Computerized Optimization of Microalgal Photosynthesis and Growth

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\section*{ABSTRACT}

We describe the use of a novel, computerized, multiple outdoor microalgal growth system to achieve conditions maximizing high photosynthesis and biomass production rates. We applied this system to determine the effects of multiple environmental and culture parameters such as light, temperature, pH, and mode of mixing and CO\textsubscript{2} supply, and their combinations on the rates of algal photosynthesis, respiration, and growth. We continuously monitored the following parameters: pH, temperature, dissolved oxygen, and light, concomitantly with algal growth and biomass production rates. In particular, we likewise monitored and determined the dramatic effects of CO\textsubscript{2} supply, mixing by paddle wheel or bubbling air modes, on algal biomass production. The isolated and synergistic effects of each factor and operation regime were measured and are discussed. We found that the diel range of maxima to minimal pH and oxygen levels strongly correlated with container performance, and were controllable by CO\textsubscript{2} supply and mixing regime. We conclude that mixing by bubble aeration was the most important growth-rate controlling factor. It limited the build-up of high oxygen and pH levels that reduce photosynthesis that were measured by O\textsubscript{2} evolution. In this study, OD measurements calibrated with biomass concentrations were used to calculate growth rates and estimate the photosynthetic rates of the microalgae. The potential of algae as a source of food, feed, biofuel, and fine chemicals has been widely recognized, but their competitiveness depends on areal yields. Our results allow us to recommend culture parameters that increase areal biomass yields, regardless of the algal species, thereby bringing microalgal mass culture closer to economic viability.

\section*{Introduction}

The use of microalgae in innovative high-tech mariculture has obvious economic and environmental advantages (Mata, Martins, & Caetano, 2010). However, as attractive as it is, it is still considerably more expensive than relying on traditional resources. Up till now, there are specific gaps in the technologies used that when addressed, could lead to increased yields and lower costs (Grubbelaar, 2010; Jo et al., 2020). Nevertheless, even though in recent years, yields have increased, thereby reducing product costs, the production costs are still prohibitively high (Grubbelaar, 2013).

The main bottlenecks in microalgal biomass production are the high culturing and harvesting costs (Gerchman et al., 2016; Jia Sen Tan et al., 2020). Therefore, our aim was to increase growth rates and yields, thereby reducing product costs. To achieve that goal, key controlling factors, such as light regime, oxygen, pH, temperature, and balanced growth media, have to be optimized (Abu-Ghosh, Fxler, Dubinsky, & Iluz, 2015a).

Microalgae, like all plants, produce O\textsubscript{2} in the light. This “by-product”, which is not used for respiration, is released into the water. At night-time algae only respire, using up some of the carbohydrates and oxygen formed during the light hours, while releasing CO\textsubscript{2}. Excess O\textsubscript{2} in the medium inhibits photosynthesis by “end-product inhibition” (Goldschmidt & Huber, 1992). Under oxygen supersaturation and relatively low CO\textsubscript{2} availability conditions, the activity of Ribulose bisphosphate carboxylase/oxygenase (RuBisco) shifts from being a carboxylase to an oxygenase (Ohad, Adir, Koike, Kyle, & Inoue, 1990). Mixing equilibrates gas pressure between culture medium and air, thereby preventing oxygen supersaturation. The metabolic activities of algal cells, photosynthesis, and respiration continuously change pH. During daytime, algae take...
up dissolved CO₂, and dissociated bicarbonate ions from the growth medium and utilize them for photosynthesis (Berman-Frank, Zohary, Erez, & Dubinsky, 1994). The removal and uptake of CO₂ raises the pH. Conversely, during night-time, the algal cells respire, releasing CO₂ into the water and acidifying it.

The effects of light on algal growth are obvious, due to the dependence of photosynthesis on it (Dubinsky, 1989; Dubinsky, Matsukawa, & Karube, 1995). In high-density cultures, light becomes limiting as a result of self-shading, resulting in slow growth as the photic depth is only a few centimetres deep (Huisman, van Oostveen, & Weissing, 1999; Lindemann, Backhaus, & St John, 2015). Under such conditions, container production can become negative as the respiratory maintenance energy requirements exceed the light-limited photosynthetic production. At the culture surface, the high light becomes inhibitory (Han, Virtanen, Koponen, & Straskraba, 2000). Mixing can prevent high-light, free-radical-induced damage by reducing the duration of algal exposure to excessive light. Furthermore, as the algae move vertically through the culture, cells are exposed to fluctuating light that, under certain conditions, could significantly enhance photosynthesis (Abu-Ghosh, Dubinsky, Banet, & Iluz, 2018; Abu-Ghosh, Fixler, Dubinsky, & Iluz, 2015b, 2016; Iluz & Abu-Ghosh, 2016). Mixing also prevents algal sedimentation that causes the settled algae to be in the dark and have no access to nutrients. The algae die and decompose creating a harmful anaerobic layer (Iluz, Yehoshua, & Dubinsky, 2008).

The importance of temperature in affecting photosynthesis and growth rates stems mainly from the different temperature-dependent reactions of photosynthesis and respiration. Photosynthesis, like most photochemical reactions, has a weak dependence on temperature; however, respiration increases markedly with temperature. Hence, net photosynthesis and biomass production rates are sensitive to temperature (Dungan, Whitehead, & Duncan, 2003; Madsen & Adams, 1989). Under extreme situations, cultures could collapse when respiration exceeds gross photosynthesis, requiring expensive cooling. Alternatively, thermophilic algal strains could be used (Dubinsky et al., 1995; Varshney, Mikulic, Vonskh, Beardall, & Wangikar, 2015).

The goals of our study were to:

(a) Monitor algal growth rates and physiochemical parameters by computer-linked sensors.
(b) Control pH by CO₂ delivery rates.
(c) Choose optimal mixing methods to avoid oxygen supersaturation.
(d) Determine the dependence of algal photosynthesis and growth rates on culture parameters.
(e) Optimize algal biomass production rates by a combination of the operational parameters maximizing net photosynthesis.

Materials and Methods

Algal Species and Growth Medium

The freshwater microalga *Chlorella vulgaris* (Chlorophyta) was grown indoors under controlled conditions as the inoculum for the outdoor containers. Temperature 23°C and irradiance at the water surface 70 μmol quanta photons m⁻² s⁻¹. The culture was grown in sterile Bristol medium (http://web.biosci.utexas.edu/utex/Media%20PDF/bristol-medium.pdf), in 20 l flasks, mixed by air bubbles up to optical density (O.D.) 1.0 at 680 nm. (total culture volume was 80 l). The algal culture was then used to inoculate the outdoor containers, 20 l culture was added to each 100 l container. Bristol medium made up with tap water was used as a growth medium. Algal growth was estimated from optical density at 680 nm (NanoDrop 2000 c Spectrophotometer, Thermo Fisher Scientific, Wilmington, DE, USA), following calibration against dry weight (Fig 1b). For statistical analysis, see Fig 1.

Computerized Algal Culture System

Four 100 l outdoor open containers were used (Fig 2). Temperature, pH, dissolved oxygen, and light intensity were monitored in situ every 30 seconds.

Light was measured above the container surface by a quantum sensor 190 R (LI-COR Instruments, Lincoln, NE). Temperature, dissolved oxygen and pH sensors (Hach-Lange GmbH, Düsseldorf, Germany) were placed inside each container ~10 cm below the culture surface. One of the containers was enriched with CO₂ on demand to preselected pH threshold values. CO₂ supply was started at pH 7.7 and shut off at pH 7.0. All data were acquired, stored, and displayed using Z-View Software (ZIVAN RDT Systems, Tel-Aviv, Israel). Integration of the control systems (including CO₂ supply) was installed by Modotec Ltd. (Haifa, Israel).

Prior to sampling, we added tap water up to the initial medium level to compensate for the evaporative loss. We sampled all containers in triplicate for O.D. measurements, seven times during each experiment. Since algae sank to the bottom of the “un-mixed control container”, the samples from that container were taken after a short manual mix that resuspended the algae. Experiments lasted for 2 weeks from inoculation to final harvesting.
Figure 1. Treatment effects on *C. vulgaris* growth, in the different containers. Standard deviation n = 3 (a) Culture densities (680 nm) in the four containers over a 14-day period. (b) Optical density versus dry biomass weight.

**Data Analyses**

The Algae container Analysis Program consists of a set of three routines written in LabVIEW® 2015 (National Instruments, Austin, TX) to analyse and display data from the algae containers. All Graphs are displayed by Python 3.7.1, and statistical analysis was applied with R 3.6.2.

(a) *Pretreat and Combine Files* – This program reads four text files containing temperature, oxygen, pH, and light data collected at 30-second intervals over an extended period (~2880 points/day). Each file can contain the specific data for several weeks for a single container. The program reduces the data to an average point per minute (1440 points per day) and corrects for any missing values by interpolating neighbouring points. The dissolved oxygen (DO) data are corrected for solubility as a function of temperature (Supplementary fig. 1) and converted to oxygen saturation. The data are combined into a single file for use in the *Container_Main* program.

(b) *Container_Main* – This program reads a text file containing data for temperature, oxygen, pH, light, and time at 120-second intervals over several weeks for a single container (ca. 21 000 data points). The data are displayed separately for each day on a double “Y-axis” graph due to the different data ranges.

For simplicity, it is possible to display every 5th point, in which case the data for each point are an unweighted average of the five data values (Savitzky & Golay, 1964).

(i) *All-in-one Graph* – This program displays graphs for oxygen, pH, light, and temperature for a given container over the entire period of the experiment in a single graph.

(ii) *Statistical analysis* – An autoregressive model fitted for the oxygen evolution or pH changes over the time-series data. Akaike information criteria used as error and outlier prediction. Sigma squared is used for the variance of the model, akaike information criterion (AIC) and standard error used for model accuracy, log-likelihood used for assessment of the fit between the model and the data. Augmented Dicky-Fuller Test was used to test the stationarity of the data (Petris, Petrone, & Campagnoli, 2009).

Data were decomposed for the seasonal trend to visualize the fluctuations in oxygen and pH. The difference in trend between containers and the randomness in the
data were evaluated, respectively (Godfrey & Gashler, 2018).

**Results and Discussion**

**Mixing Regimes Affect Growth Rates**

*C. vulgaris* cultures were grown outdoors in four 100 L open containers, each with a different mixing regime as detailed in Table 1. Light intensity, temperature, pH, and dissolved oxygen (D.O.) were continuously monitored as described. Temperature ranged between 18°C before dawn and 28°C at noon. Light intensity peaks reached ~1050–1300 μmole quanta photons m² s⁻¹. Growth rates were calculated from the O.D. measurements. All cultures went through a five-day lag period followed by a fast exponential growth phase. The biomass peaked on the 7th or 8th days. It is noteworthy that the control container #4 peaked earlier but reached the lowest yield. In all cases, the peaks were followed by a gradual biomass growth rate decline (Fig 1a). We attribute that decline to a progressive light limitation by the denser culture, combined with nutrient exhaustion.

![Figure 2. A computerized outdoor algal growth system. (a, b) 100 l open containers mixed by paddles or air bubbles. Each container was 70 cm long, 52 cm wide and 40 cm deep. Culture depth was 32 cm. Paddle size was 17.5 by 12 cm. They reached down to 10 cm above bottom and 3 cm from container sides (containers No. 1, 2). Air was supplied throw a perforated tube at the bottom of the container (container No. 3). (c) Temperature, pH, dissolved oxygen, and light intensity were continuously monitored in situ every 30 seconds by a computerized system. All sensors, except light sensor, were placed 10 cm below the culture surface; light sensor was placed above the surface.](image)

| Container No. | Mixing regime | Remarks          |
|---------------|---------------|------------------|
| 1             | Paddles       | CO₂ enrichment   |
| 2             | Paddles       | No added CO₂     |
| 3             | Air bubbles   | No added CO₂     |
| 4             | No mixing     | (control) No added CO₂ |

Table 1. Treatments of the four containers. Since the algae settled to the bottom of the unmixed container, it was necessary to re-suspend the algae before sampling in order to get a homogenous sample.

The culture mixed by air bubbling, in container #3, reached the highest density of all four algal containers. That was followed by the culture mixed by a paddle wheel and enriched with CO₂. Third, was the culture mixed by paddle wheel without CO₂ enrichment. The control culture with no mixing and no CO₂ addition suffered from the sinking of algae, discussed above.

The packed algae at the container bottom, obviously had limited access both to nutrients and to light, resulting in the lowest algal biomass production (Fig. 1).

To understand the reasons leading to the differences in algal densities, we examined the impacts of mixing...
regimes on oxygen saturation, pH levels and temperature within the containers.

The evident differences in peak biomass are due to the key role of the mixing regime on algal growth. The mixing regime’s effects stem from the differences in CO₂ availability, the mitigation of the inhibitory effects of oxygen supersaturation on photosynthesis, prevention of thermal stratification, and the exposure of the cells to light intensity fluctuation.

**Mixing Regimes Affect Oxygen Saturation in Algal Containers**

Oxygen saturation percentages were calculated from the concentration of dissolved oxygen (ppm) relative to medium temperatures (Supplementary fig. 3). Oxygen saturation values of the four containers were compared (Fig. 3). Saturation in the unmixed container 4, exceeded 400% (of its atmospheric equilibrium value) during daytime. The highest amplitude of saturation changes, ranging about 200% between day and night values, was reached in container 4. This confirms the limited gas exchange through the algal culture/atmosphere interface.

Oxygen saturation levels of cultures in containers 1, 2, and 3 reached maxima of ~150%, ~130%, and ~115%, respectively, on days 11–14. During night-time, saturation was ~105% (days 1–10) and ~100% towards the end of the experiment.

The differences in oxygen saturation between containers 1 and 2 (both mixed by paddles) were due to differences in biomass density. Container 1 was enriched with CO₂ and more biomass grew in it than in container 2; hence, it released more O₂ during daytime and consumed more O₂ for respiration during night. Therefore, oxygen saturation amplitude between days and nights in container 1 (~50%) was higher than in container 2 (~30%).

The culture in container 3, mixed by air bubbles, presented the lowest oxygen saturation level and the smallest diel amplitude (~11%). We view this as the main reason for its attaining the highest biomass of the four containers. This means that even though it produced more oxygen than other cultures, the mixing by air bubbles was more effective in the removal of excess oxygen than mixing by paddles. The results underscore the importance of the removal of dissolved oxygen from the culture, which supported higher photosynthetic and growth rates.

**Mixing Regimes Affect the pH of Algal Cultures**

The pH of algal growth media is affected by physical, chemical, and biological factors. Adding carbon dioxide to the container immediately lowers pH, since CO₂ reacts with water protons and hydroxyl ions, and forms carbonic acid, which dissociates into carbonate/bicarbonate ions, thereby lowering culture pH, as was evident in container 1 (Fig. 4b).

During daytime, CO₂ uptake by algae, raised the pH, while algal respiration during night-time, released CO₂ into the water and lowered the pH (Dubinsky & Rotem, 1974). This sinusoidal pH pattern in containers 2, 3, and 4 resulted from the diurnal, photosynthesis-driven, CO₂ limitation (Figs 5–8).

The pH in container 1 was controlled and kept within the preselected range of ~ pH 7.3 ± 0.3. As biomass increased, it utilized more CO₂ and pH increased faster so that the CO₂ enrichment was triggered more frequently (Fig. 4b).

The large differences in diel amplitude between noon pH maxima and night-time minima were clearly biomass and photosynthesis-dependent in the mixed containers 2 and 3, as was the increase in diurnal peak values (Figs 5–7). In the unmixed container 4, pH gradually increased, thereby limiting CO₂ availability for photosynthesis, and concomitantly biomass decreased (Figs 5 and Figs 8).

![Figure 3. Oxygen saturation (%) graph of the four algal containers.](image-url)
The steepest, but short increases in pH maxima were observed in the control container (container #4, Fig 5 and 8b). Peak pH levels (daytime) increased from ~6.5 to ~9.3 while pH minima (at night) varied from ~6 to ~8.7. These large pH fluctuations show the controlling importance of mixing, evident in their attenuation in the mixed containers 2 and 3.

In the algal culture mixed by paddles (container 2), pH levels followed a different pattern. Peak pH levels (daytime) went from ~8.4 to ~10.4, while at night they increased from ~7.5 to ~8.4. The pH amplitude between days and nights increased from ~0.9 on the first day of experiments to up to ~2 on the 10th day (Fig 6B). The slope of pH daytime maximal trend-line for the duration of the experiment was steeper than the night-time trend. As biomass increased, so did the pH amplitude between days and nights.

The pH values of the algal culture mixed by air bubbles (container 3) presented the most moderate trend-line slopes, although the highest growth occurred in that container. The pH daytime maxima went from ~7.7 to only ~9, while night lows increased from ~7.4 to ~7.9. The pH amplitude between days and nights increased from ~0.3 on the first day up to ~1.1 on the 10th day of the experiment (Fig 7b). We attributed this moderation to the mixing by air bubbles that resulted in a more efficient gas exchange between algal culture and air, thereby creating a “physical pH buffer”.

Both mixing regimes, bubbling and paddles, did enhance algal photosynthesis and growth rates;
nevertheless, air bubbling was far superior, probably due to the dramatically higher culture-to-gas interface created by the bubbles, thereby accelerating gas (O₂ and CO₂) equilibration processes, stabilizing and optimizing conditions for high rate photosynthesis.

Figure 6. Container 2, paddles, no CO₂ enrichment. (a) Legend as in Figure 4a. (b) Light intensity (yellow line) and pH (green line). Trend line of pH peaks (grey dotted line) and night-time minima (blue-dotted line).

Figure 7. Container 3, air bubbles, no CO₂ enrichment. (a) Legend as in Figure 4a. (b) Legend as in Figure 6b.

**Mixing Reduces Temperature Rise in Open Container**

Temperature measured 10 cm below culture surface, did not differ among containers 1, 2, and 3 throughout the experiment (Figs 4a, 6a and 7a). Temperatures in the unmixed container 4 reached higher values, approximately
+2°C above those in the mixed containers (Fig 8a). This difference is attributed to preventing the build-up of thermal stratification combined with an increase in evaporative cooling in the mixed cultures.

Our conclusions:

(a) Computer control and parameter monitoring allow the optimization of algal mass cultures.
(b) Mixing prevents algal sedimentation.
(c) Mixing prevents high-oxygen concentrations in the light, while providing oxygen for night-time respiration.
(d) Mixing prevents high-light photosynthesis inhibition at the culture surface and exposes the algae to enhancing fluctuating light regime.
(e) Mixing reduces high pH build-up that limits CO₂ supply for photosynthesis.
(f) Air bubbling is the superior mixing method.
(g) Mixing reduces warming of the cultures by increasing evaporation and preventing the build-up of thermal stratification.
(h) CO₂ addition, controlled by pH, enhances photosynthesis and accelerate growth.

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Author contributions

B. Vasker: Conception and design of the study, conducting the experiments, collection and assembly of data, drafting the article, final approval of the version to be submitted; M. Ben-Zion: Analysis and interpretation of data collected, facilitated the mathematical computerized functions of the paper and drafting the article; Y. Kinel-Tahan: Conception and design of the study, drafting and revising the article, final approval of the version to be submitted; Z. Dubinsky: Conception and design of the study, critical revision of the article for important intellectual content, final approval of the version to be submitted; J. Grobbelaar: Acquisition of data, revising the article critically for important intellectual content, final approval of the version to be submitted; H. Yudkin: Statistical analysis and graphic presentation of data collected; Y. Yehoshua: Acquisition of data, revising the article critically for important intellectual content, obtaining funding, final approval of the version to be submitted.
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