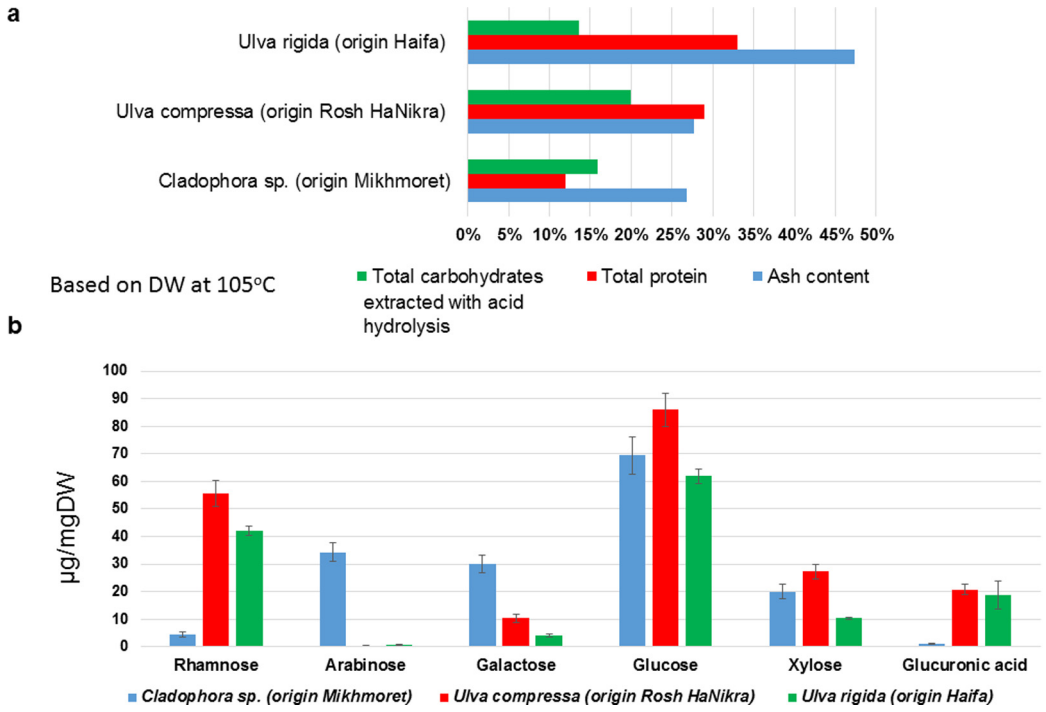

Fig. 5. a. Approximate cultivated macroalgae composition. b. Cultivated in MPBR macroalgae *Cladophora* sp., *Ulva compressa* and *Ulva rigida* major carbohydrates profile. Error bar show ± Standard deviation (n = 4).

The total carbohydrate yield was 15.91 ± 0.86%. To the best of our knowledge, this is a first report for acid hydrolysis of whole marine *Cladophora* sp with quantitative information on the individual monosaccharides release. Previous studies reported on water soluble cladophoran reported on 4:3:1 M ration of arabinose: galactose: xylose (O'Donnell and Percival, 1959). Glucose source is most probably cellulose, which is a major target for *Cladophora* derived products (Ek et al., 1998; Mihranyan, 2011, 2008, 2007). The protein content in the biomass was 12% ($DW_{105°C}$), in comparison to 15–21% reported in (Trung et al., 2013). The ash content was 26.76 ± 0.01%.

U. compressa isolated from Rosh HaNikra sampling site was cultivated for 175 days from 1 March to 23 August 2016. The growth rates changed from P_{min} −2.47% day^{-1} to P_{max} 16.79% day^{-1}, with a P_{av} 2.89% day^{-1}. The P_{max} was observed for 18 continuous days from 18 May to 5 June 2016. *U. compressa* isolated from Mikhmoret sampling site was cultivated for 172 days from 1 March to 20 August 2016. The growth rates changed from P_{min} 1.46% day^{-1} to P_{max} 5.51% day^{-1}, with a P_{av} 3.65% day^{-1}. The P_{max} was observed for 27 continuous days from 3 May to 30 May 2016. *U. compressa* isolated from Reading sampling site was cultivated for 143 days from April 14 till 23 August 2016. The growth rates were mostly negative, with the biomass loss at P_{min} −82.34% day^{-1}, P_{max} of only 0.83% day^{-1} was observed 38 continuous days from 14 April to 24 May 2016 (Fig. 4b). Previous studies reported on −10 to 35% day^{-1} at various environmental conditions (Martins et al., 1999; Potter et al., 2016).

Carbohydrate and protein profiling was done for *U. compressa* harvested at Rosh HaNikra site and cultivated in the system (Table 1, Fig. 5). The P_h was 3.5%. Rhamnose content was 55.57 ± 4.72 μg mg_{DW}^{-1}, arabinose 0.33 ± 0.17 μg mg_{DW}^{-1}, galactose 10.34 ± 1.52 μg mg_{DW}^{-1}, glucose 85.94 ± 5.93 μg mg_{DW}^{-1}, xylose 27.26 ± 2.69 μg mg_{DW}^{-1} and glucuronic acid 20.73 ± 1.87 μg mg_{DW}^{-1}. The total carbohydrate yield was 20.17 ± 8.40%. These results are lower than the results reported in (Feng et al., 2011), where the optimization of *U. compressa* (previously classified as *Enteromorpha*) was reported. In that study biomass was collected from the shore and extracted monosaccharides content was 175.2, 55.3, 183.4, and 88.5 mg g^{-1} for glucose, xylose, rhamnose and glucuronic acid at the best hydrolysis conditions for each of the monosaccharides (Feng et al., 2011). The parameter that could explain the difference between these results is the nitrogen

concentration. In our system, the nitrogen content was higher than in natural sea water and previous studies showed that macroalgae accumulate high carbohydrates at nitrogen starvation in *Ulva* sp (Gómez Pinchetti et al., 1998). The total protein content of this biomass was 29% ($DW_{105°C}$) in comparison with 17.48% reported in (Kandasamy et al., 2012) or 9.42–20.60 (depending on the location and harvesting season) (Haroon et al., 2000), 9–14% (Aguilera-Morales et al., 2005) and 21% reported in (Mamatha et al., 2007). The ash content was 27.69 ± 0.01%.

U. rigida isolated from Rosh HaNikra sampling site was cultivated for 89 days from June 7 to September 4. The growth rates changed from P_{min} −3.13% day^{-1} to P_{max} 19.51% day^{-1}, with a P_{av} 4.61% day^{-1}. The P_{max} was for 16 continuous days from 20 June to 6 July 2016 (Fig. 4c). *U. rigida* isolated from Haifa sampling site was cultivated for 175 days from March 1 to August 23. The growth rates changed from P_{min} −0.88% day^{-1} to P_{max} 8.39% day^{-1}, with a P_{av} 2.01% day^{-1}. The P_{max} was for 64 continuous days from 31 May to 3 August 2016 (Fig. 4c). Previous studies reported on 17% day^{-1} of *U. rigida* grown on effluents from fish cages (Korzen et al., 2015) and 18.7% day^{-1} in tanks (Bruhn et al., 2011) and 11.8% in spray cultures (Msuya and Neori, 2010).

Carbohydrate and protein profiling was done for *U. rigida* harvested at Haifa site and cultivated in the system (Table 1, Fig. 5). The P_h was 1.4%. Rhamnose content was 42.11 ± 1.62 μg mg_{DW}^{-1}, arabinose 0.52 ± 0.18 μg mg_{DW}^{-1}, galactose 4.07 ± 0.54 μg mg_{DW}^{-1}, glucose 61.93 ± 2.59 μg mg_{DW}^{-1}, xylose 10.29 ± 0.35 μg mg_{DW}^{-1} and glucuronic acid 18.77 ± 4.98 μg mg_{DW}^{-1}. The total carbohydrate yield was 13.70 ± 5.88%. The protein content from the biomass harvested was 33% ($DW_{105°C}$), to comparison to 12–25% (measured by the same method) as reported in (Shuuluka et al., 2012) for a 5 month study in natural and nutrient enriched cultivation. An analytical study for *U. rigida* deconstruction reported 18.3% protein, 0.7 μg mg_{DW}^{-1} arabinose, 0.1 μg mg_{DW}^{-1} fucose, 11.7 μg mg_{DW}^{-1} galactose, 1.4 μg mg_{DW}^{-1} galacturonic acid, 183 μg mg_{DW}^{-1} glucose, 62.3 μg mg_{DW}^{-1} glucoronic acid, 1.4 μg mg_{DW}^{-1} fructose, 0.9 μg mg_{DW}^{-1} mannitol, 18 μg mg_{DW}^{-1} mannuronic and guluronic acids, 91.2 μg mg_{DW}^{-1} rhamnose, and μg mg_{DW}^{-1} 38.5 xylose (Pezoa-Conte et al., 2015). The ash content was 47.36 ± 0.02%.

It is important to emphasize, that the total protein content was measured using Kjeldahl method in which the total protein content was calculated from the measured organic nitrogen content using a factor of 6.25, required by the standard for the food industry. Previous studies, however showed that this factor can be different for macroalgae (Angell et al., 2016; Lourenço et al., 2002) and a specific corrections will be needed in the future for each species.

The growth rates measured in this study had wide ranges, mostly due to the biomass sporulation. This problem can be addressed by synchronizing the age of the population and by decreasing the stress conditions, such as temperatures fluctuations. The biomass yields were also limited by the maximum illumination, which was limited by the solar systems. Additional problems could appear because of the rapid water evaporation during the summer. Although the salinity was controlled by adding the deionized water, fluctuations at the local level at each cultivation reactor during the day could take place. In addition, automation of nutrients measurements and addition will significantly improve the overall efficiency of the system.

3.4. System integration and energy balance

For maintenance and cultivation of various macroalgae species, the mechanical parts of the system were synchronized with natural solar irradiance and biomass growth rates as follows. Water exchange in all reactors was continuous and was set for two volumes (81 L) exchange in 1–2 h. Air mixing was set to operate continuously during the day time with automatic control because only during the sun light there is active photosynthesis. At night the 30 s mixing pulse was provided every 30 min to avoid anoxic conditions building up.

The energy balance of the proposed biorefinery system is shown in Fig. 6. The majority of the incoming solar energy is attenuated by the solar-thermal system existing in the building. This absorbed energy is then used for powering air condition of the building through the absorption chiller. The rest of the direct solar energy (~10% of the total flux) arrives to the MPBR (3.5–5.2 kWh d^{-1}, PAR). Additional direct energy inputs for the biomass growth in our system came from 1) the air blower (0.4 kW, operating for 12 h a day during photosynthesis) 4.8 kWh per day; 2) recirculating water pump (0.55 kW, when fully operational the pump worked for 30 min and 30 min rests, when partially operational the pump worked for 15 min and 45 min rests) 3.3–6.6 kWh day^{-1}; 3) pump for bringing the new water arrived from the sea to the 4th floor of the building (1.5 kW ton^{-1}), 0.05 kWh day^{-1} during the 6 operation months. Thus, the total direct energy consumption of the system was 11.65–12.65 kWh during the 24 h of operation. Additional, indirect source of energy for the system was the energy embedded in the nutrients (ammonium nitrate and phosphoric acid). Quantification of nutrients absorbed by the biomass in this system is currently under research and because of the high dynamics of their concentrations is not in the scope of this work.

The measured specific energy content for *Cladophora* sp was 15.042 MJ kg^{-1}, for *U. compressa* it was 14.310 MJ kg^{-1} and for *U. rigida* it was 9.879 MJ kg^{-1}. The maximum accumulated energy was calculated for the P_{max} of each species for all 14 cultivation MPBR and was 0.018 kWh day^{-1} (0.033 Wh L^{-1} d^{-1}) for *Cladophora* sp (starting cultivation density 0.05 gr_{ww} L^{-1}), 0.045 kWh day^{-1} (0.081 Wh L^{-1} d^{-1}) for *U. compressa* (starting cultivation density 2.5 gr_{ww} L^{-1}) and 0.016 kWh day^{-1} (0.029 Wh L^{-1} d^{-1}) for *U. rigida* (starting cultivation density 0.36 gr_{ww} L^{-1}). This translates to energy conversion efficiency from PAR to chemical energy of 0.35–0.53% for *Cladophora* sp, 0.87–1.3% for *U. compressa* and 0.31–0.47% for *U. rigida*. The effect of the initial cultivation density on the biomass energy accumulation on *Ulva* was investigated in

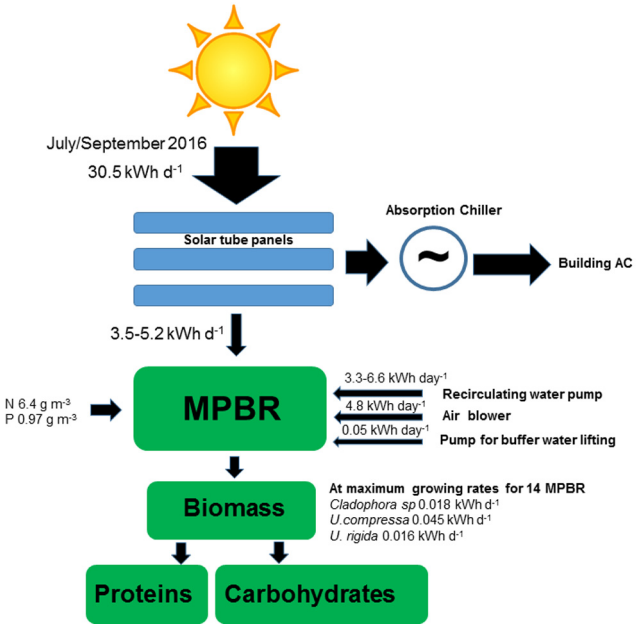

Fig. 6. Energy balance on the MPBR system inside the solar-thermal system of the PSES building. Solar energy was taken from global illumination data for August-September 2016 from the Israel Meteorological Service Database (http://www.ims.gov.il/). Solar energy input was calculated for the total area of 14 cultivation MPBR (4.48 m^2). Biomass energy accumulation was calculated assuming maximum growth rates of species in all 14 reactors with 0.15: dry: 1 wet weight ratio.

(Bruhn et al., 2011) and showed to have an optimum at 4 g L^{-3}, and is the subject of the future work for other species.

To summarize, MPBR operation has been demonstrated for 6 months on maintenance and cultivation of three green macroalgae species *Cladophora* sp., *U. compressa* and *U. rigida* isolated from 3 sampling points in the costal areas of Israel. Although the P_{max} for *Cladophora* sp., was 95.5% day^{-1}, for *U. compressa* was 16.79% day^{-1} and for *U. rigida* was 19.51% day^{-1}, very high fluctuations in the growth rate led to P_{av} of 3.47–95.47% day^{-1} for *Cladophora* sp., -40.76–8.03% day^{-1} for *U. compressa* and 2.01–4.61% day^{-1} for *U. rigida*, depending on the origin site. These large fluctuations cannot be explained by variations in illumination, mixing and nutrients supply and future studies are needed to understand the impact of the biological parameters, such as strains background, ploidy, age and sporulation on the growth rate fluctuations. Although the protein content for investigated biomass was high in comparison with other plants, 12% for *Cladophora* sp., 29% for *U. compressa*, and 33% for *U. rigida*, further studies are needed for protein content factor determination. Further work is needed for profiling of the residual, unhydrolysed polymers in the biomass.

4. Conclusions

In this work, we reported on the indoor, laboratory scale system that enables maintenance and cultivation of macroalgae in the building environment. MPBR operation has been demonstrated for 6 months on maintenance and cultivation of three green macroalgae species *Cladophora* sp., *U. compressa* and *U. rigida* isolated from 3 sampling points in the costal areas of Israel. The maximum measured growth rates for *Cladophora* sp., was 95.5% day^{-1}, for *U. compressa* was 16.79% day^{-1} and for *U. rigida* was 19.51% day^{-1}. The system provides a convenient platform technology that enables fundamental and applied macroalgae studies in the off-sea, urban laboratory environment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.06.061.

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