TEL AVIV UNIVERSITY

The Iby and Aladar Fleischman Faculty of Engineering

The Zandman-Slaner School of Graduate Studies

The effect of flashing light on macroalgae growth and energy conversion efficiency: the case study of *Ulva sp*.

A thesis submitted toward a degree of Master of Science in Mechanical Engineering

By

Oz Habiby

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This research was carried out in the

School of Mechanical Engineering

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Prof. Alex Liberzon & Dr. Alex Golberg

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Abstract

The world of bio-products is growing while the need for clean and green resources gains attention as part of new environmental awareness agenda and limited oil resources. Algae as a marine biomass product have a positive influence on the environment and a variety of byproducts for the cosmetics, fertilizers, and energy market. For bio fuels, the production of algae biomass today is not efficient enough; the costs are too high and the productivity is too low compared to oil barrel price and the energetic yield for investment. One of the limiting factors is the productivity per installed unit area due to low light utilization. Significant part of the light reaching the surface of the water is not being used for the photosynthesis because it is in excess to what current photosynthetic machinery can process. Addressing this problem by increasing the algae density is possible but still, in dense cultures the light does not penetrate through the first layer of algae and the lower layers are in the dark. One of the suggested solutions is mixing the culture. Mixing enables to bring lower layers to the surface and sink the upper ones after the dosage of light was absorbed. Mixing creates a light regime similar to flashing light in addition to other effects such as nutrient mixing, mechanical response of an algae and increased mass transfer. Every time a thallus reaches the surface it gets an amount of light and when it sinks down light is blocked and until the next time it surfaces. Similar methods of mixing were shown to work in microalgae photo bioreactors but not yet in macroalgae.

The goal of this research is to examine the influence of averaged outdoor intensity flashing light on the growth of the macroalgae in order to assess the effectiveness of the mixing. The technology for growing macroalgae under lab conditions or under field conditions exists but premature. The first objective of this research is to establish a new lab system for the study of macroalgae. The second objective is to examine the macroalgae productivity and utilization of light efficiency under flashing light regime in comparison to constant light.

A new design has been developed and built in our lab enables to examine time varying light influence on macroalgae. The data collected from the control group experiments show reproducibility of the results with up to 21% deviation from average, compared to 323% in similar macroalgae system in PSES, which means the

system supply uniform conditions and enables comparative experiments. The flashing light experiments show that under radiation of 1000 μ-mol photons/$m^2 \cdot s$ and with only 50% of the photon flux, up to 84% of the product can be achieved in comparison to constant light. As for the efficiency of light conversion to biomass, the results show a significant enhancement by an order of a magnitude under flashing light with 1% of the photon flux. For the same conditions, similar numbers were observed for the estimated potential of productivity which was higher by 10 times in comparison to constant light. Eventually, the results support the hypothesis that the productivity per area and efficiency of light conversion can be enhanced in dense cultures of macroalgae by sharing the constant light to flashes between several layers of algae.

For future directions we suggest the model can be implemented in offshore cultivation and we believe it will enable similar enhancement up to an order of magnitude in the efficiency of converting light to biomass. Different methods of converting constant light to flashing light should be examined and attenuation of the intensity can be taken into consideration too in order to reach higher efficiency.

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List of symbols

A – Area [m²]

ALR – Airlift reactor

AVG – Average

C – Carbon

DC – Duty cycle

DGR – Daily growth rate [1/day]

DW – Distillated water

DW – Dry weight [g]

E – Enhancement in efficiency

EI – Energy investment

Exp – Experiment

EY – Energy yield

Freq – Frequency [Hz]

FW – Fresh weight [g]

H – Depth [m]

I – Intensity [μ-mol photons/m²·s]

I_{opt} – Optimal intensity [μ-mol photons/m²·s]

I_{Amp} - Intensity amplitude [μ-mol photons/m²·s]

I_{avg} – Averaged intensity in time [μ-mol photons/m²·s]

L – Diameter of a single thallus [m]

L – ideal path length [m]

LHV – Low heating value [MJ/Kg]

N – Nitrogen concentration [PPM]

NDGR – Normalized daily growth rate [dimensionless]

NPP – Net primary productivity

P – Phosphate concentration [PPM]

P_A – Productivity per unit area [g/day·m²]

P_G – Gross growth rate [1/day]

P_m– Maximal net growth rate [1/day]

PAR – Photosynthetic active radiation

PBR – Photo bioreactor

PI – Photosynthetic irradiance

PNDGR – Potential normalized daily growth rate [dimensionless]

PSES – Porter school of environmental studies

PPM – Particles per million

PPT – Particles per thousand

R – Radius [m]

S – Salinity [PPT]

STD – Standard deviation

T° – Temperature [C°]

V – Velocity [m/s]

W – Weight [g]

d – Diameter of algae group [m]

f – Frequency [Hz]

k_E – Saturation parameter

k_s – Intensity influence constant

q_r – Radiated energy flux [W/m²]

r_{resp} – Respiration

t – Time [sec]

t_d – Duration of darkness [ms]

t_l – Duration of flash [ms]

x^*– The fraction of the functional activated PSU

yr – year

Γ – Ratio of integration of light [dimensionless]

α – Initial slope of the PI curve

ε_c – Efficiency of conversion light to chemical energy

ε_N – Net efficiency

ε'_N - Net efficiency under flashing light

ε_t – The efficiency of transforming the constant light to flashing light

μ – Net growth rate [1/day]

μ_{ctrl} – Control group growth rate [1/day]

μ_{max} – Maximal growth rate [1/day]

τ – Dimensionless time coefficient.

υ – Frequency [Hz]

ϕ – Duty cycle [dimensionless]

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1. Introduction

Sustainable production of food and energy is one of the major challenges for the next decades. The integrated production of food, fuels and platform chemical from biomass is coined bio-refining. Some of the key issues for biomass production are land availability, net energy balance, potable water use, environmental hazards and processing technologies. On shore biomass yields do not answer the problems of land availability and potable water use. Marine biomass does not rely on these sources and for that might bring a better solution to the problem. The on-shore cultivation of microalgae for energy, though promising, is currently impossible due to the costs of required land preparation, infrastructure and negative net energy balance on the entire systems [1] However, an expanding body of evidence has demonstrated that marine macroalgae can provide a sustainable alternative source of biomass for sustainable food, fuel and chemical generation [2]–[3].

Macroalgae, which contain very little lignin are promising candidates for future sustainable food and transportation fuel feedstock. One of the limiting factors in the macroalgae to bio-fuel value chain is the biomass yields affected by the process known as the light/dark reactions [4]. Plants have evolved the ability to harvest almost 100% of the arriving protons [5]; however, the total photosynthetic efficiency is only around 5% due to the low utilization of photons. In the natural illumination, at the outdoor environment, the rate of photosynthesis is not limited by a number of photons but by internal process in the photosynthetic reactions [6]–[8].

Several works has already proved which different species, microalgae, tomatoes, soybean and potatoes [9]–[12], can grow under flashes using the light more efficiently in conversion light to biomass [10], [13], [14]. The amount of photons being supplied to the leaf should be not exceeding to the saturation zone and not limiting the ideal growth rate. During flashing light it is suggested that he intensity, which the leaf experiences, is averaged in time [10]. High intensities can be shared between several groups of algae and attenuated to efficient levels [10].

We suggest that by external intervention, the photon flux arrives the culture of algae, can be manipulated in order to increase the photon utilization and save energy.

External mixing exposing different layers of the cultivation to the light or other geometrical solution should be examined to allow the various thallus layers to receive light for a certain time interval [5]. This approach is expected to increase the yields per installed area using deeper volumes for cultivation.

The effect of mixing on the energy gain was estimated before [5] on *Ulva sp*. an interesting feedstock due to its high growth rate [15]–[17]. The ethanol productions estimations for Ulva are 17% of the dry weight [16]. Previous articles have already suggested the enhancement of photosynthetic efficiency for micro-algae by flashing light [14], [10], [18]; however, there is no research on Ulva macro-algae. For these reasons we suggest to study the cultivation of *Ulva sp*. as a case study for the macro-algae cultivation under pulsed light.

This study focuses on the flashing light influence on the growth rate of Ulva. The results of the experiments enable to design a reactor supporting the photosynthetic process with optimal illumination. Sharing the flux of photons arriving to the surface between the algae, in denser and deeper cultivation more efficiently will lead to higher productivity per installation area.

The final purpose of our research is to improve the areal productivity of the raw material for the bio-refinery. Ulva crops could create an economic added value in the fields of food, cosmetics and agriculture. Off shore bio-refining as an innovative industry can provide new employment opportunities for coastal countries and assist the deduction of reliance on petroleum products on the road to energetic independence.

2. Literature review

2.1. Biomass for the fuel market; general model, pros and cons

The general model of biomass to bio-fuel procedure is similar the standard refinery model and coined bio-refinery [19]. The raw material is being collected and transported to a center where it is converted and processed to different products and eventually distributed. The difference is the source of the raw material and the converting process; in the oil industry the source is a well field located in one place, while for biomass, the source is distributed [16] and can be different organisms as switch grass, corn, sugarcane and algae. Oil wells are available only in certain areas around the world but for the bio-refinery, farms and fields of crops are common wherever settlements exist. The conversion of oil includes separation of the materials by refining while the conversion of bio-mass can include many stages from extracting sugars and proteins to digestion of bacteria in the fermentation process of bio-fuels.

Biomass sources has a variety of pros; the biomass source is renewable, it is not related to specific areas and can be grown almost everywhere around the globe; different types of biomass in different areas. Another major advantage is the reduction in emission of greenhouse gasses. During the life of the organism it absorbs CO_2 equal to the amount emitted while it is being burnt. This is based on the assumption that the biomass is 100% renewed and while a unit of biomass is being used another is being cultivated. That is relevant for SO_2 emissions too[20]. Another advantage is the contribution of biomass industry to the regional development. The energy density of bio fuels can reach up to 45 [MJ/kg] [21] but in most of the reports the values are between 15- 43 [MJ/kg] [22] . Due to the limits of the energetic value of bio-fuels the transportation is also limited (will be discussed on the cons section) the solution is to grow and treat the biomass on local range. This concept creates a self sustaining system which produces, process and use the product in a limited region [16]. One example is the power generation system in India [23]. In national scale, biomass source for bio fuels can create economical security as not many countries are reach is fossil fuels.

The first major disadvantage of biomass sources is the competition of bio-fuels with food on the land and tap water. This competition can create a balance

which is pushing higher prices on the food markets [24]. An example is the rising corn prices during 2000-2008 while it was widely used for ethanol production under government subsidies in the US [25]. Another disadvantage is the complicated logistics in order to collect biomass to the bio-refinery and the distribution of the bio-fuels to the market. The lower the energetic value of the fuel, the more units needed for the customer and so the transportation costs. With lower energy density the efficient transportation range also decrease. Some additional risks related to the fact that intensive cultivation demands fertilizers and pesticides; these can contaminate surface water. So as use of genetically modified organisms, this can result in spread over natural habitats and cause extinctions and mutations.

2.2. Macroalgae; Pros & cons, Productivity, limiting factors

Marine biomass sources have 2 substantial advantages over the terrestrial biomass sources. First, it does not require potable water which is often in need in developing countries. Second, it does not require ground which is already in high demands for agriculture, industry and residence. The marine area has an unfulfilled potential in terms of space, resources and innovativeness. It also does not have lignin which is a major issue in biomass processing.

Different challenges limit the production of bio fuels from macroalgae. First, the economical aspects: the total cost which is depend on the labor costs and the materials. Other limits are the total efficiency of the procedure, bio fouling and nutrients availability. One of the most significant parameters is the biomass productivity. In comparison with other biomass crops; the productivity of algae (micro and macro) is relatively high [26], and yet that’s not enough. A reference number for the productivity of Ulva can be used from offshore cultivation near Tel Aviv which returned an annual average of 5.8 g dry weight per square meter a day [27]. A target value for productivity, 25 g $dw/m^2 \cdot d$, was defined in Ref. [26] for a full scale bio fuels production facility. The United States DOE (Department Of Energy) defined target value for 2020 as 3,700 gallons of algal bio-fuel intermediate (or equivalent dry weight basis) per acre per year (gal/acre/yr) on an annualized average basis which is equivalent to 151 g $dw/m^2 \cdot d$. The gap between the results of the offshore cultivation and the target values displays the need in enhancing productivity.

Biomass productivity is defined as the generation of mass per unit surface (or volume) per unit time. The productivity can be defined in relation to the final product such as sugars or proteins. For example, using the chemical composition of the biomass the energy yield can be determined.

The (Net Primary Production) NPP is defined as the rate at which all plants produce net useful chemical energy. It is an important to estimate and predict the production of a biomass source. The measurement in terms of carbon mass fit our goals because carbon is the important element in the composition of bio fuels (and other carbon based fuels). In the following table presented the values for different biomass sources with a special part focusing on macroalgae [27].

Table 1. NPP of different biomass sources.

Biofuel Crops	NPP (gr C m^{-2} $year^{-1}$)
Switchgrass	622
	624
Miscanthus	1546
	1489
Rice	631
Corn	408
	713
Wheat	378
	320
Sugar cane	1721
Food crops	613
Middle East (C_4, perennial, leguminous and woody)	290
Macroalgae	
Laminaria-Ascophyllum (Nova Scotia)	1900
Macrocystis (Kerguelenn archipelago)	2000
Laminaria (South-West England)	1225
Macrocystis (California)	400-820
Codium fragile (Long Island)	696-4700

Ulva sp. (Ria Formosa Lagoon (estimation))	190
Ulva compressa (Minicoy Atoll)	1460
Ulva rigida (Venice lagoon)	358
	646
Ulva sp. Reading Tel Aviv (measured). Grown in a single layer photobioreactor	838

It can be seen from **Table 1** that the NPP of macroalgae is relatively high. Other results support the argument such as the high productivity of cultivated macroalgae [28].

In order to enhance productivity several approaches may be considered. Some of the solutions are:

1. Enhance CO2 and nutrients supply using industry exhaust gases and artificial fertilizers or upwelling of rich nutrients water from the depth [29]–[31].
2. Improve reactors design in order to enhance CO2 and nutrients uptake and the light exposure to each one of the unit in the reactors [31]-[32].
3. Genetic engineering [34].
4. Improving sunlight conversion to biomass by limited flashing light regime [5].

In this project, we will focus on the basic question whether it is possible to improve light utilization per installed unit area by shortening the exposure time of algae and exchanging between layers of algae. We would try to enhance yields using manipulation of light/dark sequence for algae. The energy conversion in the photosynthetic procedure is being described by ratio between the energy invested in photons to the energy yield in biomass heating value. For the cultivation of Ulva near Tel Aviv the annual average was 0.35% for global radiation or 0.7% for PAR radiation only (calculation detailed in materials and methods).

2.3. Ulva; Characteristics and life cycle, cultivation and applications

Ulva, a green marine macroalgae of worldwide distribution found in the intertidal and shallow waters within the Israeli Mediterranean shores. In addition to

the seaweed high growth rates, glucose is a major monosaccharide in the Ulva and can be fermented to bio ethanol [35].

Ulva is characterized by wide leafs and may refer sometimes as sea lettuce. It usually grasps to rocks along the sea shore but can also grow in open water with no grip [27]. Ulva can reproduce in 3 different ways [36]. The first, vegetative reproduction, a thallus is being torn in 2 pieces; each one will grow as an independent unit. The second is asexual reproduction, where zoospores are being formed in every cell and released gradually to the water through the cell wall. At the end of the process nothing is left of the thallus but a thin film of empty cell wall. After swimming for a while, the zoospore finds a substrate and grasp to it to form the holdfast and later the blade which will grow to be the leaf itself. The third is sexual reproduction, where zoospores are being developed into sexual plant with 32 to 64 biflagellate gametes in each cell. When a fusion occurs, zygote is formed and moves to find a substrate and holds to it. It forms a holdfast and later a blade and develops into a full lead [37].

Cultivation methods vary from unattached cultivation in open ponds or plastic bags to attached cultivation on ropes and carpets from the seeding, through the nursery phase to the full leaf development [35], [38]. The literature about cultivation of macroalgae in lab scale photo bio reactors is limited [40]- [41]. Like other types of algae the Ulva requires light, nutrients, carbon and a range of environmental conditions as salinity, temp and pH. The specific values for *Ulva sp.* are not yet known but for *Enteromorpha* (*Ulva compressa*) which is a macroalgae of the same family most of the values have been estimated. The optimal temperature is about 25°C and the limits of positive growth rate are 5-35°C [41]. The optimal salinity is about 18 PPT and the frame is 0-45 PPT. The optimum light intensity is 600 μ-mol photons/m^{2}·s, the lower or upper limits are unknown (previous research show positive growth rate with 0.6 μ-mol photons/m^{2}·s) [41]. For the nutrient levels the model was based on previous research [19].

The Ulva chemical composition enables diverse usage in applications from pharmaceutical, cosmetics and food and energy production. Just few of the examples: Proteins being extracted from Ulva is suggested to support anti aging treatments [42]. Fertilizers made of Ulva is recommended in combination with chemicals for the growth of vegetation [43]. Ulva can be used as added value products for human

nutrition purposes [44]. Ulva biomass can be used also for bio fuels applications. Production of bio ethanol or bio methane can be made by extraction of sugars and fermentation [27], [45].

2.4. PBRs; the need, concept and previous designs

A PBR is a system producing biomass using a light source and supplying specific conditions to the organism. It enables high growth rate, usually higher than in the natural habitat. PBRs are widely used in the micro algae industry enabling to produce very pure biomass. The light source, the growing media, nutrients source and the flow source, can each be natural or artificial. Usually the systems are being distinguished as open or closed systems [46]. The open systems are exposed to the outside world by water contact or atmosphere and the closed systems are almost isolated from the environment. Anyway, the product is similar biomass of the same species. Offshore PBR will probably provide slightly different characteristics in the product in comparison to lab PBR due to less controlled environment.

The need of PBRs is both for scientific research and for industrial use. In order to test different influences on the biomass during experiments all variables must be uniform besides the studied parameter. This type of experiment cannot be implemented offshore because the lack of control in the environmental conditions, the potential for contaminations and the operational challenges comes with offshore experiment. Artificial illumination, media and constant nutrients flux can be provided in lab conditions and with a proper design almost any variable can be tested.

The simplest PBR is a race pond. It is a circular pool with a partition in the middle and a pedal creating circulation of water along the pool. The illumination arrives from the sun and the system is open to environmental influences [47]. The conditions in the PBR are approximately equal to all the thallus.

More complex design are the tubular PBR or flat panels for micro algae [46]. The PBR should be able to control environmental conditions as: flow, temperature, pH, salinity illumination and contaminations. Some includes self cleaning systems to handle the bio-film being accumulated on the surface of the panels. This type of PBRs can be places outdoor with natural illumination or indoor with artificial illumination. Positioning such a system (tubes/panels) indoor enables to control environmental

parameters more easily but requires resources of space and illumination which lead to greater expenses.

Another type of system is the hanged plastic bags which is a type of tubular reactor but with partitions between the cells. One of these systems can be found in Porter School of Environmental Studies in Tel Aviv University [40]. In this system there is no ability to control the position of the algae and in adjacent, the illumination. Lab scale experimental systems testing light influence for micro algae exist but the scale of the reactors is too small for macroalgae [18], [14]. Another system for macroalgae was suggested as recirculation metabolism chamber [48] where the algae is attached in the growth chamber and a pump circulates the water from the tank around it. The constant position of the algae in the chamber enables to control the illumination. This design is an improvement of a previous PBR suggested for tests on corals [49].

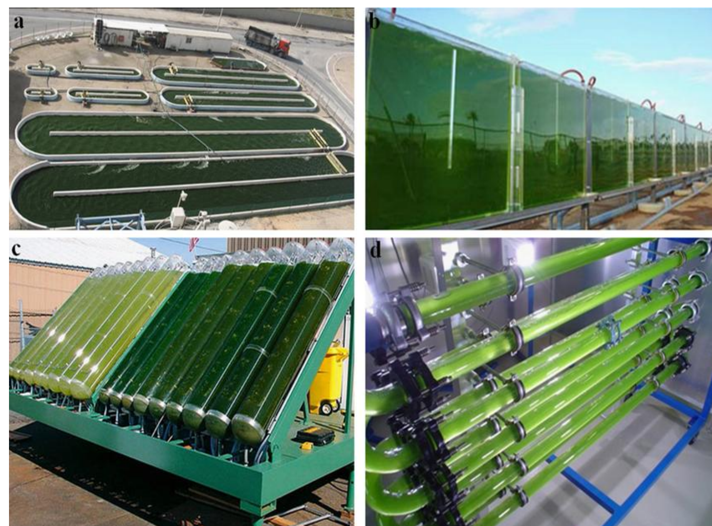

Figure 1. PBR designs: a- open ponds, b- flat panels, c- outdoor tubular PBR, d- indoor tubular PBR [50].

Our experiment requires uniform conditions to all reactors and total separation in illumination. Some tubular reactor has been suggested, similar to micro algae reactors, but a system to examine flashing light influence on the growth rate which fulfills these demands has not been found.

Figure 2. Macroalgae Indoor tubular PBR design (Reef builders Inc. https://reefbuilders.com/)

Many different aspects create a set of design criteria that will influence the design of a PBR; we will try to examine few. The product characteristics can affect the design, what is expected to have at the end of the process? Is it a batch or stream system? The species will create a system of consideration about the conditions we want to provide. The cost of creating these conditions will stand against the benefit and the profitability of the conditions effect on the product. The light source will be taken into consideration in the design in order to maximize efficiency and usually enhance the growth rate. If the light source has intensity stronger than needed it can be dispersed in a form or plate or tubular PBR. Another consideration is the expected density of the biomass in the reactor. Dense population might need illumination from several directions and in this case a race pond will not be suitable.

In different PBRs the artificial illumination is different according to the product [51]. The influence of wavelength selection has been tested on the cultivation of *Ulva fasciata* [52]. The research focused on the PAR spectrum which is the part of spectrum being used for photosynthesis. The results show picks in the absorption on the blue and red areas of the spectrum. Not surprisingly, the green didn't show any picks because the color of the algae is green and most of the green light is being reflected.

Mixing and flow rates are another factors in the design [46]. CO2 and nutrients availability so as the removal of waste from the surrounding of the organism are all depends on the mixing in the reactor. Low mixing might function as a limiting factor in the biomass reproduction. Mixing will also influence the fluctuation in

illumination in dense cultivations. Most of microalgae cultivation using CO2 or air bubbles for mixing. In attached cultivation, which is a platform that the algae grasps, the water moves on the surface of the algae instead of moving the algae in the water; the mixing is replaced with the movement of the water on the surface.

These are only few aspects of the design criteria of PBRs; the full description of the system and the consideration is described under materials and methods section.

2.5. Photosynthesis; components and stages

Photosynthesis is a process of carbon fixation in which water and CO2 are being used with light energy to create organic compounds. This process is being conducted in plants, algae and some bacteria [71]. It is the basic process which enables all living creatures to exist and responsible for the gas balance in the atmosphere. The general reaction of the process is: $12H_2O + 6CO_2 + \text{Light} \Rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$. In this process organic compound; glucose, is being created from light and non organic compounds; oxygen and water. The photosynthesis takes place in an organelle called the chloroplast [54]. It contains high concentration of chlorophyll which is a pigment that allows the plant to absorb light energy. Two types of chlorophyll exist in green plants; a and b. These two types of chlorophyll are the most common and adjusted to visible light, the difference between them is the wavelengths which they absorb most [71].

The process can be divided to 2 stages. The first, which is the light dependent reaction, where molecules of ATP and NADPH are being created using energy of light, and the second, which is the light independent reaction, where these molecules are being used to capture and reduce carbon dioxide [54].

The light dependent reaction can occur in 2 paths; the cyclic or non cyclic, and occur in the tylakoid which is membrane compartment inside the chloroplast. Photo system I is where the cyclic path starts in the chlorophyll. A pigment in the chlorophyll absorbs a photon and release an electron which continue to an electron acceptor molecule and return to photo system I with the creation of the ATP molecule [71]. The non cyclic path starts with the electrons from last stage which does not return to the photo system but being used to create NADPH molecules. Photo system II is where photons are being captured in the chlorophyll and used to disassemble

water to oxygen and hydrogen. Electrons from the water take the place of the missing electrons in the photo system and the remainder energy from the disassembly is being used to create more ATP molecules.

The independent stage starts with carbon dioxide being absorbed from the environment; it is attached to the enzyme RuBisCO where the cyclic chain reaction called Calvin Benson cycle starts and eventually creates glucose. This stage requires only ATP, NADPH and CO2 and can occur in total darkness [71].

2.6. Flashing light effect; PI curve and the integration of light effect

The PI curve (Photosynthetic Irradiance curve) describes the relation between photosynthetic rate and light intensity [53]–[54], similar to the example in **Fig. 5.** The chart starts with a sharp climb followed by saturation area and beyond decrement can be found due to photo inhibition.

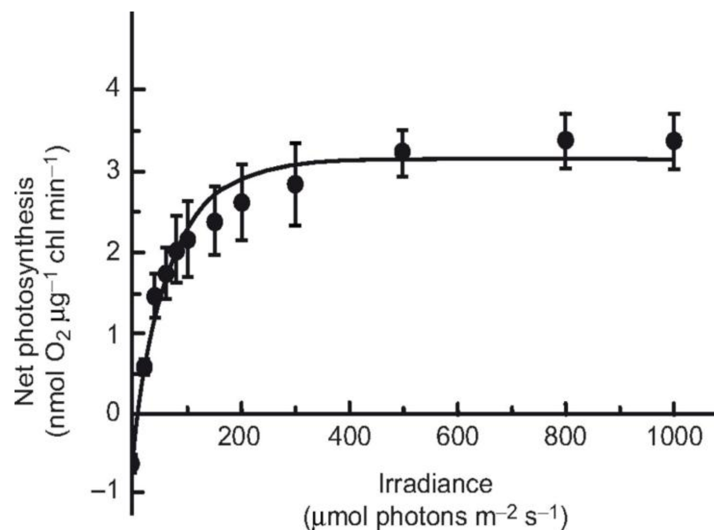

Figure 3. PI curve of micro algae living in scleractinian corals [43].

The units of photosynthesis can be described in mol of O2 being released [14] or mol of CO2 being absorbed [55], either way it represents the metabolism and chemical energy creation in the organism. The units of illumination are µ-mol photons/m²·s which is special unit describing only radiation in the PAR spectrum. The adjust growth rate can be measured either by optical density, cell number or biomass accumulation [14].

The area of the chart where saturation and photo inhibition takes place represents inefficient process. The irradiance above certain levels goes to waste and these values are not rare in the Israeli climate [56] and also in other areas around the world as India and the United States [57]–[58]. More than that, while the intensity is too strong for the organism and reach above a certain value, part of the photosynthetic system in the cell is damaged which leads to decrease is the biomass photosynthetic activity and productivity [59], [54]. This effect also known as photo inhibition and familiar as the need to partially shade some of the crops in sunny areas like Israel in order to protect them. For the same averaged intensities the photo inhibition effect is as twice higher for continuous light rather than flashing light [54] and that is another reason to avoid strong intensity with constant light and consider flashing light.

Another phenomenon that should be introduced is the integration of light. When a plant receives flashing light in high frequency it is suggested that the influence equivalent to the averaged intensity in time [10][13].

$$I_{avg} = \frac{1}{T}\int_{t_0}^{t_0+T} I\, dt \qquad \textbf{(1)}$$

In the following example, for a tomato leaf, the black columns represent the constant light and white columns represent the flashing light [55]. The average intensities in time are equal in both and so as the grow rate.

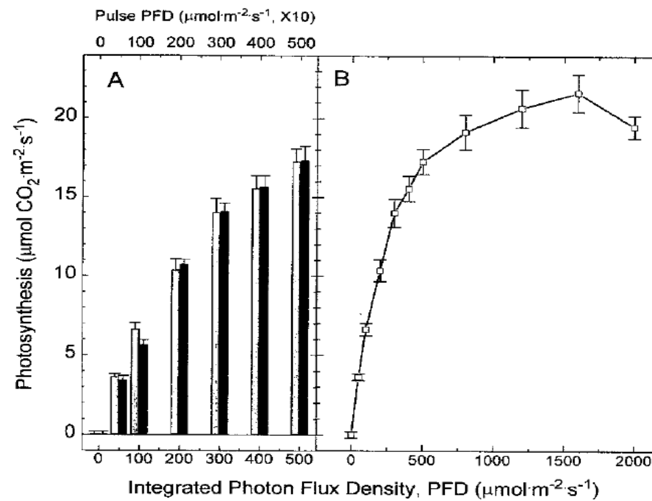

Figure 4. A- The photosynthetic response of tomato leaves to continuous light (black) and pulsed light (white). B- PI curve of tomato leaves [46].

The growth of microalgae under flashing light have been proven to be successful by several researchers before, all taking in to consideration the integration of light under high frequencies. The strong intensities with the flashing light was suggested as a new method for cultivation [10], [60].

In the following **Table 2** we present a list of previous experiments with flashing light on microalgae and other plants. It can be seen from the papers that the flashing light effect have returned successful results with several of organisms and with equal productivity compared to constant light. From each paper the best setup of parameters can be taken in order to be tested with macroalgae.

Table 2. Previous experiments with flashing light.

Subject	Researcher	Reference	Year	Most productive setup
Chlorella	J. N. Phillips & J. Myers	[60]	1954	Freq 4, DC 20%
Phaeodactylum tricornuturn	K. L. Terry	[10]	1986	Freq 7, DC 3%
Dunaliella salina	S. Abu-Ghosh	[14]	2015	Freq 50, DC 50%
Tomato	D. J. Tennessen	[55]	1995	Freq 100, DC 1%
Soybean	T. L. Pons	[11]	1992	NR
Potato	R. C. Jao	[12]	2004	Freq 720, DC 50%
Nannochloropsis salina	E. Sforza	[18]	2012	Freq 10, DC 10%

Freq represents frequency and DC represents Duty Cycle, with both its simple to describe flashing light.

The limit of intensity can be coined as the photon flux tolerance and was observed to be especially high for algae. The flux tolerance in open pond is typically 200-400 μ-mol photons/m²·s but it has been proved that algae tolerance is higher, up to 2000 μ-mol photons/m²·s [61] which is equivalent to the outdoor natural peak of intensity. Other studies, with tomato leaf, have proved that under flashing light even

higher intensities up to 5000 μ-mol photons/m²·s [55] can be implemented under the tolerance limit.

A new research suggested enhancement of the growth rate by combination of constant and flashing light [14]. This idea simulates what actually happens in mixing of macroalgae in a pond, constant weak light arrives from dispersion and flashing light hit the thallus when it reach the surface during the mixing cycle.

An interesting question is what is the lower limit of the frequency for full integration of light? A research on Nannochloropsis salina shows that its value is between 5-10 Hz [18], yet other research points to around 1 Hz [10]. We can asses that for different types of algae the number will be in the same magnitude.

From these two phenomena above, high intensities and integration of light, we can see a problem and a possible solution [59]: high levels of intensities might go to waste (saturation) or harm the algae (photo inhibition) but instead, it can be used more efficiently by sharing the photon flux by mixing to different groups of algae and using the integration of light method. The averaged intensity can be reduced to the efficient levels (on the climbing slope of the PI) and eventually grow more biomass per unit area.

2.7. Growth rate models; *Ulva compressa*, flashing light and mixing

When we want to examine the procedure for engineering applications, a model must be suggested in order to describe the process. A comprehensive model for *U. compressa*, a was described before [41] and mentioned here because it is suitable for the description of light influence (additional models will be reviewed during the chapter). The model is governed by the following equation

$$\mu = \mu_{max} \cdot f(I) \cdot f(T°) \cdot f(S) \cdot f(N) \cdot f(P) - r_{resp} \quad \textbf{(2)}$$

It is described as a function of maximum theoretical growth rate (μ_{max}), at the optimum light (f(I)), temperature (f(T)), salinity (f(S)) and internal concentration of nutrients (f(N,P)) minus the respiration (r_{resp}) [41]. When all environmental conditions are in the optimum level the growth rate is the maximal in relation to the theoretical value. In order to describe the light influence an optimum function was

used, simulating the slope on one side of the x axis and photo-inhibition effect on the other side of the axis:

$$f(I) = \frac{I}{I_{opt}} e^{\left(1 - \frac{I}{I_{opt}}\right)} \quad \textbf{(3)}$$

Where I is the measured photon flux density and I_{opt} is the optimal intensity which is suggested to be 600 for this type of seaweed [41].

The next step in describing the light function is understanding the integration of light influence using another implementation [10]. If we assume all other environmental conditions are constant and their functions is being taken into consideration in the f(I) function, the gross growth function (P_g) can be described as

$$P_g = f(\phi I) \quad \textbf{(4)}$$

Where $\phi = \frac{t_l}{t_l + t_d}$ is describes Duty Cycle which is the ratio between the light period (t_l) to the total cycle of flashing light ($t_l + t_d$) . If the integration of light does not takes place the equation form changes to

$$P_g = \phi f(I) \quad \textbf{(5)}$$

And because the PI is a monotonous linear function at the beginning and later reach saturation, the relation between the two equations will always be

$$f(\phi I) \geq \phi f(I) \quad \textbf{(6)}$$

In order to examine the integration of light the authors [8] were using the following equation

$$\Gamma = \frac{P_g(I, \phi, \upsilon) - \phi P_g(I)}{P_g(\phi I) - \phi P_g(I)} \quad \textbf{(7)}$$

$P_g(I, \phi, \upsilon)$ is the observed growth rate at intensity I, flash ratio ϕ and frequency υ. If the value of Γ is 1 then the integration of light is full. If less than 1 it is only partial or none exist when Γ equal 0. This model will not be implemented in our work because it requires the full PI curve but it gives the important understanding that also partial integration of light can take place.

The model we used for the light influence function is the PI curve in the form of a derivation of the Michaelis–Menten curve. Disregarding the decrement of photo inhibition in high intensities the equation takes the following form [62]

$$\mu = \mu_{max} \frac{I}{k_s + I} \tag{8}$$

Where μ represents the growth rate, μ_{max} is the theoretical maximal growth rate under optimum intensity, I is the measured intensity and k_s is the value of intensity which will return growth rate of half μ_{max}.

In order to translate the flashing light to mixing regime a mixing module should be defined also. The cultivation model for our work is based on Liberzon & Golberg paper [5] suggesting an offshore deep cultivation of algae including mixing which creates a cyclic movement of the algae to the surface and back to the depth. With every cycle the layers rise to the surface, absorb photons and later sink to the depth into the darkness. When the cycle is being repeated, it is a sort of flashing light for the thallus group.

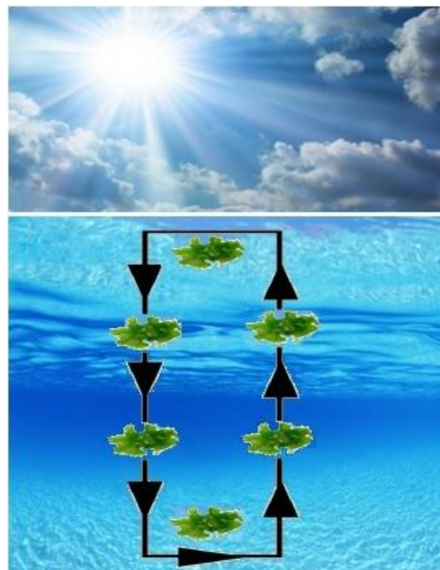

Figure 5. Schematic sketch of the mixing model for offshore cultivation and flashing light regime [5].

According to the path length, streamline and mixing velocity the light and dark period are defined. The ideal path length L is a function of the light independent

reaction t_d, the maximum time requires the receptors absorb maximum photons t_l, averaged mixing velocity V and the diameter of a single thallus l.

$$L = V \cdot (t_l + t_d) \quad \textbf{(9)}$$

Assuming an air lift mixing device with a square streamline with surface length R and depth H, the light, dark period and the ratio can be calculated as

$$t_l = \frac{R}{V} \quad \textbf{(10)}$$

$$t_d = \frac{R + 2H}{V} \quad \textbf{(11)}$$

$$\phi = \frac{R}{2R + 2H} \quad \textbf{(12)}$$

Combining these models creates a growth rate equation as a function of intensity I, light/dark ratio ϕ calculated on the base of the geometry of device and the velocity. The relation between geometry and light dark reaction is brought by Liberzon & Golberg [5], the form of the PI equation by Theor [62], and the relation between integration of light and PI curve by Terry [10].

$$\mu = \mu_{max} \frac{\phi I}{k_s + \phi I} \quad \textbf{(13)}$$

The questions we are trying to address here will be examined also by the model. Will the Ulva grow under flashing light and follow the integration of light effect? Can we estimate the parameters for the model based on similar species of macroalgae? And what is the potential in terms of productivity of sharing the constant light to several groups of algae by flashes.

2.8. Efficiency models; enhancement of converting light to biomass

The efficiency of energy conversion in this case is being defined as the ratio between the photons energetic investment to the chemical energy yield of the biomass. The efficiency changes as a function of intensity, in the low-flux regime photosynthetic efficiency is greatest, where the photons input rate is low enough to

avoid bottleneck effects, so each absorbed photon can be fully exploited for photosynthesis [59].

The theoretical limit for the efficiency was calculated on the base of 8 moles of photons per a mole of hexose synthesized [59]. For the whole PAR spectrum the theoretical efficiency can be estimated as 17%. Previous research showed results of 15% as expected from the theoretical model [59].

The net efficiency (dimensionless number) of the photosynthetic process in relation to the illumination was defined as [10]:

$$\varepsilon_N = \frac{P_G(I) + r_{resp}}{I} \quad \textbf{(14)}$$

While $P_G(I)$ is the gross photosynthetic rate in as function of intensity I and r_{resp} is the respiration value (negative in this display). And in adjustment the net efficiency of the photosynthetic process under flashing light:

$$\varepsilon'_N = \frac{P_G(\phi I) + r_{resp}}{\phi I} \quad \textbf{(15)}$$

While ϕ represents the duty cycle. These expressions have units of photosynthetic rate ($\frac{\text{fmol } o_2}{\text{cell} \cdot \text{h}}$) to intensity ($\mu - \text{Eins}$) what means it cannot be used as efficiency expression which is dimensionless, but this is not the final expression, later; the author defined the enhancement in the photosynthetic efficiency as:

$$E = \frac{\varepsilon'_N}{\varepsilon_N} \quad \textbf{(16)}$$

And with this expression the multiplication in photosynthetic rate can be assessed. With this method of calculation [8] enhancement of the efficiency in constant light compared to flashing light demonstrated increase of up to 15 times, based on data from Phillips and Myers research [60].

An efficiency of 15% was also reached with *Spirulina* micro algae using a thin rectangular bifacial PBR and double sided illumination and a high dense cultures [61]. Unfortunately, most of the published data on flashing light effect lack the engineering perspective of efficiency calculation and will force us the suggest our own calculation that will be detailed under Materials and Methods section. Yet, based on the data that

was displayed above comparison in enhancement can be made and the range of values can be compared.

3. Goals and objectives

One of the bottlenecks in the bio fuel production is the productivity per unit of the cultivation area. The yields are too low for efficient energetic cycle and cost effective production. The yield per unit area can be increased by developing more efficient methods for cultivation.

A study of the effect of flashing light on macroalgae and the potential it has on enhancing conversion efficiency does not exist and this is the gap we will be filling in this research.

It is hypothesized here that higher yields per unit area can be achieved by using the light more effectively and increasing the photosynthetic efficiency.

4. Materials and Methods

4.1. Approach

In Ulva cycle from biomass to final product many stages are included: cultivation, harvest, drying, pre-process and final process to a desired product. The factors influencing on the algal growth rate are described in the following Figure:

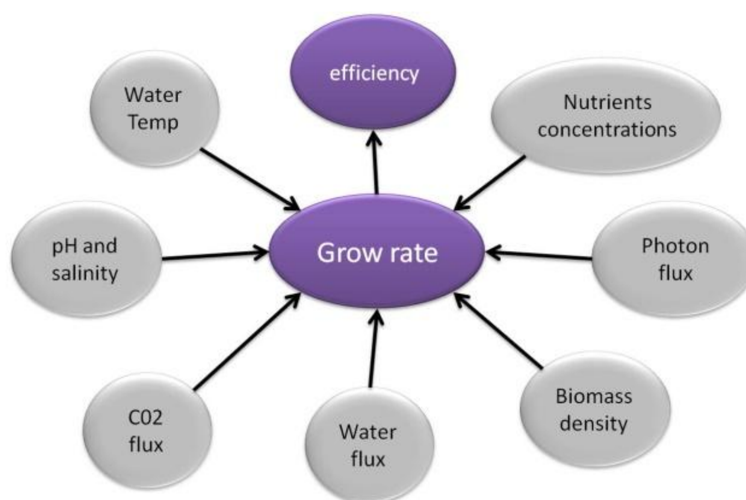

Figure 6. Algal growth rate contributing factors.

The growth rate is based on many different independent parameters. Our experiment focused on the influence of light on the growth rate. Lack or excess of light leads to poor growth rate or mortality of the algae.

The question of this research is can we enhance the yields of the biomass by using these excess photons? Separating this problem to smaller sub problems will be the aim of the following paragraphs.

First, what is the maximum number of photons per unit area per time period the algae can use converting to chemical energy? The number of photons will be parallel to the intensity strength of the illumination. The definition of intensity is how many photons hit a unit area per unit of time.

Second, what is the rate of the absorption of photons or what is the time period between these photons being supplied to the algae? The mechanism of absorbing the

photons and using it in the photosynthetic cycle has a time frame. One option is that while a certain unit (thylokoid) is handling the last absorbed photon it is not free to absorb the next one, another option is that this process might be limit in a later stage that prevent from the cell to use photons being absorbed. This "busy" period is followed by absorption period in a cycle. These two periods together can be described as flashing light cycle parallel to the light-dark reaction. The algae absorb photons in the light period and busy during the dark period. This process can be also described with flashing light characterized by the frequency and duty cycle.

We will discuss specific flashing light properties: intensity, frequency and duty cycle.

Intensity will be described as the number of PAR (photo-synthetic active radiation) photons arriving to a surface in a finite time period. Units of μ-mol photons/m^2·s (sometimes referred as μ-Einstein) will be used in the following pages.

Frequency will be defined as the number of cycles repeated in one second. One cycle starts at the moment the first photon hit the surface and finish at the end of the dark period.

Duty cycle will be defined as the ratio of light and dark periods within one cycle. The light period is the time section that photons hit the surface and the dark period is the time section no photons arrive.

The basic idea is to share the constant light flux between several layers or groups of algae in form of flashing light. It is suggested that after algae received the needed photons for its growth, during it busy period the following photons will go to the next algae. With this idea, the excess light can be used effectively. The next algae will arrive from a deeper layer of water or just from behind the first algae, after being hidden in the shade of the previous.

Back to the working factors in the cultivation process, each one can function as a limiting factor. In order to test the influence of illumination properly it is needed to create uniform conditions for all other parameters. More than that, a certain parameter in absent may lead to incorrect picture of the results. That means that all parameters must be at level of optimum or in the saturation area. This project will not try to find the whole field of parameters and optimums but to create a picture of a

certain area of the map. The independent parameters will be defined according to the targets of the project and the results will reflect the investigated environment of the illumination parameters. The variables that will be tested are the frequency and duty cycle, the intensity will be kept constant and equal to ambient in Israel with 1000 μ-mol photons/m^2·s [56].

4.2. Lab experiment

The experiments were built relying one on another. The first step was a control group experiment in order to test the experiments system and understand the growth rate under nominal conditions of constant light. The 2nd step was testing several flashing light regimes according to previous studies on other types of algae. With this data compared to the control group, the 3rd step was decided to focus specifically on DC and its influence. Eventually on the 4th step additional data was collected to support the different stages.

Table 3. Experiment program stages.

Step	Topic	Number of experiments	Repetitions
1	Constant light	3	18
2	Flashing light	3	2
3	Duty cycle influence	3	3
4	Additional data	6	3

4.2.1. Step 1 – control

During these set of experiments, we have tested the growth rate of the algae in the system under constant light with intensity of 1000 μ-mol photons/m^2·s. Because the experiment is being conducted along several weeks, the subjects are different by age and genome between one week and another. In addition, due to the fact that the experiment system is a prototype, a preliminary data has to be collected in order to understand the growth rates of the algae under nominal conditions. With this data, we were able to determine either certain weekly results are different than normal, lower or higher. Lower growth rate can be a result of age, biological conditions or imply on

a problem with the setup of the experiment or contaminations in the system. Eventually the data collected during this part of the experiment was analyzed and used to assess the success of the following parts. This part lasted 3 weeks, each week 1 experiment of 3 days with 6 reactors.

4.2.2. Step 2 – flashing light tests

During this part, several combinations of light parameters were tested in order to check the influence on the algae growth rates. The light parameters sets were based on previous data of flashing light experiments with other organisms [10], [13], [14]; frequency between 1 to 50 Hz and DC between 3 to 50%. During every week 2 of 6 reactors used as control with constant light and intensity of 1000 µ-mol photons/m²·s. The rest of the reactors were being tested with the different flashing light sets and equal intensity as the control. Testing the data collected in this part; we tried to identify trends and influence of the grow rate potential. This part lasted for 3 weeks, 1 experiment per week, with 2 repetitions for each set of a flashing light, total of 6 different sets. Using the conclusions, the last part was designed to focus on DC influence.

4.2.3. Step 3 – flashing light focusing on DC

In these set of experiments the frequency of the flashing light was fixed to be 1 Hz and the DC was changing between 1-50%. During every week 2 of 6 reactors were being used as control with constant light and intensity of 1000 µEinstein. The rest of the reactors were being tested with the different flashing light sets. This part lasted for 3 weeks with 3 repetitions for each set of a flashing light, total of 4 different sets.

4.2.4. Step 4 – additional collection of data

In the last part, additional data was collected in order to give strength to the previous parts. A saturation experiment was performed with constants light and intensities of 400, 700 and 1000 µ-mol photons/m²·s. Additional experiments of flashing light with frequency of 1 Hz and DC of 5, 60 and 75% and negative control (dark reactors) were performed. This part lasted for 5 weeks with 2 repetitions for each set, total of 4 different sets.

4.3. The experiments program

Along the 4 parts of the research described above the experiments protocol was constant. Only the flashing light parameters were modified between the experiments. Each experiment lasted 3 days and 2 nights. Each day included 9 hours of illumination and the night lasted for 15 hours of darkness. For the convenience, we will be referring to the time of the experiment as 3 days and so as the results will be analyzed. The meaning is that the daily growth rates that will be displayed are actually 2.5 days growth rate divided by 3. The algae biomass was measured at the beginning and end of each experiment in order to calculate growth rates. The initial biomass weight was constant through all of the experiments 0.6 g. During the experiments morning and evening measures were performed to adjust flow rate and measure temperature in the water.

4.4. The experimental system

The influence of different time periods and duty cycles of illumination in the light/dark regime was examined on group of thalli. The system supply uniform conditions to each one of the 6 reactors (Flow rate, pH, temperature, salinity, nutrients concentration) with simple control system. In each reactor, a group of thalli in uniform weight and characteristics were positioned. The reactors were seated in dark chambers, separated from each other by partitions which allow testing each one with different light parameter. The subjects were weighted at the beginning and at the end of the experiment to examine the growth rate.

Figure 7. The experimental system in operation with flashing lights visible in the background.

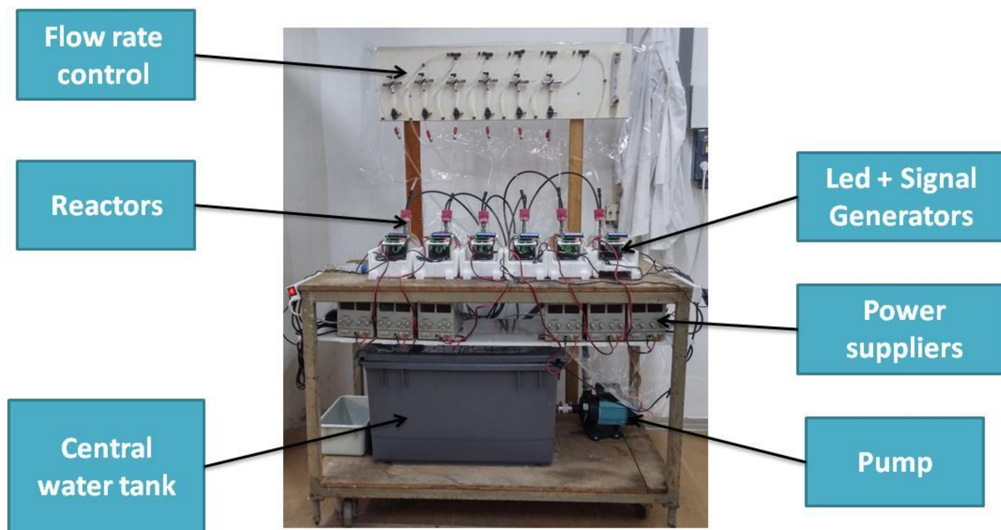

Figure 8. The experimental system main components

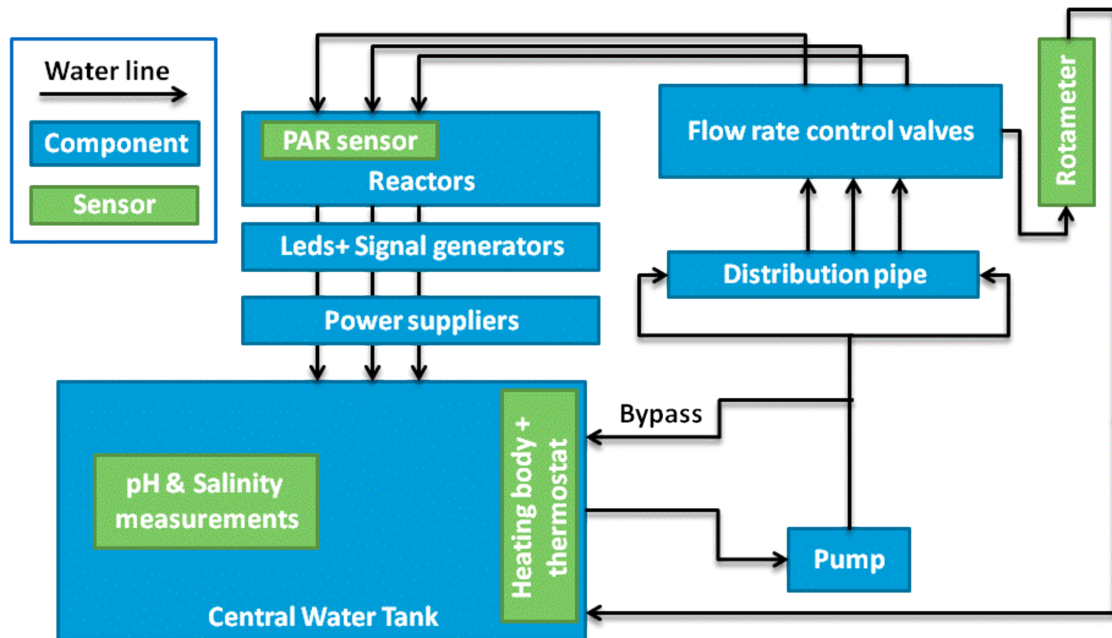

Figure 9. The block diagram of the experimental setup.

4.4.1. Flow rate control

The experiment system includes 130 L tank (Plasson, Israel) of water with centrifugal pump (ESPA, PISCIS1, Spain) circulating the water in closed loop through 6 test tubes where the thalli were positioned. The water flows from the tank to the pump through 32 mm PVC flexi tube (pal-yam, Israel) and to a distribution pipe (PVC, length 1m, width 5mm, Plasson, Israel). From the distribution pipe 6 thin pipes (Legris 8x5.5 mm polyurethane polyether) are connected to a series of valves including bypass system and a rotameter (50-800 ml/min, error 1.25% FS, Emproco Israel). Each tube is connected to 3 valves in a row, first – a simple ball valve to start or stop the flow, second – a sensitive needle valve to control the flow rate, and third – a 3-way valve to direct the flow to the rotameter or to the test tube (fast connectors and valves, Ilan Gavish, Israel). The valves system enables to direct each of the 6 pipes to the rotameter separately or to the matching test tube. This set of devices enables to fix the flow rate for each test tube 850 ml/min. The reactor is a round bottomed flask (1 L, 13 cm diameter, Duran group) with one inlet tube and one outlet tube drilled through the rubber cup. From each test tube the water flow out through the outlet pipe returning the water to the tank. Within the system, a filter of 130

microns was integrated on the bypass line in order to collect organic waste from the water.

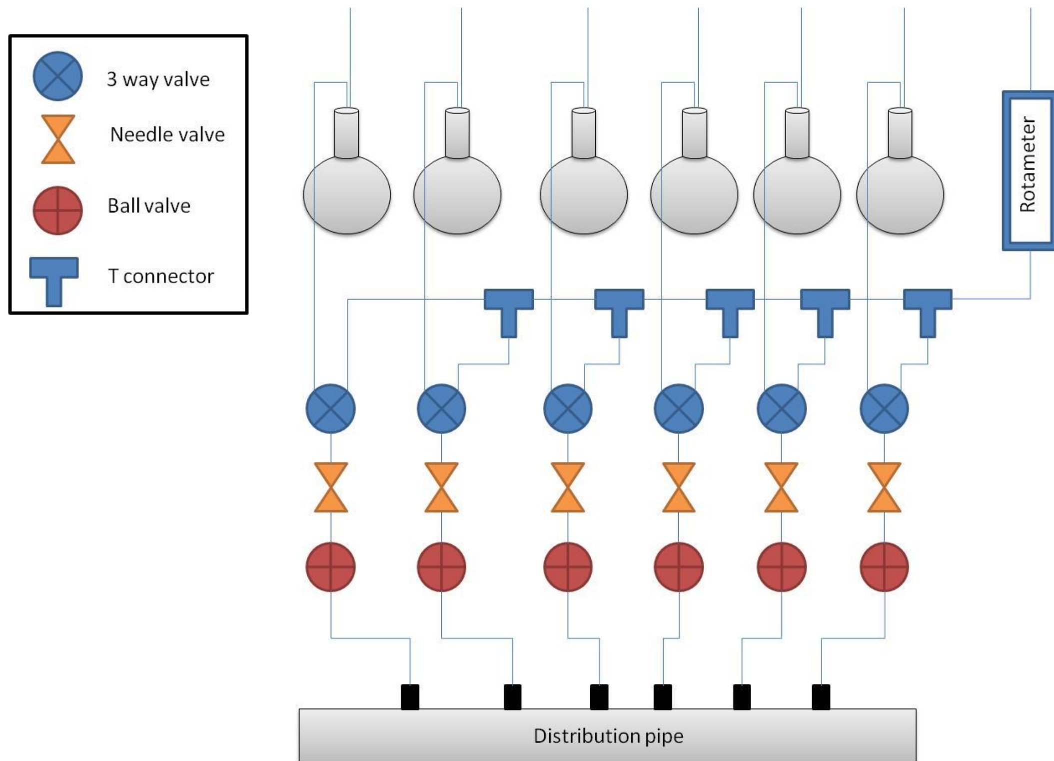

Figure 10. Schematic diagram of the valves system.

Figure 11. The valves system.

4.4.2. Temperature

Within the water tank a 300W heating body and a thermostat (JEBO 2010, China) were integrated. The thermostat was programmed to maintain water

temperature over 23 C°. The room temperature was adjusted by the local air condition system to 20-25 C°. Twice a day water temperature measurements were taken. The temperature was measured in the tank near the inlet of the pump using thermometer ((-10°)-150°, 1° resolution, Livingstone).

4.4.3. Illumination system

Each test tube was positioned with a matching LED system (60W PAR grow light LED, Flora Photonica, Israel) enabling to control the illumination parameters for each test tube. The LED light includes 6 colors in the PAR wavelengths (380, 430, 460, 630, 660, 740 nm) 3 blue and 3 red. Each led was connected to a signal generator (1-3000 Hz, 1-99% Duty Cycle) and a power supplier (MCH-303A, 30V/3A, Lion electronics, Israel) controlling illumination intensity (up to 4000 µmol-photons/m²/s).

The wavelengths were measured with MK350 spectrometer (360-750 nm, UPRtek, USA). The LED light radiates as a point source. All intensity measurements were taken at the center of the tube using Li-Cor 192 PAR radiation sensor (400-700 nm, error 5%, Li-Cor USA). The test tubes were separated with simple cartons partitions to prevent mutual influences. The tests for the illumination system are described in appendix C.

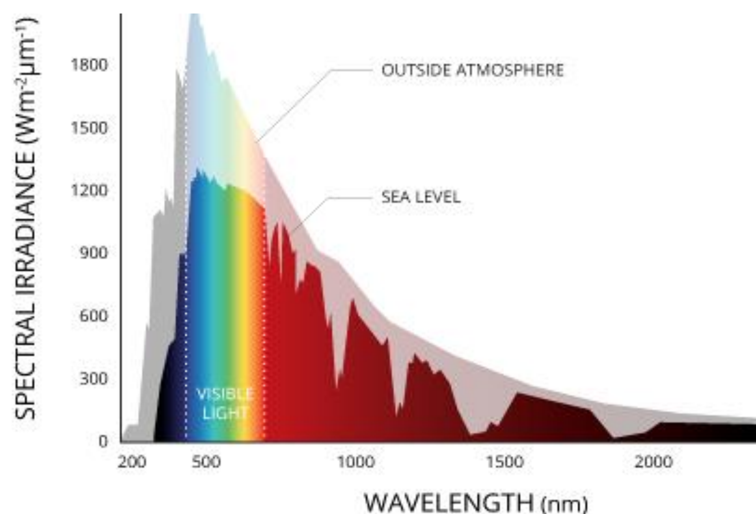

Figure 12. The sun spectrum.

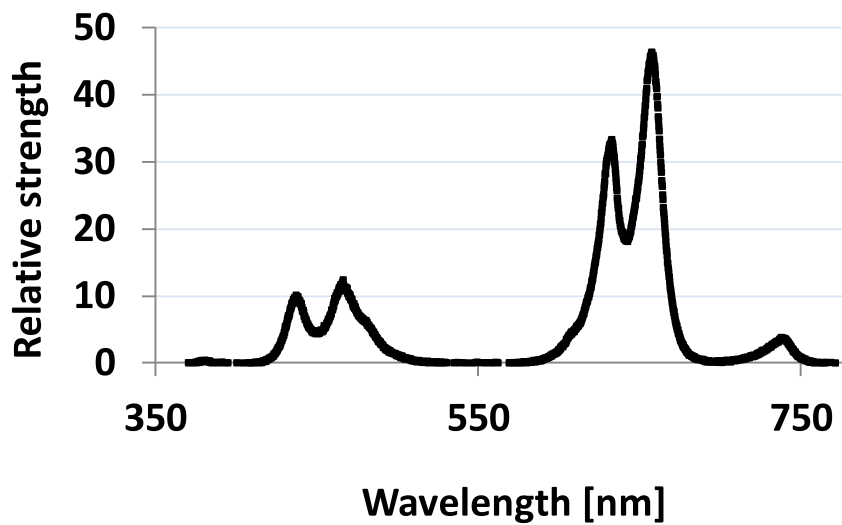

Figure 13. The Led spectrum

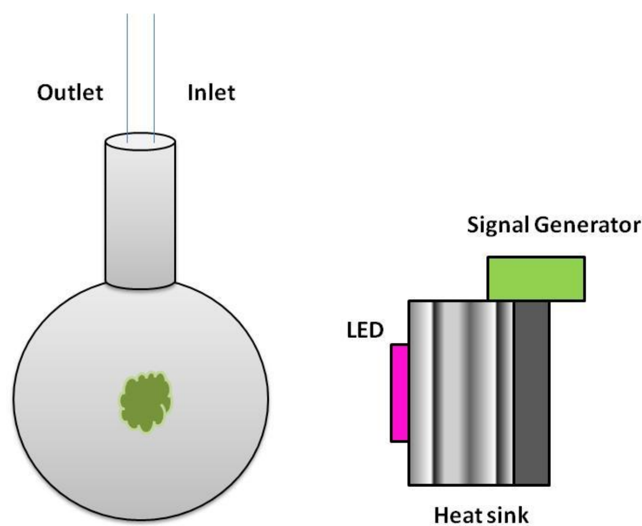

Figure 14. The LED and reactor setup

4.4.4. The media

For each experiment the media was replaced with fresh clean water. 75 L of DW was mixed with 2.865 g of Red Sea salts (Red Sea, England). And fertilizers salts (detailed next paragraph). The water was mixed for 12 hours before the experiment.

4.4.5. Nutrients concentration

For PO_4^{-2}, NaH_2PO_4 fertilizer was added to the water and for NH_4^+, NH_4Cl fertilizer. 1.855 g of NH_4Cl and 0.282 g of NaH_2PO_4 fertilizers salts were measured with XS105 analytical scales (0.01 mg error, Mettler Toledo, Switzerland). Both fertilizers were added using nutrients calculator (Appendix D.) Uniform flow rate (maximum 2% difference) supplied to the different test tubes guarantee equal nutrients flux to thalli. The Initial concentrations of nutrients in each experiment were: Phosphate (P) 0.97 PPM, Nitrogen (N) 6.47 PPM.

4.4.6. pH and salinity

Water pH and salinity levels were measured before every experiment in the tank. The experiment started with values of 8 ± 0.5 pH measured with pH tester 30 (error 0.01, Oakton, USA). Salinity of 35.5 ± 0.5 PPT measured with refractometer (0-70 PPT, error 0.5, I.P.L, Israel).

4.5. The subjects

The algae were taken from Israel Oceanographic and Limnological research center in Haifa (IOLR), where it being cultivated for research. The algae were kept in a pond in Porter School of Environmental Studies (Tel Aviv University) for a few days and later moved to the experiment system in Wolfson Building of Mechanical Engineering. The species is assumed to be Ulva rigida which is a local algae type that grows along the sea shore of Israel.

The subjects of each weekly experiment share the same genes as they are being taken from the same thallus. At the beginning of each experiment 3-4 different thallus were divided to 6 test tubes with total fresh weight of 0.6 ± 0.03 g.

4.6. Weighting the thallus

The dry weight is defined as the constant weight that a thallus will approaches after sufficient time drying in the oven. The water on the surface and from within the cell evaporates and only dry mass remains. The ratio between the dry weight to the fresh weight was already measured in our lab – 15%.

In this experiment, we need to record the fresh weight of the thallus. This parameter is not fully defined in the literature. Weighting a living thallus demands keeping the water within the cells, only the water from the surface should be removed quickly from the thallus to avoid evaporating from within. Few short experiments (appendix B) proved that a drying method using paper towels in a two minute time frame since the thallus was taken from the water returns constant values for repeated tests. A simple protocol was defined as the standard weighting procedure for the experiment: First the thallus is being dried using lettuce dryer for about 30 second and later the thallus is being dried with paper towel to remove leftovers of water from the surface in a period of no longer than 2 minutes. The measurements were taken using analytical scales BBA-600 (error 0.01 g, MRC, Israel). With this method and the relation to the dry weight the procedure is fully defined.

4.7. The growth rate model

The suggested model, based on a previous model used with *U. compressa*, is a function of theoretical maximal value μ_{max}, environmental conditions (temperature, salinity, nutrients flux and concentration) in the system and respiration as was introduced in the literature review in Eq. 2 [41].

Neglecting respiration and setting the environmental parameters to specific values gives a factor out of the environmental conditions functions. The illumination function is being represented only by intensity in the base model. We will assume the Intensity is in saturation level but the light is not constant, it is a flashing light. The flashing light will be implemented as a square wave characterized by amplitude (I_{Amp}) ·, frequency and duty cycle.

$$\mu = \mu_{max} \cdot a_1 \cdot f(I_{Amp}) \cdot f(freq, DC) \quad \textbf{(17)}$$

One last step in order to simplify the model is the assume frequencies is high enough for the effect of integration of light [63]. If this assumption is true and the frequency can be neglected, than the meaning is that for the algae the light is constant and only DC governs the PFD (Photon Flux Density). When the frequency influence is being neglected another factor goes out and the theoretical maximum for lab conditions is being determined and coined as μ_{ctrl}.

$$\mu = \mu_{ctrl} \cdot f(I_{Amp}) \cdot f(DC) \quad \textbf{(18)}$$

If the algae see the flashing light as constant light than the function $f(I)$ will act like a saturation curve. In this case the averaged intensity in time can be used for the growth equation. For a square wave the averaged intensity can be calculated simply by the duty cycle and amplitude:

$$I_{avg} = \frac{1}{T} \int_{t_0}^{t_0+T} I \, dt = I_{Amp} \cdot DC \quad \textbf{(19)}$$

T represents the time period. Eventually the final equation reduced to:

$$\mu = \mu_{Ctrl} \cdot f(I_{avg}) \quad \textbf{(20)}$$

Several examples for equation of the illumination function were already introduced in the literature review. If the integration of light takes place the function should act as a PI curve. We chose to use Michaelis Menten equation, and in order to assess its constant, Ks, we have used the optimum intensity function suggested for *U. compressa* (Eq. 3). Ks constant represents the value of intensity that will grow half of the maximal growth rate. Using the optimum intensity from the model and with the function of intensity, using numerical solution Ks can be calculated to be 140 [μ-mol photons/m²·s]. Thus, the function of illumination in the form of Michaelis Menten finally gets the form of:

$$f(I_{avg}) = \frac{I_{avg}}{140 + I_{avg}} \quad \textbf{(21)}$$

From the second stage all of the results will be displayed in normalized growth rate value according to

$$\frac{\mu}{\mu_{Ctrl}} = \frac{I_{avg}}{140 + I_{avg}} \quad \textbf{(22)}$$

μ_{Ctrl} represents the growth rate on the control group of each experiment. This is the final form of the model that will be used to predict results.

4.8. Equations and calculations

4.8.1. Growth rate

The following equations are being used through the report. The daily biomass accumulation as we defined it:

$$\mu\left[\frac{g}{day}\right] = \frac{W_2 - W_1}{N_{days}} \tag{23}$$

The growth rate is being measured by the difference between final weight W_2 and initial weight W_1. In order to express the results with numbers in relation to the initial biomass we define μ_{rel} or DGR as was previously suggested [27]:

$$\mu_{rel}[\%] = DGR = \frac{W_2 - W_1}{W_1 \cdot N_{days}} \cdot 100 \tag{24}$$

The error in assessing the biomass accumulation is dependent on the weight error (W_{error}):

$$DGR_{Error}\,[\%] = \frac{\left(\frac{2 \cdot W_{error} + W_2 - W_1}{W_1 - W_{error}} - \frac{W_2 - W_1}{W_1}\right)}{N_{days}} \cdot 100 \tag{25}$$

When the comparison will be made between different groups of flashing light parameters the results will the displayed as normalized growth rate in relation to the control. This method enables to compare different experiments results and keep one standard value:

$$NDGR[\%] = \frac{DGR}{DGR_{Ctrl}} \tag{26}$$

4.8.2. Productivity per unit area

On the same basis, the productivity per unit area a day (P_A) is defined according to the cross section area of the test tubes, which is A=0.00432 m².

$$P_A\left[\frac{g}{m^2 \cdot d}\right] = \frac{\frac{W_2 - W_1}{N_{days}}}{A} \tag{27}$$

Examining an ALR (airlift reactor) model; the DC can be expressed in terms of geometry (**Fig. 25**). For a certain average velocity V, frequency f, ALR radius R and depth L, the DC can be calculated as:

$$DC = \frac{R}{2R + 2L} = \frac{f \cdot R}{V} \quad \textbf{(28)}$$

Also the depth L can be expressed in terms of the right side of the equation using the next relation:

$$L = \frac{1}{2f} \cdot V - R \quad \textbf{(29)}$$

That means that in order to create a certain DC and determine depth of the ALR we need to define less parameters or in other words the problem has been reduced from 5 variables to 3.

Assuming that with deeper ALR the algae groups can be multiplied, this means that the yields can be greater per installation area until a certain limit of minimal growth rate. The results can be displayed as PNDGR (Potential NDGR) which is a function of the depth L and R or the DC. The number of the groups (or layer) is:

$$N_{groups} = \frac{100}{DC} = \frac{2R + 2L}{d_{group}} \quad \textbf{(30)}$$

d_{group} represents the length of 1 algae group and we will choose $d_{group} = R$ in order to determine DC according to Eq. 28 and have 1 group of algae on the surface at a time during the mix. The Potential of NDGR is:

$$PNDGR = NDGR \cdot N_{groups} \cdot \varepsilon_t \quad \textbf{(31)}$$

ε_t is the efficiency of transforming the constant light to flashing light. On the same basis the potential productivity can be calculated for several flashing light groups.

$$P_A\left[\frac{g}{m^2 \cdot d}\right] = \frac{\frac{W_2 - W_1}{N_{days}}}{A} \cdot N_{groups} \cdot \varepsilon_t \quad \textbf{(32)}$$

4.8.3. Energy conversion

In order to estimate the efficiency of the process we would like to estimate the ROI (return on investment) for the energy balance. The energy investment (EI) is the number of photons that hit the surface of the algae in the reactor in terms of PFD (photon flux density [μ-mol photons/m²·s]) and the energy yield [EY] is the biomass accumulation with it heating value.

$$\varepsilon_c = \frac{EY\left[\frac{\text{biomass LHV}}{m^2}\right]}{EI\left[\frac{\text{photons}}{m^2}\right]} \quad \textbf{(33)}$$

This ratio for offshore cultivation of Ulva, as was introduced in the literature review is 0.35% with annual averages in outdoor conditions. For the bio-reactor in the lab the calculation is presented in the following paragraph.

The energy yield is calculated using the biomass accumulation ΔW , the dry to fresh weight ratio $0.15 = \frac{FW[g]}{DW\,[g]}$ and the LHV=11 $\left[\frac{MJ}{Kg}\right]$ (both measured in our lab)

$$\Delta W[g] = W_2 - W_1 \quad \textbf{(34)}$$

$$\Delta E\ [KJ] = \Delta W[g] \cdot 0.15\left[\frac{g}{g}\right] \cdot LHV\left[\frac{MJ}{Kg}\right] \quad \textbf{(35)}$$

The energetic yield (EY) in terms of KJ/m² is calculated according to the cross section area of the test tubes, which is A=0.00432 m².

$$EY\left[\frac{KJ}{m^2}\right] = \frac{\Delta E}{A} \quad \textbf{(36)}$$

The radiation flux (q_r) is being calculated according to sun spectrum with intensity of 1000 μ-mol photons/m²·s and the DC determine the fracture of light that being used. Transforming μ-mol photons/m²·s to Watt/m²·s is being calculated using the factor of 0.219 and the total energetic investment (EI) is calculated using the time of illumination in order to receive the results in Joules.

$$q_r\left[\frac{W}{m^2}\right] = 1000 \cdot 0.219 \cdot \frac{DC}{100} \quad \textbf{(37)}$$

$$EI\left[\frac{KJ}{m^2}\right] = q_r\left[\frac{W}{m^2}\right] \cdot \frac{t\,[sec]}{1000} \quad \textbf{(38)}$$

Eventually, the efficiency of conversion photon energy to chemical energy within the algae:

$$\varepsilon_c = \frac{EY}{EI} \quad \textbf{(39)}$$

In this chapter we have described the materials, methods and calculation that have been used through the research and the next chapter relies on this work.

5. Results and analysis

The results are being displayed in daily growth rate (DGR). The mass was recorded at the beginning and the end while the illumination hours were recorded along the experiment. The data was processed as daily growth rate, taking the total growth rate and dividing by 3.

5.1. Step 1 Control group results

These set of experiments were conducted in order to examine the nominal growth rate in the system under constant light conditions and understand the abilities and performances of the system. Can the system return uniform results under uniform conditions? Are there any differences between the reactors that influence the results? And how similar are the algae groups between one week and the other in their growth potential? These are the questions we will try to answer in the following pages.

In order to answer the first question – can the system return uniform results under uniform conditions? We want to examine the distribution of results. In the first 3 experiments groups the algae grew in all 6 reactors with nominal conditions and constant light. In the following 6 experiments groups only 2 reactors used as control while the rest where used as flashing light reactors. The results of the total control groups are being displayed in the following Figure.

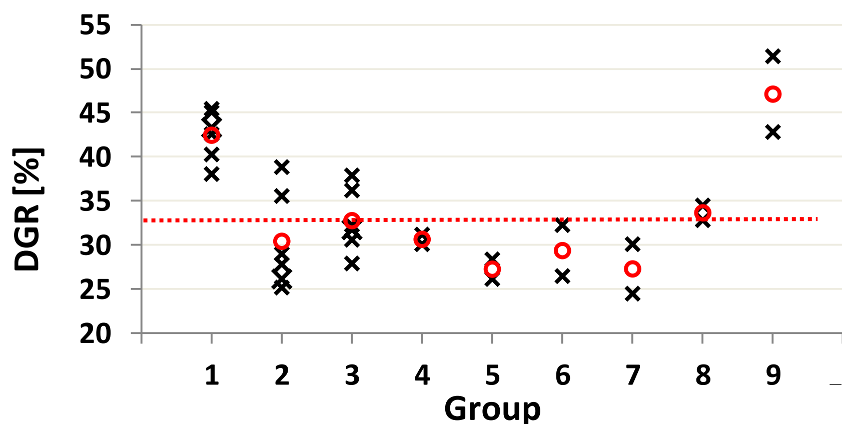

Figure 15. Control reactors results by DGR. (x) Repetition, (o) Group average, (---) General avg.

The general AVG of the 30 repetitions is 34.1% and the STDV is 7.04%. It can be seen that the differences between the groups is significant. The AVG of group 5 (the lowest) is almost half of the AVG of group 9 (the highest); only 57% of its value. This inconsistency demands a series of experiments to understand the reasons and forming a solution; a Taguchi experiment design can be used in this case [64] but averaging method was selected to reduce the noise and variations as every following experiment was repeated at least once, while for the control group; every experiment included 2 reactors.

In comparison, *Ulva sp*. cultivated offshore next to Tel Aviv showed annual average of 4.5% DGR [27] and in indoor PBR system located in PSES experimental data showed averaged growth rate of *U. rigida* between 2.01-4.61% and of *U. compressa* between 2.89-3.65% [40]. The reasons for the differences between our lab results and the offshore cultivation can be in the lack of nutrients offshore in the east coasts of the Mediterranean [27]. For the differences between our lab results to the lab system results in PSES the fertilizing is similar, so the reason can be linked to the length of the experiments or the method of cultivation. The experiments in PSES last for 89-175 days while in our experiments the cultivation last for only 3 days. The growth rate curve tends to be sharper during the first days of cultivation [18]. The method in PSES is hanged plastic bags with intensive aeration which leads to harsh shear stress while in our system the thalli were kept and protected in the reactors while the water was moving around them in circulation. Another possible reason is the temperature of the water in PSES. During the experiments the temperature varied between 18-33°C[40], this range can be harmful for the algae.

The inner group variation is not negligible; was up to 21% of the average (31.5±6.5% DGR in group 2). But in comparison, the variations in the results of PSES system were up to 323% of the average with *U. rigida* for 89 days. For *U. compressa* the variations were up to 480% of the average for 175 days experiment [40]. The meaning is that the system results are stable within the experiment group in comparison with similar systems.

The next step is to examine the results on the distribution chart in **Fig. 16**. The assumption that the results should distribute as a Gaussian is being supported by the

Shapiro-Wilk test for normality which returns value of 0.98. Yet, this assumption can be questionable as from visual inspection it seems like a double humped distribution.

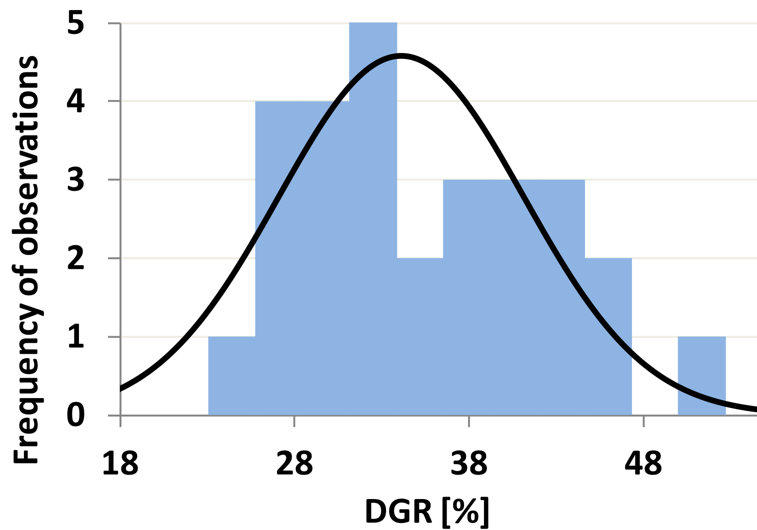

Figure 16. General distribution of the results by groups of DGR.

One possible explanation for the distribution shape is that within the general test sample we can find two sub-populations. If we will separate groups 1&9 from groups 2-8, the distributions of the sub-populations seem to be closer to Gaussian with the largest amount of samples in the middle as can be seen in **Fig. 17**. We can refer the groups 2-8 as "Normal" and the groups 1&9 as "High" – referring to a higher growth rate. Week 1 began on the 12 of December 16 and week 9 on the 6 of February 17.

Taking a closer look at the results of weeks 1 & 9 (**Fig. 15**) it's possible to see the results are higher than the average (34.1%) of the total sample group. While the weekly average of weeks 2-8 is below 35%, the weekly average of 1&9 is above 40%.

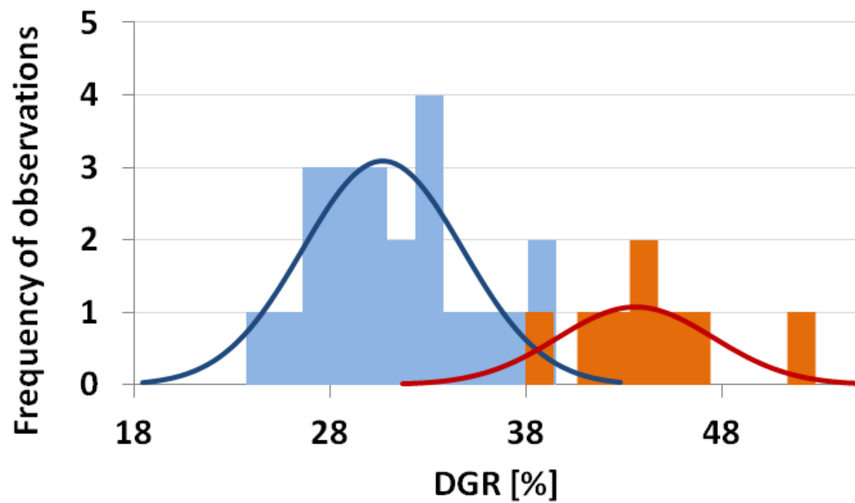

Figure 17. Control group results divided to different classes and Normal/High categories.

The theoretical curves were calculated according to the group's means and standard deviations. Shapiro-Wilk test for normality stands for both groups with values of above 0.95.

The groups were divided to 2 categories according to the weekly averaged control growth rate; High and Normal, as described earlier. The P value for the 2 categories is 10^{-8} , this result is not trivial. Testing every other random combination of 2 groups' results (in the "High" category) in comparison to the rest of the groups (in the "normal" category) does not return similar result for the P Value which means this result is significant.

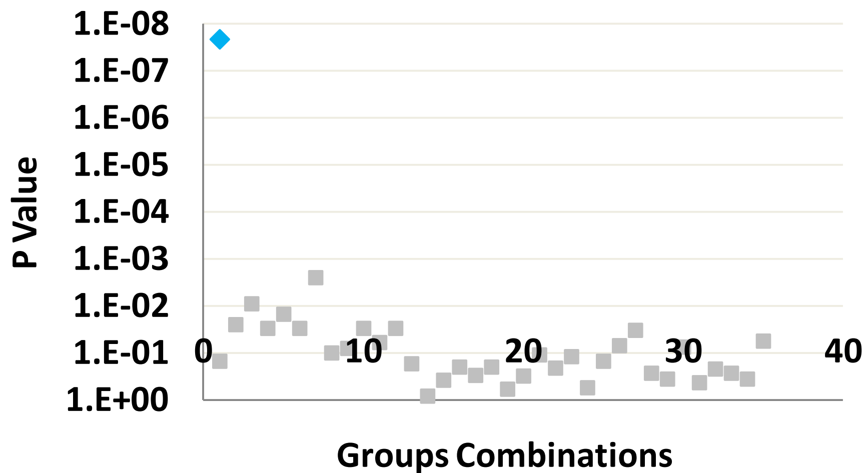

Figure 18. Different combination of Normal/High categories and their P Values.
◆ The tested category (1&9) ■ The rest of the combinations

One assumption is that during these weeks ("High") there was a bloom that leads to higher growth rates. For week 9 there are measurements from offshore cultivation supporting the idea of a bloom during March and April and in adjust to the result in the lab [27].

In relation to the results and assumption discussed in the last paragraphs the control group AVG and (standard deviation) STD are displayed in the following table:

Table 4. Control group AVG and STD in the different week.

	Average DGR [%]	STD [%]	Repetitions
Groups 1-9	34.11	7.04	30
Groups 2-8	30.67	4.08	22
Groups 1&9	43.58	3.97	8

It can be seen from **Table 4** that when taking all groups into consideration the general STD is the highest. We can see that for "Normal" and "High" the STDs of both categories are similar and lower than the general STDV. In the general sample group the STD is 7% but when the groups were divided to "Normal" and "High" the STD dropped to 4% what implies on 2 different population as being assessed in a T test.

Another question is what is the inner-group difference? This time only groups 1-3 will be examined because the later groups included only 2 control reactors which do not provide enough data for STD calculation. The data is in the following table:

Table 5. Control group AVG and STDV in the first 3 groups.

	AVG DGR [%]	STD [%]
Group 1	42.41	2.85
Group 2	30.38	5.51
Group 3	32.73	3.68

As it can be seen from the table there is a difference between the weeks by the AVGs. A normalizing method must be implemented in order to compare between different weeks' results. The differences in the STD support the need for repeating and averaging all results. According to these results and the previous analyze it have been decided that every week will include 2 control reactors and all results will be normalized in relation to the control reactors AVG as NDGR (Eq. 26).

A relation between specific reactor and higher/lower growth rate was examined in order to examine possible influences on the results.

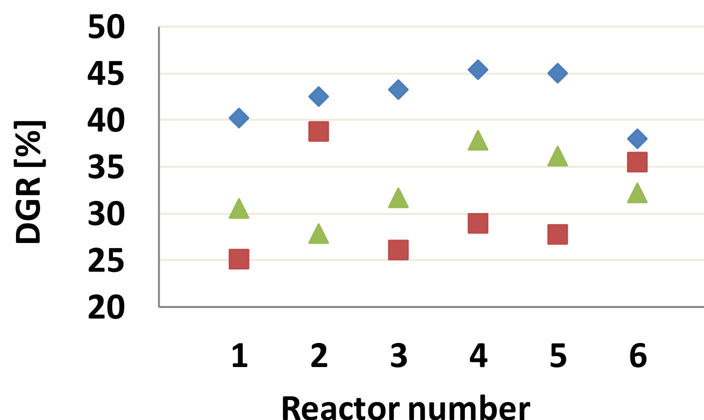

Figure 19. Daily growth rate as function of reactor during the control experiments.

◆ Group 1 ■ Group 2 ▲ Group 3

A set of tests were performed: normalizing each reactor to the highest weekly DGR and normalizing each reactor to the weekly AVG. with these 2 tests none of the

reactor performs constant trends but as it can be seen from the **Fig. 19** and **Table 6** below reactor 1 tends to be the last and reactor 4 tends to be first in growth rates. The reactors were ranked from the higher to the lower growth rate in each experiment.

Table 6. Reactors DGR ranking in first 3 groups.

Reactor	Exp 1 rank	Exp 2 rank	Exp 3 rank
1	5	6	5
2	4	1	6
3	3	5	4
4	1	3	1
5	2	4	2
6	6	2	3

With this set of results and the following **Fig. 20** the conclusion was that repetitions on the experiment must be randomized in relation to the reactor. With this method of averaging the noise of the reactor should be attenuated.

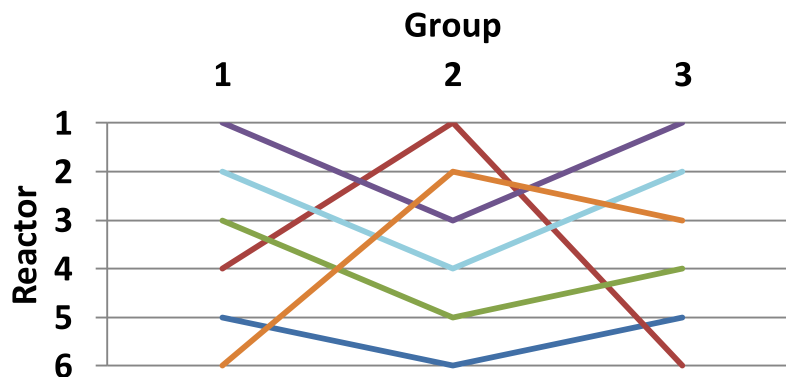

Figure 20. Rank of reactor as function DGR during the control experiments.

Reactor 1 Reactor 2 Reactor 3 Reactor 4 Reactor 5 Reactor 6

The last task from this part is to determine the error in the results. Taking into consideration the ratio of the STD to the group AVG the averaged value is 0.13 in terms of NDGR.

In this part of the research it has been proven that the system return stable results under constant condition within the weekly group. A range of results was collected and created a reference for following experiments. Normalizing method in relation to the control reactor and randomizing method between the reactors through the weeks have been suggested.

5.2. Step 2 Flashing light results

This part of the experiment is designed to explore the space of flashing light. We will define this space with 3 dimensions: Intensity, frequency and DC. In our experiments the intensities are fixed to 1000 μ-mol photons/$m^2 \cdot s$ and the rest of the parameters were based on previous research with different plants. This stage purpose is to collect initial data to suggest a direction to focus on in the next stage.

All results are displayed as NDGR (normalized daily growth rate), while every result was normalized by the weekly control group AVG. The following table displays the results:

Table 7. Results of Step 2 flashing light.

Setup	Frequency [Hz]	DC [%]	DGR [%]	NDGR
A	40	20	16.39	0.54
B	4	20	17.93	0.59
C	7	3	5.83	0.21
D	50	50	22.78	0.84

Every setup of flashing light is characterized by frequency and duty cycle. During every experiment 2 reactors were being used as control, 2 reactors were being used for one setup and last 2 reactors for another setup; total of 4 setups in 2 weeks. Additional 2 set ups of random flashing light parameters were tested and will not be displayed in order to keep analyze on 1 parameter at a time. The results vary between 5-23% DGR with no specific trend. The NDGR results vary between 0.21-0.84 and it can be seen that setup D have achieved growth rate almost as high as the control group value.

The flashing light parameters in the table were chosen according to previous studies on different microalgae and other plants. Group A parameters were chosen in similarity to previous study on *Chlorella* and returned value of 1 NDGR in the reference [13]. Group B parameters were chosen in order to examine the influence of the frequency (larger by magnitude). Group C parameters were tested on *Phaeodactylum tricornuturn* and returned NDGR of 0.98 for mean of 4 light intensities in the reference [10]. Group D parameters were tested on *Dunaliella salina* and returned values of 1.14 NDGR in the reference [14], the reason can be the different integrated intensities values ratio (which is half for the strength for the continuous light group) in this experiment. It would be impossible trying to compare the results to the reference because the subjects are different, microalgae and macroalgae, so as most of the experiment parameters: the nutrients levels, flow rate etc.

Carefully reviewing the results of group C reveals an interesting phenomenon. While the DC is only 3% the NDGR is 0.21. The meaning is that only 3% of constant light was invested but the harvest is about 21% of the constant light product. And now we would like to coin a new term PNDGR (Potential Normalized Growth Rate) which express the potential of using the flashing light on several groups instead of using constant light on 1 group. 3% DC means the light can be shared between 100/3 groups, that's 33 groups of algae, and the potential of yields will be the NDGR multiplied by the number of group. 21% multiplied by 33 groups equal total product of 6.93 NDGR. That is almost 7 times higher than the constant light yield. With this insight, we moved on to the next stage in order to examine it in details.

The next question is what has stronger influence, the frequency or the duty cycle? Comparing the results on the same chart and checking for a trend lines gives the following **Fig. 23**.

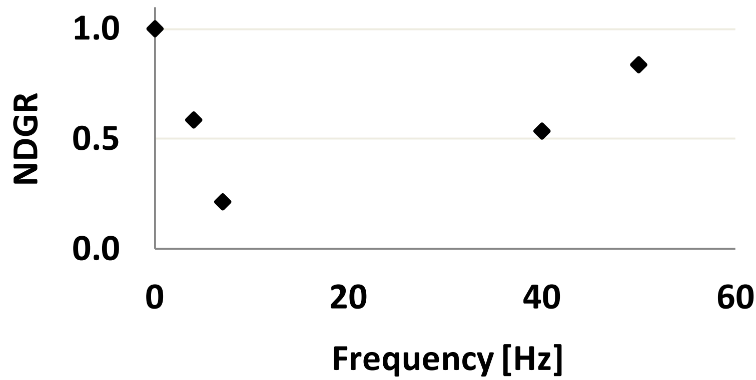

Figure 21. Step 2 Flashing light NDGR as functions of frequency.

It is hard to see any correlation from **Fig. 21**. We can say that in this small group of results the relation between frequency and growth rate could not be found. If we examine setups A and B we can see the duty cycle is equal and the frequency is different by a magnitude, yet, the NDGR is different only by 10%. The assumption is that integration of light start at a certain frequency and anything higher than that value is within the integration zone. In comparison to other researches in the field of microalgae; one claims that the integration zone of the frequency starts at 1 Hz [10] and another claim its higher between 5-10 Hz [63]. The differences can be a result of experimental conditions or the subjects being tested.

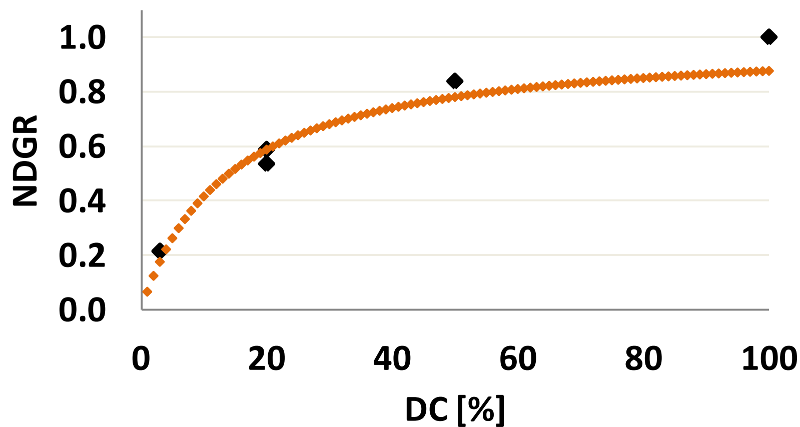

Figure 22. Step 2 Flashing light NDGR as functions of Duty cycle.

◆ Measurements ◆ Theoretical PI curve

In **Fig. 22** the correlation between the parameters is rather clear. The Spearman Correlation for monotonous relation between the variables returns a value of 0.96. It is obvious that the duty cycle has much stronger relation to the growth rate than the frequency in this group of results. We added the theoretical PI curve according to Michael-Menten equation for enzymatic activity (Eq. 22). The average intensity is displayed as function of DC and amplitude as was shown previously and the K value represent the intensity gives half of the maximum growth rate. K has been calculated from previous research about similar macroalgae, *U. compressa.* The full development of the theoretical model is described in the methods chapter. The trend is according to the expectations. Influence of DC was mentioned in previous studies, Phillips and Myers research demonstrated similar results with 0.82 result in Spearman test for DC and only 0.6 for frequency [60]. Terry has displayed decrement in the integration of light for high duty cycles which implies on growing saturation of light as can be seen in our chart [10].

A research about *Postelsia palmaeformis* and *Hedophyllum sessile* macroalgae in very long flash durations showed an enhancement in photosynthetic rate until a limit of about 40 sec flash period [48]. For flash durations of 0.5-100 and 6-48 sec in adjustment (for each type of algae) and duty cycle of 0.5 the results of the NDGR were 1-1.7 with a pick around 10 sec flash durations. The difference in the experiment design was that in our experiments the intensity stayed 1000 μ-mol photons/m^2·s and the averaged intensity varied while in this experiment the averaged intensity was constant. Another interesting argument is that these algae grow under light fluctuation of about 5-10 sec due to wave's regime. This can be related to some kind of adaption of the algae to the light regime in the habitat. The frequency seems to have an influence on the growth rate in these subjects and area of parameters.

The purpose of this part was accomplished as it was found that the DC tends to have stronger influence on the growth rate in the area of parameters which we have researched and that the lower DCs can provide higher efficiency or energetic ROI (return on investment). With this conclusion and the suggested model of PNDGR we move to the next stage.

5.3. Step 3 + 4 Flashing light focus on DC results

This part focused on the DC influence. All setups frequencies were 1 Hz and the DC varied between 1-75%. 1 Hz frequency was selected as a relatively low frequency, which is relevant for offshore mixing, but on the edge of integration of light limit. The purpose of this part is to examine the DC influence curve and find an optimum for the product yield.

As we have seen in the last part, using low DC can achieve higher efficiency of light utilization. We also assume that under a certain DC value, the total photon flux density supplied to the algae is not sufficient and the growth rate will drop in a manner that no longer enables yields higher than constant light.

All results are displayed as NDGR as every result was divided by the weekly control group AVG. The following **Table 8**. displays the results:

Table 8. Results of Step 3+4 flashing light.

Frequency [Hz]	DC [%]	NDGR
1	1	0.10
1	5	0.29
1	10	0.26
1	25	0.48
1	50	0.69
1	75	1.05

Every setup of flashing light is characterized by different duty cycle. During every experiment 2 reactors were being used as control and 1 reactor for each duty cycle; total of 5 setups.

According to last stage rising monotonous connection between DC and NDGR the results fill the expectations but for DC 5% and 75% which is higher than DC 10% and 100% in adjust. For DC 5%, this result is possible because in the low area of the DC axis the biomass accumulation is low and the error (up to 0.13 in terms of NDGR) can be significant. For the same reason, it can be seen that for DC 75% the value of NDGR is higher than 1. The relation between DC and NDGR can be seen from the table and better at the following figure:

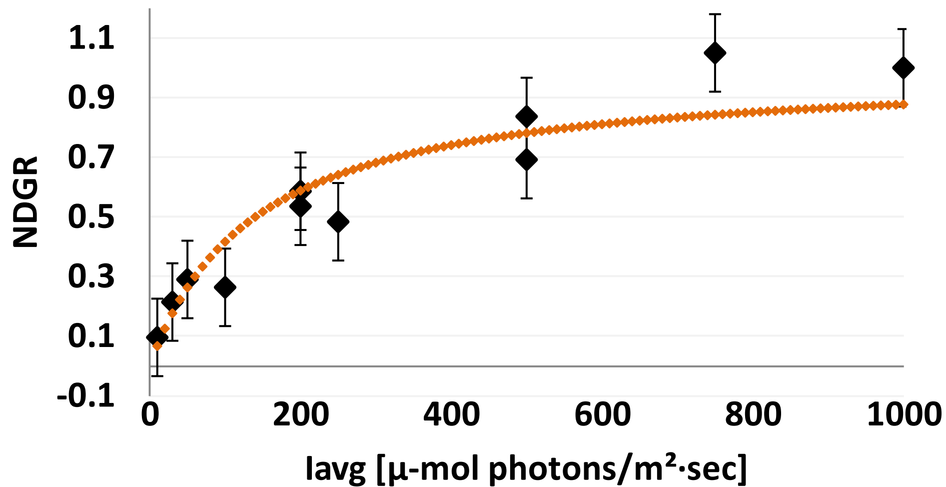

Figure 23. Step 3+4 Flashing light NDGR as functions of Iavg.
◆ Measurements ◆ Theoretical PI curve

In orange is the theoretical PI curve (Eq. 22) as described in the last **Fig. 22**. This time DC is being displayed as Average intensity (Iavg). The trend is filling the expectations and the error bars which have been determined by the STD of the initial control sample cover the curve almost in every point. The results of the 2nd stage support the results of the 1st stage, confirming that the increase in DC increase the productivity and in addition, eventually leads to saturation.

As for the DC 75% or 750 µ-mol photons/m²·s point it can be also a possible maximum point which is followed by photo inhibition and decrease in productivity according to the full description of the PI curve [54]. The value of 750 is not far the 600 mentioned in the literature review for *U. compressa* [41]. A saturation experiment was performed with constant light based on the same principals of the last experiments with values of 400/700/1000 µ-mol photons/m²·s. The results are presented in **Fig. 24** and do not support this hypothesis. It can be seen that between 400-1000 µ-mol photons/m²·s the NDGR results are close to 1 which means it's a saturation zone. Previous studies on Ulva show 30-100 µ-mol photons/m²·s [65] for saturation according to acclimatization.

This saturation curve, flashing light results of 1 Hz and flashing lights results of above 4 Hz are displayed separately on the following figure.

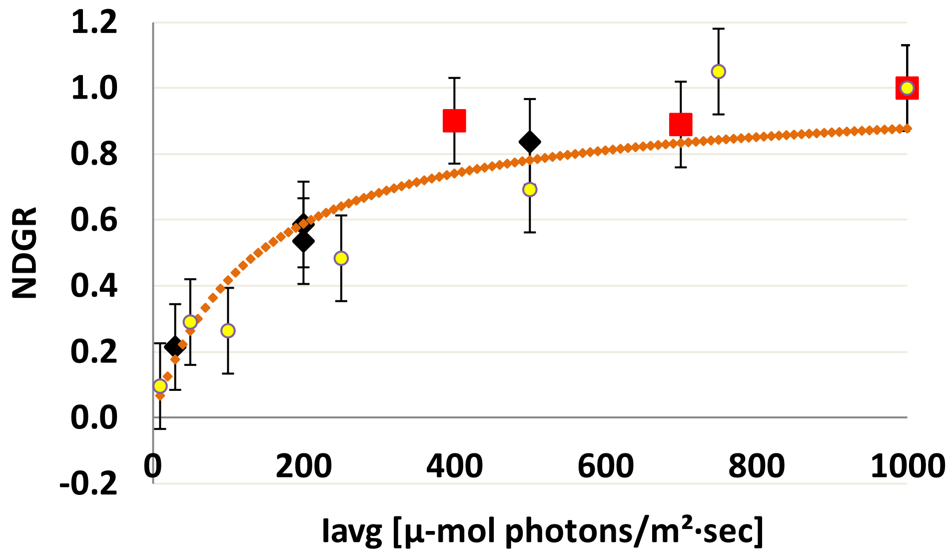

Figure 24. Step 2-4 Flashing light NDGR as functions of Iavg.
○ 1 Hz ◆ >4 Hz ■ saturation ◆ Theoretical PI curve

In this **Fig. 24**, we can see that for our *Ulva sp*. we expect the theoretical PI curve (discussed before) to be slightly higher according to the saturation zone. Another interesting thing we can see is that for 1 Hz results of 100/250/500 μ-mol photons/m²·s the values are consistently lower than in higher frequencies. The explanation might be that with 1 Hz the integration of light is only partial [10][18]. In the literature review we have seen a microalgae, *Nannochloropsis salina*, with frequency limit for integration of light around 5-10 Hz. In another paper the different possibilities for partial integration of light is being presented [13].

Another topic that should be discussed in the results section is the "dark reaction". In some of the experiment a special "negative control group" was positioned in order to examine if there is any growth rate in the dark. The results varied between 0 to 0.2 NDGR and no reason have been found to the questions why in some cases a growth in the dark was observed and in others it didn't and what determines the extent of the growth in the dark. Two simple explanations should be examined in farther research:

1. Light penetration to the dark reactors.
2. Growth in the dark, heterotrophic growth using stored starch. [66].

These parts of research will be left for the following researchers in the group.

5.4. Potential of productivity

In terms of P_A (productivity per unit area) the results for constant light show improvement under lab conditions (Eq. 27). In comparison to Ulva growing offshore in Israel the productivity is higher by 31% [27] but compared to kelp it is lower by 50% [67]. The results in the lab compared to offshore cultivation are not surprising as the conditions in the lab support high productivity, with high nutrients concentrations and high level of light for each thallus in addition to optimum temperature.

In order to estimate the potential productivity for ALR (airlift) bio reactor the number of groups should be determined. Assuming the desired DC, frequency and the velocity are given the radius R can be calculated (Eq. 28). These define also the number of the groups N and depth L (Eq. 28, 30). The efficiency is determined according to the energy invested in mixing the water in relation to the solar energy input. The adjust sketch illustrates the model:

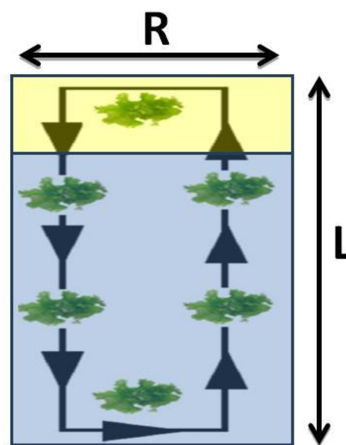

Figure 25. ALR model of flashing light

In the figure the yellow upper part represents the light area and the blue lower part represents the dark area. The PNDGR (Eq. 31) express the amount of biomass which can grow (normalized values) if the flux of constant light will be shared between several groups of algae as flashing light. Assuming the frequency can be decreased (to relevant values for ALR water velocity) without affecting the results from the lab, the productivity estimation for offshore cultivation can be calculated. Using flashing light of 1% DC with 100 groups in adjust, and estimated efficiency $\varepsilon_t = 0.5$ (converting constant light to flashing light) the outcome is 28 g/m²·d (Eq.

32), higher than 25 g/m²·d target value which was mentioned in the literature review [26]. All results in the following table displayed with DW and referring groups 7-9 results (dry weight).

Table 9. Potential productivity of Ulva under different conditions.

Location	Light regime	$\boldsymbol{\varepsilon_t}$	$\mathbf{P_A}$ [g/m²·d]
Lab	Constant	1	7.6
Lab	Flashing	1	73.3
Offshore	Constant	1	5.8 [27]
Offshore	Flashing	0.5	28 (Estimated)

Another possible application is a system which collects light from the surface with solar panels and shares it in flashes with LED in the depth to the surrounding algae cultivation. Assuming the desired flash DC is 1% and the efficiency of the conversion is 1 so the relation between the surface area and the distribution area is 100 times. As we are discussing productivity per unit area and the vertical dimension is not limited (theoretically) we would like to demonstrate the results in terms of depth. In this example the light source is LED systems which illuminates in depth and the algae are not being mixed. The cultivation does not need to move towards the light on the surface as in the ALR solution. The next chart, **Fig. 26**, describes the PNDGR as function of depth L, which is determined by multiplication of the number of groups and the algae group length, $\mathrm{d}[m]$. The units of L are length units in terms of d. The number of groups defined according to the DC as before and the efficiency of converting constant light to flashings equals to 1.

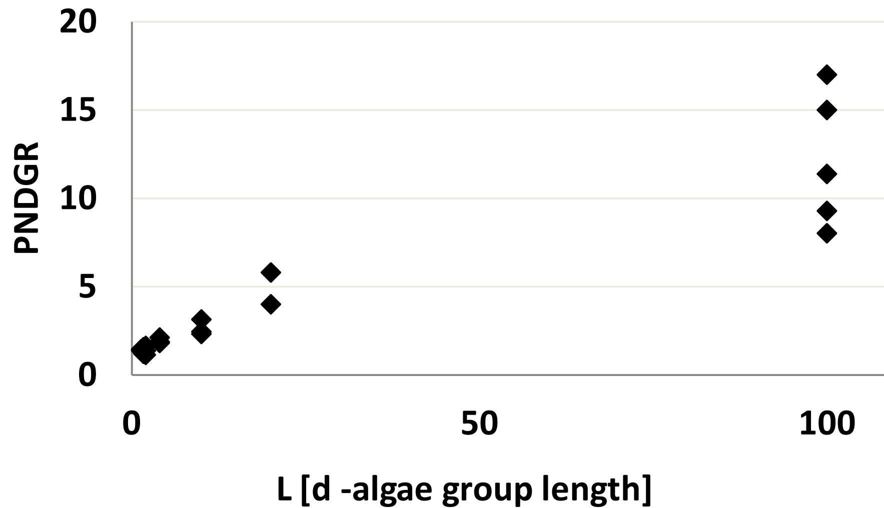

Figure 26. The PNDGR as function of Depth.

It can be seen from the figure that with the increase in depth L, the PNDGR grows up to 10 times higher (average) for the depth of 100 algae groups. With existing technology of LED light, the efficiency of using solar panels and converting constant light to red flashing light is 0.2 [59]. Red LED light is 5 times as effective as full spectrum solar radiation in deriving algal photosynthesis, which means the enhancement is not being decreased in total [59]. In terms of productivity, with this system the cultivation offshore can reach up to 58 [$g/m^2 \cdot d$], ten times higher compared to previous field results [27].

It can be seen in **Fig. 26** that the results are more concentrated for the low values of L and scattered for the high values. L is a function of DC and goes higher as the DC is lower. A sample under low DC received less light in total and in adjust the accumulation of the biomass was low. These small changes in the biomass is harder to measure what creates less stable results. For more accurate results with low DC the experiment time should be extended.

As was demonstrated in the previous paragraphs and **Fig. 26** the yields per unit area can be enhanced, and the answer to the question of research is positive; by sharing the constant light flux between several groups of algae the outcome can be greater. More efficient distribution of the solar input can provide higher productivity. The implementation can be in a form of ALR or a system of solar panels with LED lights array.

5.5. Efficiency

An interesting aspect of the results is the efficiency of converting the light energy to chemical energy in the biomass. As it was introduced through the material and methods the efficiency can be calculated as the chemical energy value outcome in relation to the photon invested (Eq. 39).

Table 10. Step 3 Efficiency of light conversion to biomass.

Frequency [Hz]	DC [%]	ε_c
1	1	11.36
1	10	3.05
1	25	2.25
1	50	1.61
1	100	1.17

The results show a clear trend of increase in efficiency with decrease in DC. For the control group the averaged efficiency was 1.17%. The results can be seen in **Fig. 27** below.

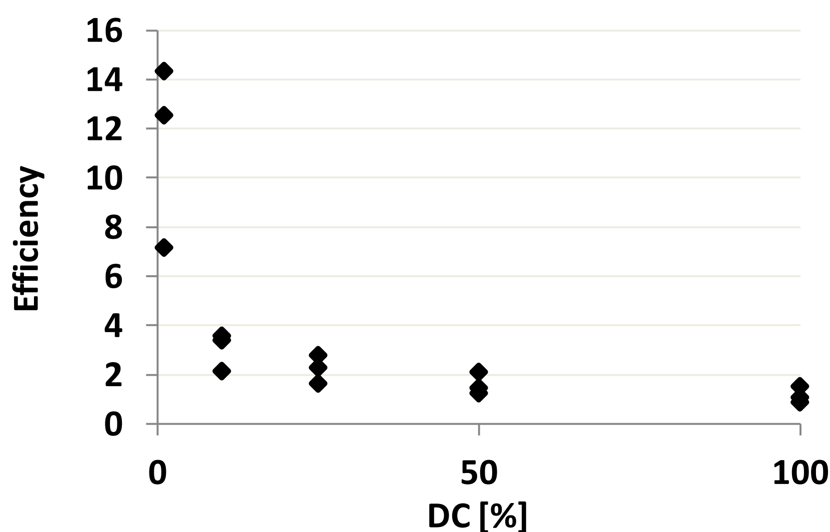

Figure 27. Step 3 Efficiency of light to chemical energy conversion as functions of DC.

The results are consistent in the higher DC values due to the high biomass accumulation and less consistent in lower DC in adjust. Between DC 100% to DC 1%

the efficiency has climbed in average by a magnitude, 10 times higher, which means the potential for light harvesting under flashing light is substantial. The highest efficiency achieved in our lab is 11.36% for flashing light and 1.17% for constant light (considering only the PAR radiation). The latter value can be compared to Ulva offshore cultivation under constant light with 0.7% efficiency [27]. For the flashing light, in comparison to other research discussing efficiency of microalgae photosynthetic activity; similar values, up to15% have been reported [59]. As for the enhancement in efficiency, reports show 15 times higher efficiency in comparison to constant light [10] which suit our results of enhancement in 10 times.

For conclusion, our results showed a positive growth rate of the Ulva under flashing light. The efficiency of converting sunlight to biomass was improved by a magnitude in comparison to constant light. The productivity per installed area was improved by a magnitude and the estimation shows it can reach above the predefined target value.

5.6. Future directions

The assumptions that the growth rate can be kept in lower frequencies should be examined, for the possible application in the ALR model. The movement of the algae in the water is slow and will create long periods of darkness in correlation to the ALR depth. The advantage of the ALR is that intensive cultivation requires pumping CO2 into the water in order to keep carbon levels high so the energetic investment in the circulation is only partial. Other possible applications keep the algae still in the water and moving the light with solar panels and LEDs or optic fibers.

For the research directions, an important test will be the PI curve; a setup of experiments in order to examine the intensity influence on the growth rate. For most of the plants the light intensity is higher than needed and most of the time the arriving light is in the saturation zone. Understanding the intensity influence will enable to share the flux not just by flashes but also by deploying the growing platforms in inclination in order to attenuate light and increase illumination area.

6. Summary and conclusions

The first conclusion from this series of experiments is that the *Ulva Sp.* as a case study for macroalgae, can grow under flashing light with similar growth rate in comparison to constant light. 84% of the constant light product was achieved with only 50% of the photon flux under flashing light regime. The setup of frequency and duty cycle, 1-50 Hz and 1-100% in adjustment, has a significant influence on the results. Some setups return similar growth rates to constant light (Freq 50, DC 50) and others were lower significantly.

The second conclusion is that the efficiency of converting light to biomass is higher under limited flashing light regime. Decrease in the photon flux leads to increase of conversion efficiency. 1.17% with constant light and 11.36% with flashing light (Freq 1, DC 1%). An interesting observation was that the growth rate and efficiency of conversion does not go hand in hand. The higher the growth rate was, the lower the efficiency and vice versa.

The third conclusion was that the productivity per area can be enhanced by sharing the constant photon flux to several layers or groups of algae. The potential for the productivity per unit area can be larger by a magnitude; from 5.8 $g/m^2 \cdot d$ to 58 in offshore cultivation. The productivity and the efficiency can go together if the algae cultivation would grow under limited light regime sharing the flux in flashes to several groups to the depth. With this method higher efficiency and higher productivity can be achieved.

The limitations of this work are caused by the lab conditions. The comparability of the results was not easy to manage. The experiments last for 3 days each and only 1 experiment per week what lead to months of testing in the lab. In order to overcome this challenge, all results were normalized in relation to the control reactors of the experiment. Other issues are the influence of LED light spectrum instead of sunlight that should be assessed and the effect of the artificial water on the results. Long term effects should be also taken into consideration as it is known that algae growth changes over time.

For future work the following should be taken into consideration: first, low frequencies, below 1 Hz, should be examined. Second, moving the light towards

deeper cultures and the effect of denser culture on the productivity should be considered. Third, the PI curve should be measured to understand the intensity influence in order to share the flux of light more accurately to the culture. Hopefully, this work will help to improve the understating of light influence about the cultivation of macroalgae in the future.

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Appendix A – Density measurements – Gil Kroin

Introduction

The goal of this part was to determine the properties of the cultivation which can be described as 2 phase solution of seawater and algae. In order to simplify the measurement an assumption of homogenous solution was made instead of measuring the algae density and water density separately. A series of experiments was designed in order to measure the desired characteristics.

Materials and methods

Effective density

These set of experiments were done by weighting a volumetric cylinder with different levels of algae density in sea water. The fresh weight of the thallus was weighted separately and later sea water was added to the algae in the cylinder to complete the desired volume.

Experiment 1:

Thallus in small-medium size (up to 5 cm in diameter) was measured.

Experiment 2:

Thallus in medium-large size (above 7 cm in diameter) was measured at the end of the day after photosynthetic activity under the assumption that the effective density will climb due to high oxygen content in the algae. 2 levels of density were measured.

Experiment 3:

Thallus in medium- large size (above 7 cm in diameter) was measured at the beginning of the day after photosynthetic activity under the assumption that the effective density will drop due to low oxygen content in the algae. 2 levels of density were measured. The algae densities in water were about 80 kg/cub which is much higher than the optimal density for cultivation which is about 8 kg/cub.

During all experiments the size of the volumetric cylinder was 100 ml and resolution of 1 ml, and the control group was the seawater without algae in the cylinder. The resolution of the weight was 0.0001 g. Each experiment repeated 3 times.

<u>Effective viscosity</u>

These set of experiments were designed with different methods in order to measure viscosity for different levels of algae density in sea water. The fresh weight of the thallus was weighted separately and later sea water was added to the algae to complete the desired density.

Experiment 4:

A system of concentric cylinders from the lab of Prof. Liberzon was used. The full description of the system can be found in the course "הנדסת ניסויים ומדידות" files. A solution of Glycerol, sea water and algae in was measured. The total volume was 150 ml, temperature 20 C°, weight resolution 0.01 g, and viscosity with 1 cp resolution. The control group was the seawater without algae in the cylinder. Each experiment repeated 3 times. The algae densities in water were about 30 kg/cub.

Experiment 5:

The last experiment focused on the added energetic investment needed in order to mix algae in the water. A mixer with a tank of 16.2 liter was positioned and was operated in 70 rpm. 310 g fresh weight of algae was added to the water and the difference in the energetic consumption was measured. The rpm resolution was 1 RPM, the wattmeter 0.1 W, the weight 0.01 g and the meter 0.1 cm. the control group was the seawater without algae in the tank. Each experiment repeated 3 times. The algae densities in water were about 19 kg/cub.

Results

Experiments 1,2,3:

Table 1. Effective density results, 1 – small medium size no photosynthesis, 2 – medium large size after photosynthesis, 3 – medium large size before synthesis.

Exp	Seawater density [g]	Algae density in water [kg/cub]	Seawater density Error [g]	Algae solution density [g]	Algae solution density Error [g]	Maximal possible difference [%]
1	1023.43	79.8	10.89	1018.36	10.19	2.5
2	1024.97	75.3	8.54	993.08	8.28	4.6
3	1024.97	77.2	8.54	998.14	8.87	4.2

The results show a trend that supports the assumption. Comparing the 2nd and 3rd groups it can be seen that after photosynthesis the density decrease, yet, the difference is negligible. The more important is that anyway the difference between sea water and the solution is smaller than 5%.

Experiment 4:

Table 2. Effective viscosity results for sea water and algae solution.

Algae fresh weight [g]	Effective viscosity from lit. [cp]	Measured viscosity [cp]	Error [cp]	Relative error[%]	Maximal possible difference [%]
0	60.1	57.11	5.31	9.3	13.8
1.5	N/A	64.33	5.29	8.2	13.7

According to the results the additional viscosity to the as a results of the algae presence is between 0-25%. The uncertainty in the results is due to the large error and the similarity between the results of the groups.

Experiment 5:

Table 3. Effective energetic investment results for sea water and algae solution mixing.

Algae fresh weight [g]	Measured Consumption [W]	Error [W]	Relative error [%]
0	13.39	0.11	0.82
310	14.26	0.12	0.84

According to the results the additional energetic investment in mixing the solution with this tank, stirrer and density is between 4.5-7.7%.

Conclusions

The properties of mixture solution of algae and sea water in high densities are hard to asses because the low differences between the control and the test group. The effective density has been proven to be similar to the seawater with maximal 5% error. The effective viscosity in the conditions of the experiment was assessed between 0-25% and the additional energetic investment between 4.5-7.7%.

The conclusion at this point can be that under optimal densities the change in the properties will be much lower and can be neglected.

Appendix B – drying technique comparison

Introduction

The goal of this part was to determine the most accurate technique to dry the algae for weight. The comparison has been done between lettuce dryer and paper towel for group of thallus and later for single thallus.

Materials and methods

Paper towel drying

Using simple paper towel, by swiping the water of the face of the thallus, the drying was done. The towel gives a good indication when the thallus is dry because no more water accumulates one it.

Lettuce dryer

The lettuce dryer spines the thallus in the centrifuge and due to density differences the water drains off the face of the thallus and pass through peripheral notches. The disadvantage of this technique is that it's hard to determine if the leaf is dry or not.

Experiment 1:

A group of thallus of about 6.5 g mass was dried with each one of the techniques and measured in order to determine the accuracy, for each method 4 repetitions. The resolution of the weight was 0.01 g.

Experiment 2:

Single large thallus, above 0.25 g, was measured using paper towel drying in order to determine the accuracy of the method. The experiment repeated for 6 different thallus, for each one 3 times. The resolution of the weight was 0.01 g.

Experiment 3:

Single small thallus, under 0.12 g, was measured using paper towel drying in order to determine the accuracy of the method. The experiment repeated for 6 different thallus, for each one 4 times. The resolution of the weight was 0.0001 g.

Results

Table 1. different drying methods results.

Methode	Avg [g]	Error [g]	Rel Error[%]
Paper towel	6.255	0.89	14.76
Lettuce dryer	6.16	1.3	23.55

Drying with paper towel returned lower relative error. Apparently due to the ability of seeing the water drain on the paper towel it leads to more accurate drying. With these results we move the next stage and check the ability of drying and measuring a single thallus.

Table 2. Paper towel method results for different sized thallus.

	Max Rel Error [%]	Avg Rel Error [%]
Large thallus	8.33	5.08
Small Thallus	9.36	6.61

The results are similar for large of small thallus. The small differences are probably due to the limited ability of weighting lower masses. It can be seen from the results that for single thallus the error size is lower in comparison to the groups of thallus (exp 1). Probably due to the attention each thallus receives separately.

Conclusions

On the 1st experiment the paper method have been proven to be more accurate. In the 2nd and 3rd experiments; testing single thallus lead to higher accuracy. Beyond the quantitative results, a qualitative argument should be taken into consideration. The lettuce dryer remove big amounts of water from the surface more efficiently than the paper towel. A combined method has been defined: 1st preliminary dry using lettuce dryer, and 2nd final dry using paper towel to achieve accuracy.

Appendix C – illumination system

Introduction

The objectives of the experiment were to determine if this LED answers the needs of the illumination system for Ulva growing in lab conditions. The system was tested in nominal, near edge conditions, and in continues work. A special attention was given to the ease of work. Eventually the accuracy of illumination was measured and calculated in order to understand the dispersion inside the tube.

Materials and methods

Intensity measurements

The intensity measurements were conducted Li-Cor 192 PAR radiation sensor (400-700 nm, error 5%, Li-Cor USA). The sensor was positioned in different points around the test tube to estimate light behavior and attenuation due to the range from the LED.

The intensity decreases as a function of the squared range.

$$I_2 = I_1 \left(\frac{R_1}{R_2}\right)^2$$

The range as a function of deviation from the center of the tube is

$$r = \sqrt{x^2 + y^2 + z^2}$$

Growth rate measurements

The suitability of the LED and the selected wavelengths to the algae were tested by biomass accumulation test. The mass was measured at the beginning and the end and during every day of the experiments which lasted 13 days. During the experiment the temperature of the room and the water was monitored in order to determine the effect of the LED light on the temperature of the algae in the system. The weight resolution was 0.01 g and the resolution of the thermometer 1 C°.

Results

The results shows that in 13 days, that includes an uncertain period of adaptation, the mass grew by 90%. The averaged daily growth rate was 5% with a pick of 18% after nutrients was added to the water.

Table 1. The results of the growth rate along the experiment. The additional "f" under the intensity refers to flashing light.

Date	time	Weight [g]	Growth rate %	Temp [C]	Room temp [C]	Intensity [μ-mol photons/m²·s]
01/03/2016	13:00	2.23	-	22		-
02/03/2016	20:00	2.21	-0.9	23	20	1400
03/03/2016	15:00	2.35	6.3	23	20	1400
04/03/2016	19:00	2.37	0.9	22	19	1400
05/03/2016	21:00	2.5	5.5	21	19	1400
06/03/2016	21:00	2.46	-1.6	21	19	1260
07/03/2016	23:05	2.55	3.7	21	19	1250
08/03/2016	18:05	2.75	7.8	21	19	1260
09/03/2016	20:21	3.26	18.5	22	20	910
10/03/2016	19:10	3.63	11.3	22	19	930
11/03/2016		3.74	3.0			930f
12/03/2016	23:10	3.85	2.9	20	19	930f
13/03/2016	20:00	4.09	6.2	20	19	930f
14/03/2016	22:00	4.24	3.7	23	19	930

The heating effect of the LED was tested. While the room temperature was 19-20 C, the water temperature reached only 22-23 C, always 2-3 degrees above room temperature. Important to mention, there was no circulation during the experiment.

The decrease in light intensity in relation with deviation from the center of the light source beam was measured. While the test tube was beside to the light source still the effect of radius on intensity decrease was functioning as the LED was a point source.

During the experiment the light measuring point was changed in order to increase accuracy. Light intensity, nutrients addition and air flow rate was adjusted

according to visual examination and consultation with Alex C. All adjustments are mentions in the results table.

Table 2. Measurements around the test tube in comparison to theoretical point source.

Point	x	y	r	Measured I [μ-mol photons/m²·s]	Point source I [μ-mol photons/m²·s]	Error [μ-mol photons/m²·s]	Relative Error [%]
LED	0	0	0	-	-	-	-
A	0	0.145	0.145	977.6	977	-	-
B	0	0.08	0.08	3237.2	3209.6	27.6	0.85%
C	0	0.21	0.21	479.6	465.8	13.8	2.88%
D	0.065	0.145	0.1589	792.6	813.5	20.9	2.64%
E	-0.065	0.145	0.1589	795.2	813.5	18.3	2.30%

In can be seen from the upper table that the deviation of the measurements from the theory is smaller than 2.9% and for that it can be assumed as point source. The measurements were taken in 2 dimensions only but that is enough to prove the behavior of the source.

Conclusions

The algae can grow under the illumination of the LED system. The intensity is the only parameter that has to be treated with special attention while setting up the system and should be assessed every few months in designated test.

The heating effect of the illumination on the water (no circulation) can be neglected under less than 1400 μ-mol PAR with constant light.

The system behaves as a point source and all following assessments for intensities can rely on the theory of illumination attenuation as a function of range.

The system can work continuously for days under defined parameters. No events of evolving failures were observed, neither deviation in the parameters.

During the experiment it was observed that different drying methods can lead to different results and uncertainty in the validity of the results. A short protocol was defined and extracted but farther work must be done on the measuring tools (see appendix B drying methods).

Appendix D - Nutrients calculator

Introduction

In order to determine the levels of nutrients concentrations in the water a special calculator was designed. The calculator can estimate the amount of chemical salts needed to be added to a specific volume of water in order to achieve target levels of concentrations. It can work with either DW water (0 nutrients) or water reached with pre-known levels of nutrients. It can also calculate the adjust combination of sea water from 2 sources, DW and chemical salts in order to achieve target levels of nutrients concentrations, Salinity and pH.

Materials and methods

Calculations for nutrients addition

The salts being used for the P was NaH_2PO_4 and for the N was NH_4Cl. The calculator is based on the following equations:

For every g of salt the P or N addition to the media will be:

$$\text{Nutrient ratio } \left[\frac{\text{g}}{\text{g}}\right] = \frac{\text{Nutrients mollar mass}}{\text{Salt mollar mass}}$$

For a tank volume of 1 m³ with DW (no nutrients) the addition to the media will be:

$$\text{Cub Nutrient addtion } \left[\frac{\text{g}}{\text{m}^3}\right] = \frac{\text{Nutrients ratio}}{\text{Target concentration [ppm]}}$$

For a tank volume of V with DW (no nutrients) the addition to the media will be:

$$\text{Specific addtion [g]} = \frac{\text{Cub nutreint addition}}{\text{V [m}^3\text{]}}$$

In order to create a buffer solution of 1 L bottle concentrated 100 times higher:

$$\text{Buffer x100 addition } \left[\frac{\text{g}}{\text{L}}\right] = \frac{1 \cdot \text{Cub nutreint addition}}{10}$$

For pre-known concentrations in the water, P/N measured the gap must be calculated:

$$\text{Nutrient gap [ppm]} = \text{target value} - \text{measured}$$

The specific addition of salt will be:

$$\text{Specific addtion [g]} = \frac{\text{Nutrient gap } \cdot \text{V}}{\text{Nutrient ratio}}$$

And eventually the potion of 1 L solution buffer x100 for the tank will be:

$$\text{Buffer portion [ml]} = \frac{\text{Specific addtion } \cdot 1000}{\text{Buffer x100 addition}}$$

Calculations for combination of several water sources

The following equation describes the procedure in order to create a new media with several sources of water. First, the following parameters of all the sources must be measured: P [ppm], N [ppm] Salinity [ppt] and pH. The final volume of water will be defined as V [L] which means eventually 5 equations will be solved, 1 for each parameter.

The matrix of measured parameters can be defined as:

Table 1. The matrix of target and measured parameters.

	Target values	Source 1	Source 2	DW
P [ppm]	D3	G3	J3	M3
N [ppm]	D4	G4	J4	M4
Salinity [ppt]	D5	G5	J5	M5
pH	D6	G6	J6	M6
Volume [L]	G7	x	y	z

The following set of equations describes the problem. The solution vector is [x,y,z] and with 3 parameters but 4 equation we need to choose a one equation to neglect and solve later. Salinity and volume will be always satisified and one of the rest of the parameters will be selected to be fulfilled in each of the following paths.

Phosphate path:

The set of equation to be solved is

$$\mathrm{D5} = \frac{\mathrm{G5} \cdot \mathrm{x} + \mathrm{J5} \cdot \mathrm{y} + \mathrm{M5} \cdot \mathrm{z}}{\mathrm{G7}}$$

$$\mathrm{G7} = \mathrm{x} + \mathrm{y} + \mathrm{z}$$

$$\mathrm{D3} = \frac{\mathrm{G3} \cdot \mathrm{x} + \mathrm{J3} \cdot \mathrm{y} + \mathrm{M3} \cdot \mathrm{z}}{\mathrm{G7}}$$

The target vector B is [D5, G7, D3] and the coefficient matrix A is

$$\mathrm{A} = \begin{vmatrix} \frac{\mathrm{G5}}{\mathrm{G7}} & \frac{\mathrm{J5}}{\mathrm{G7}} & \frac{\mathrm{M5}}{\mathrm{G7}} \\ 1 & 1 & 1 \\ \frac{\mathrm{G3}}{\mathrm{G7}} & \frac{\mathrm{J3}}{\mathrm{G7}} & \frac{\mathrm{M3}}{\mathrm{G7}} \end{vmatrix}$$

X=[x,y,z] will be the volumetric vector so

The set of equations can be represented as B=A·X and using inversed A the solution can be found easily $X=A^{-1} \cdot B$. Using the solution of X the rest of the parameters can be determined.

$$\mathrm{N\ [ppm]} = \frac{(\mathrm{G4} \cdot \mathrm{x} + \mathrm{J4} \cdot \mathrm{y})}{\mathrm{G7}}$$

$$pH = \frac{(G6 \cdot x + J6 \cdot y + M6 \cdot z)}{G7}$$

The same procedure can be repeated for the Nitrogen path or the pH path. After calculating the 3 options, the most suitable and easiest for implementation can be used. Not necessarily the set of equation has a feasibly solution what might lead to negative results of volume and a failure to comply the targets

Nitrogen path:

With the same method introduced in the last section the target vector B is [D5, G7, D4] and the coefficient matrix A is

$$A = \begin{vmatrix} \frac{G5}{G7} & \frac{J5}{G7} & \frac{M5}{G7} \\ 1 & 1 & 1 \\ \frac{G4}{G7} & \frac{J4}{G7} & \frac{M4}{G7} \end{vmatrix}$$

X=[x,y,z] will be the volumetric vector and he rest of the parameters can be determined by

$$P\ [ppm] = \frac{(G3 \cdot x + J3 \cdot y)}{G7}$$

$$pH = \frac{(G6 \cdot X + J6 \cdot y + M6 \cdot z)}{G7}$$

pH path:

With the same method introduced in the last section the target vector B is [D5, G7, D4] and the coefficient matrix A is

$$A = \begin{vmatrix} \frac{G5}{G7} & \frac{J5}{G7} & \frac{M5}{G7} \\ 1 & 1 & 1 \\ \frac{G6}{G7} & \frac{J6}{G7} & \frac{M6}{G7} \end{vmatrix}$$

X=[x,y,z] will be the volumetric vector and the rest of the parameters can be determined by

$$P\ [ppm] = \frac{(G3 \cdot x + J3 \cdot y)}{G7}$$

$$N\ [ppm] = \frac{(G4 \cdot x + J4 \cdot y)}{G7}$$

Conclusions

The creation of a media can be easily calculated and designed through a set of equations to support the process. Several approaches may be considered to find the feasible or the easiest way for the implementation. The next step after mixing the media will be to fix the remained parameters using the nutrient calculator and adding the portion of buffer solution.

Appendix E - Challenges

The experiment design

The equation of growth rate contains a large number of variables, some has had very strong influence and others are negligible. Disassemble this problem to smaller problems was the first task in the research work. How to examine the specific variable with no undesired influence of the others variables is the question needed to be answered. The approaches were used here are: saturation, predefined limits and neglect. For certain variables like ammonia concentration the saturation approach was adopted. High levels of nutrients were added to the water in order to prevent a situation where the growth rate is being limited by nutrients instead of light. Other variables like temperature and pH were handled under the predefined limits approach. While these types of variables are harder to determine at sea a frame values for each one was predefined, adjust to conditions offshore, and all experiments were kept in under these limits. The last approach was neglect and it was used in cases the variable was assumed to be less affecting the results or it was out of the lab and funding scope. For these case one of the examples are CO2 values in the water.

With these approaches we tried to bring closer the experiment to the desired results and insights. Not necessarily the results from this experiment will appear offshore but we hope the insights will.

The experiment system

The design of a prototype combining so many variables is an interesting mission. Derived directly from the experiment design; different variables needed to be controlled or monitored in different levels. The balance between the available budget (6000$) for the prototype and the uncertainty of system to work and the algae to grow in lab conditions creates a very conservative approach. Expensive components were being purchased only after a test.

The research time is limited but to design a system and conduct an experiment can take a long time. There is urgency to start the system design as early as possible in order not to create a bottle neck later in work while waiting for the office bureaucracy

to place the order or the parts to arrive. More than that, the knowledge level rise with time and the ability to change the system drops, that is called the paradox of design. This can be a major mistake to start the design too early; you can find yourself with an experiment system that cannot test your research question. The most recommended thing to do is to sit and search for similar experiment system. Maybe you will not find exactly the system you need but you will gather a big amount of insights along the search.

Another aspect of the system design is a component which acts as a bottle neck. Some components are widely used and the only thing needed to be done is to choose the right one for the experiment. Sometimes some components are rare and not easily can be found. In our case the flashing led light was as such. Many suppliers can provide PAR led light system but none can create flashing light system. More than that, none of it adjusts to such electrical characteristics as the flashes demand turning on and off the led. That was a big bottleneck in the system design, luckily a supplier was found after few months. Still the solution was not perfect while the led was functioning as a point source and as a result we had to compromise on the intensity error size.

While designing an experiment system lots of assumptions are being taken into consideration. One must rely on the work of others in order to integrate different devices and create a comprehensive system. This approach allows progressing and building complicated systems but also demands a certain of careful and attention. The system must be tested in order to assure it stands all requirements. The assumption that uniform conditions will create same growth rate in different reactors is an example to assumption that proved to be wrong. The assumption that there will be no difference between one week results to the other did not stand either though all conditions were equal.

The nutrients measurements

At the beginning of the research the approach was that every variable must be measured to assure the uniformity in the experiments conditions. A test kit of LaMotte, USA was purchased and initial testing had been conducted for phosphorous and ammonia concentrations. The P values were stable, satisfied the expectations and

behaved according to the calculations. The ammonia values were uncontrollable and changed from one measurement to the other, sometimes settling with the calculation and sometimes not. A short research was made in parallel to checks with the supplier. Measuring ammonia is not a simple task; the ammonia changes constantly in the water. One example is the carbon cycle in the water, where bacteria change the ammonia to nitrite and nitrate in natural process. Another interesting aspect is that the measurement tool effect immediately on the ammonia values. Sampling with plastic cap once and glass cap in the second will lead to different results. The supplier was not familiar with these issues and did not provide answers to the questions about the testing kit. After few weeks of unsuccessful attempts to control ammonia concentration in sea water it was decided to create semi artificial water. Adding ammonia and phosphorous salts to DW enabled to create standard conditions in the beginning of each experiment under the assumption that the DW water is being filtered uniformly. A new filter arrived to the beginning of the experiments and was used along the research. Periodic checks were made to examine the intactness of the filter. The nutrients levels were not measured as the test kit loosed its reliability but the results gave steady growth rates eventually.

Water composition stability

Another aspect of the media composition stability was pH levels and contaminations. The first was unstable during the seawater experiments period mentioned in the last paragraph and the latter was not measured at all. During the research for pH influencers the first suspect was the air pump. Over aeration of the water may lead to decrease in pH level. The air pump was disconnected but the pH level kept dropping with no apparent reason. A pH buffer (bi-carbonate) was added in order to prevent the dropping but did not help. Eventually after changing to semi-artificial water and using red-sea salts the initial pH values were stable. However it can be seen from the measurement that with time initial pH tend to drop at the beginning of the experiments. Contaminations in the system may be the reason. The central tank and reactors were washed between every experiment with bleach but the connecting tubes were not. Possibly, a small amount of bacteria cultivated in these tubes and created this effect.

The scale of the system

The experiment system containing up to 120 liters was designed in order to provide "sea" conditions to the tested algae. By saying "sea" conditions meaning not just the environmental conditions but also the characteristics of a stable reservoir not affected by the biomass. If the amount of water will be too small than for example; the nutrients level will drop quickly and thus will create a limiting factor. Temperature is more stable in big systems and less changes within it due to the water high heat capacity. From the operational point of view big systems demands lots of work and create an operational tale that sometimes is at first seemed to be longer than the experiment itself. A small system enables short period of setup and usually less labor before and after the experiment. The balance between the scale of the system, its similarity to a reservoir and the ease of operation is important for the quality of results on one hand and the amount of results on the other. Small system may be easier to operate and provide lots of results in a short time but not necessarily keeps stable conditions during the experiment. Big system maybe not needed in certain occasions but will take a lot of power of the researchers and resources of the lab.

Control

One of the first steps of research was to examine the experiment system and assure it supplying uniform condition to the reactors. It was tested by 2 approaches: the first – measuring directly the parameters, and second – testing the growth rate results in uniform conditions. As it was detailed under the control group results section, between different weeks the growth rate was different even if the conditions were not changed. The reason is that the algae of one week were different than the other in genome, the age, the former conditions and the time of the year. The conclusion was that every week had to include control reactors which all of the results will be compared to and normalized. Using this method the results could be compared between one week and another while it is all normalized.

Appendix F – Light influence models

For review, many others forms were suggest since 1905, for example, it can be described also as an exponent function or hyperbolic tangents function, for microalgae or sponges [68] [69][70]:

$$\mu = \mu_{max}\left[1 - \exp\left(\frac{-I}{k_E}\right)\right]$$

$$\mu = \mu_{max}\tanh\left(\frac{\alpha I}{\mu_{max}}\right)$$

Where μ represents the growth rate, μ_{max} is the maximal growth rate under optimum intensity, I is the measured intensity, Ke is a saturation parameter and α is the initial slope of the curve (empiric). These models require specific data about the PI curve which is not available yet on Ulva.

Rubio [54] has suggested a comprehensive mechanistic model for the photosynthesis in microalgae. Taking into consideration continuous light, intermediate light, photo inhibition and photo adaptation the authors have suggested a set of equations to be used together and adjust model for the estimations of efficiency. The model includes also evaluation for the expression of the integration of light equation as it was formulated by previous research [8]. The equation governing the flashing light effect is the following:

$$\frac{P}{P_m} = \frac{I}{\alpha}\int_0^{\phi}(1 - x^*)d\tau$$

Where P is the measured productivity, P_m is the maximal productivity, I is the intensity, α is a constant related to maximal rate energy consumption, absorption coefficient, and concentration of functional photosynthetic units (PSU) in the cell. x^* is the fraction of the functional activated PSU, ϕ is the duty cycle and τ is dimensionless time coefficient. Unfortunately, these parameters are not measureable in our lab. Eventually it has been decided to use the Michaelis Menten equation for the module and the integration of light because these are the only combination of models our data supports.

Appendix G – Offshore cultivation system

Introduction

In order to start and learn the challenges waiting offshore for cultivation systems a prototype was built and positioned in the Reading power station harbor, Tel Aviv. Based on previous work the experiment system was designed and tested first in our lab and later offshore.

Materials and methods

System design

Based on the work "Improve the performance of airlift reactors" by Yusuf Chisti and Murray Moo-Young an airlift (ALR) bio reactor. The system is built out of external wide cylinder open in the upper face, which called the down comer, and internal cylinder open in both the upper and lower faces, which called the riser. The air is being released in the bottom of the riser using external pump. At the bottom of the down comer a weight is sitting in order to help balancing the system in the water. At the bottom and top of the external tube an additional transparent domes were added in order to create smoother flow around the edges and still enable sunlight to come in.

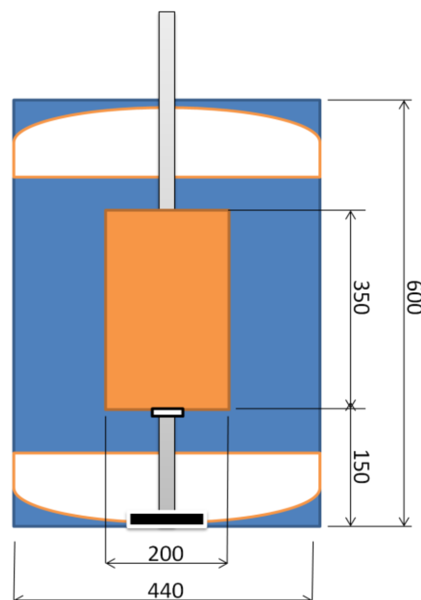

Figure 1. design of the ALR system. Length size - mm.

Figure 2. Picture of the ALR system.

Figure 3. Picture of the ALR system in the water.

Lab experiments

Before deploying the system offshore it was tested in the lab in order to assess the frequency and duty cycle in the system and to adjust the measurements. Above the system a camera was positioned and the video was later analyzed. The goal of this stage was to determine the optimal height of the riser in the design.

Offshore experiments

The system was deployed offshore and initial biomass was positioned in the system. The goal of this part was to measure biomass accumulation in the system. The measurements were taken once a week. During the last experiments a camera was positioned in order to record activity around the system and learn about interaction between the environment and the ALR. Next to the system flat cages with algae were positioned in parallel experiment to measure differences.

Results

Lab experiments

5 experiments with 33 repetitions in total returned unstable results. There were many events of stacked algae on parts of the ALR and other or algae stuck in turbulences on the surface. With air flow rate of 30 liter per minute the model predicted cycle time of 25.7 seconds and Ton (time of exposure to sun) of 3.98 seconds. For air flow rate of 6 liter per minute the model predicted cycle time of 51 seconds and Ton of 7.89 seconds. The air flow rate during the experiments was not measured due to lack of equipment but can be assessed with visual inspection to be in between these limits. It can be seen in the following table that the results are different than the predictions of the model.

Table 1. Results of the lab tests for the main parameters.

	Cycle time [sec]	T on [sec]	DC
Average	13.33	1.79	0.19
STDV	8.71	0.96	0.14

In order to receive similar results in the model the air flow rate needed to be 300 liter per minute.

Offshore experiments

5 experiments over 12 weeks were performed with different initial biomass and different positions of the ALR in the water. Almost every week a decrease in the biomass was measured between 4-100%. Positive growth rates of 15% were measured

only during one week and the algae was very muddy these weeks what might affect the results. The averages of growth rate during all the time of the experiments was -45% (decrease in biomass). During this period of experiments the flat cultivation cages performed positive growth rate with average of 20% (weekly increase in biomass). Between the experiments several approaches was tested but all failed: with and without the riser, with and without the upper transparent cover, close and far from the dock.

Conclusions

<u>Lab experiments</u>

For this specific measurement of ALR the optimal height of the head of the riser from the bottom of the system is 49 cm. It was observed that over aeration (no measured value) create very turbulent environment and damage the desired streamlines. The algae tend to accumulate on every crossing element in it way, a mixing device should not include any object inside the mixing volume. Positioning the aeration point at the bottom of the riser instead of at the bottom of the ALR shows better performances and fewer events of stucked algae on the bottom of the ALR. The results showed high instability which means this design is far from optimal and it would be hard to reproduce test with it.

<u>Offshore experiments</u>

The reason for the loss of biomass in the reactor is not clear. Possible explanations are over aeration of the system and algae escaping from the top of the system. Another possible explanation is that the external cylinder windows for water exchange with the sea were too small and the level of nutrients inside the vessel was too low. Additional reasons can be high pH and temperature environment inside the ALR. From the pictures that were taken during the experiments it can be seen that there was animals activity around the system what may lead to loss of algae.

עבור כיווני מחקר עתידיים אנחנו מציעים ליישם את המודל בגידול בים ואנו מאמינים שתוצאות דומות יכולות להתקבל עם שיפור של עד סדר גודל ביעילות ובפרודוקטיביות. שיטות שונות של המרת אור קבוע למהבהב צריכות להיבחן על מנת להשיג תוצאות טובות יותר.

תקציר

עולם הביו מוצרים הולך וגדל יחד עם הדרישה למקורות אנרגיה נקייה וירוקה כחלק ממודעות סביבתית חדשה וכתגובה למקורות הדלק המוגבלים. אצות הן ביו מסה ימית בעלת השפעה חיובית על הסביבה ומגוון רחב של מוצרים בתחום הקוסמטיקה, הדשן ושוק האנרגיה. כיום, עבור ביו דלקים הפקת ביו מסה של אצות אינה יעילה מספיק; העלויות גבוהות מדי, והפרודוקטיביות נמוכה מדי בהשוואה למחיר חבית נפט והתשואה האנרגטית עבור ההשקעה. אחד הגורמים המגבילים הוא הפרודוקטיביות ליחידת שטח כתוצאה מניצול לא מיטבי של התאורה. חלק נכבד מהאור המגיע לפני השטח לא מגיע לידי שימוש בגלל שהוא בעודף ליכולת הניצול של המנגנונים הפוטוסינטתיים. ניתן לנסות לפתור את הבעיה ע"י הגברת הצפיפות של האצות אבל בגידולים צפופים האור אינו חודר את השכבה העליונה והשכבות התחתונות נשארות בחושך. אחד הפתרונות האפשריים הוא ערבול של בריכת הגידול. הערבוב מאפשר לשכבות התחתונות לעלות לפני השטח ומשקיע את השכבות העליונות לאחר שאלה קיבלו את מנת האור הדרושה להן. הערבול יוצר משטר תאורה הדומה לאור מהבהב בנוסף לאפקטים כמו הנגשה של נוטריינטים וגירוי מיכני של האצות. בכל פעם שאצה מגיעה אל פני השטח היא מקבלת מנת אור וכאשר היא שוקעת היא חוזרת לחושך עד לפעם הבאה שהיא צפה. שיטות דומות של ערבול הוכחו כיעילות במתקני גידול למיקרו אצות אבל עוד לא נבדקו במקרו אצות.

מטרת המחקר לבחון את ההשפעה של אור מהבהב בעוצמת תאורה חיצונית ממוצעת על גידול מקרו אצות במטרה להעריך את אפקטיביות הערבוב. הטכנולוגיה לגידול מקרו אצות בתנאי מעבדה או בתנאי שדה קיימת אך אינה מפותחת מספיק. היעד הראשון של מחקר זה היה להקים מערכת ניסוי מעבדתית לחקר מקרו אצות. היעד השני היה לבחון את הפרודוקטיביות וניצול האור ע"י האצות תחת אור מהבהב בהשוואה לאור קבוע.

מערכת ניסוי חדשה עוצבה ופותחה במעבדה שלנו על מנת לבחון את השפעת האור המהבהב על מקרו אצות. הנתונים שנאספו מקבוצת הבקרה מראים הישנות של התוצאות עם 21% סטייה מהממוצע, בהשוואה ל 323% במערכת מקבילה בבית ספר פורטר, מכאן שהמערכת מספקת תנאים יציבים ומאפשרת לבצע ניסויים השוואתיים. הניסויים באור מהבהב מראים שתחת עוצמה של 1000 מיקרו-אינשטיין ועם 50% בלבד משטף הפוטונים ניתן להשיג 84% מהתוצר בהשוואה לאור קבוע. באשר ליעילות ההמרה של אור לביו מסה, התוצאות מראות שיפור משמעותי בסדר גודל, פי עשרה, תחת אור מהבהב עם שטף פוטונים של 1% בלבד. עבור תנאים אלה תוצאות דומות נרשמו גם עבור הפרודוקטיביות, עד פי עשרה לעומת אור קבוע. בסופו של דבר התוצאות אוששו את השערת הניסוי שהפרודוקטיביות ליחידת שטח ויעילות ההמרה של אור לביו מסה יכולות להשתפר בגידול צפוף של מקרו אצות ע"י חלוקה של האור בהבהובים בין מספר קבוצות של אצות.

אוניברסיטת תל אביב

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על-ידי

עוז חביבי

העבודה נעשתה בבית הספר להנדסה מכנית

בהנחיית פרופ' אלכס ליברזון וד"ר אלכס גולברג

טבת תשע"ח

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