Impact of Cell Size, Light Wavelengths, and Intensities on Growth, Oxygen Production, and Consumption Rates of Chromochloris zofingiensis and Haematococcus lacustris.

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Article

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Impact of Cell Size, Light Wavelengths, and Intensities on Growth, Oxygen Production, and Consumption Rates of *Chromochloris zofingiensis* and *Haematococcus lacustris*.

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Abstract

Chromochloris zofingiensis (*C. zofingiensis*) and *Haematococcus lacustris* (*H. lacustris*) were cultivated in an autotrophic conditions. The algae cell sizes were measured to determine their size and light absorption for oxygen production and consumption rates. Methodologies were developed to measure the oxygen production and consumption rates of both species using different light wavelengths (blue and red) and intensities (20–80 μmol m^{-2} s^{-1}). *H. lacustris*, with larger cells, potentially exhibited slower growth rates (0.09 d^{-1}) at 20 μmol m^{-2} s^{-1} in blue-light and high O_2 consumption in the red-light, which resulted in an increase in pH (11.8) at 80 μmol m^{-2} s^{-1} in red-light. Conversely, *C. zofingiensis* was distinguished by smaller, non-motile cells, which streamlined oxygen production, transport, and responded favorably to changes in blue-light intensities, with a higher specific growth rate of 0.19 d^{-1}. Both algae species had high oxygen production and consumption rates in blue-light environment due to its short wavelength. While in the blue-light environment, *C. zofingiensis* optimized its photosynthetic quotient (2.3) at 60 μmol m^{-2} s^{-1}. This study underscores the biotechnological potential of *C. zofingiensis* as an oxygen source and high-value products.

Keywords: *Chromochloris zofingiensis*; cell size; oxygen production; oxygen consumption; photosynthetic quotient; light.

1. Introduction

Photosynthesis, is the fundamental process by which organisms convert light energy into chemical energy, and this process is critical in sustaining life on Earth. This natural phenomenon not only drives the production of oxygen but also forms the basis of the food chain. Among the diverse organisms capable of photosynthesis, microalgae stand out for their exceptional efficiency and versatility[1]. Microalgae are a diverse group of unicellular and multicellular fast-growing microorganisms that use light and CO_2 to grow and produce biomass[2].

Attention has been drawn to the essential nutrients and compounds found in algae[3] and these organisms are often overlooked in discussions of oxygen production in our planet's ecosystem. As primary producers, algae utilize photosynthesis to convert carbon dioxide and sunlight into energy, releasing oxygen as a byproduct [4]. This process not only sustains the algae themselves, but also fuels the oxygen levels essential to supporting life in our environment. In recent years, the study of algae and their photosynthetic capabilities has received increased attention due to the potential production of high-value substances that could be applied in various fields, including environmental science, biotechnology, agriculture, and medicine[1].

H. lacustris is recognized as the leading microalgal producer of astaxanthin[5]. It comprises of green ovoid spherical biflagellate cells containing chloroplasts, which are motile and free-floating in the initial green vegetative stage. The accumulation of astaxanthin in this microalga is approximately 1.9–7 % of its dry weight[6] with cell walls that are relatively thick[7]. However, several challenges need to be addressed with regards to this algae, such as its complex life cycle, relatively low cell yield, sensitivity to adverse conditions, thick and rigid cell walls, and efficient pigment extraction[8]. In contrast, *C. zofingiensis* is a unicellular, non-motile, freshwater chlorophyte microalga with a spherical or oval-shaped cells. The cell size varies depending on the life stage of the cell, and the range of sizes observed is 2–17 µm. The increase in cell size is due to the accumulation of lipids and starch[9]. *C. zofingiensis* is capable of autotrophic, mixotrophic, and heterotrophic growth at high densities (121.5 g L^{-1}), astaxanthin productivity (111 mg L^{-1} day^{-1})[10], and tolerance to a wide range of environmental conditions[11]. As stated by[12], the advantages of *C. zofingiensis* as an alternative

alga for natural astaxanthin production include superior tolerance to adverse culture conditions, a higher growth rate and lipid productivity, and a more fragile cell wall, which results in enhanced extractability of the target compounds. These features renderers it a highly desirable species for biotechnology applications.

There are limited studies that have monitored and controlled the oxygen production and consumption rates of *C. zofingiensis*, particularly in relation to its ecological benefits and biomass production. Additionally, the photosynthetic quotient (PQ) of this microalga is crucial for assessing CO_2 reduction into carbohydrates for biomass accumulation. In this study, we introduced an innovative, low-cost and effective system method of monitoring the oxygen production and consumption in a closed system using probes, Arduino and Raspberry Pi. This system allows for the rapid measurement of light respiration and post-illumination oxygen uptake immediately in a dark environment without disturbing the cultivation and growth of the microalgae in the reactor.

This study aimed to determine the algae cell characteristics, oxygen production and consumption rates of *C. zofingiensis* and *H. lacustris* in response to different wavelengths and intensities of light, within a controlled and monitored environment. Understanding these responses is crucial for optimizing algae cultivation systems and ecological benefits, as light plays a key role in photosynthesis and overall biomass production. The findings from this study can inform strategies to enhance algae growth for applications in carotenoid production, carbon capture, and sustainable agriculture.

2. Materials and Methods

2.1 Algae Cell

C. zofingiensis (synonym *Muriella zofingiensis*, culture collection No. 2175 of the National Institute for Environmental Studies, Tsukuba, Japan) and *H. lacustris* (culture collection No. 144 of the National Institute for Environmental Studies, Tsukuba, Japan) were subcultured autotrophically in a 500 mL flask with modified Bristol medium. The medium was referred to as CZ-M1[13] which consisted of the following (per litre) 0.75 g $NaNO_3$; 0.175 g KH_2PO_4; 0.075 g K_2HPO_4; 0.075 g $MgSO_4 \cdot 7H_2O$; 0.025 g $CaCl_2 \cdot 2H_2O$; 0.025 g NaCl; 5mg $FeCl_3$; 0.287 mg $ZnSO_4 \cdot 7H_2O$; 0.169 mg $MnSO_4 \cdot H_2O$; 0.061 mg H_3BO_3; 0.0025 mg

87 $CuSO_4 \cdot 5H_2O$; and 0.00124 mg $(NH_4)_6Mo_7O_{24} \cdot 7H_2O$. The culture was continuously supplied
88 with filtered air and the temperatures were maintained at 25 °C, controlled by regulating the
89 air temperature in the chamber. The culture was illuminated with white light, Toshiba
90 FL40SS, 37 watts (20 µmol m^{-2} s^{-1}, 12L/12D) and was finally prepared for experiment 1 and
91 2.

92 2.2 Culture Experimental Set-up

93 i. Experiment - 1

94 The subculture in subsection 2.1 yielded the algae samples, which were then diluted with
95 distilled water to a final volume of 10 mL per tube. The dilution was achieved by adding 1
96 mL of algae sample to 9 mL of distilled water. The samples were then homogenized using a
97 vortex mixer to ensure accurate cell counting and measurement. 10 µL of the algae sample
98 was introduced into the 0.1 µL JHS standard Thoma hemacytometer (0.100 mm depth ×
99 1/400 mm^2 width, Sunlead Glass Corp., Tokyo, Japan) for enumeration. Another 10 µL of
100 the algae sample was utilized to determine the algae cell size using an Olympus BX50
101 microscope with a ToupCam IP103100A camera and ToupView software for analysis. Both
102 the counting and measurement were performed with a 40X lens magnification. A total of five
103 samples were analyzed, with each sample counted ten times and were measured twenty times.
104 The microalgae cell concentration were calculated using the following equations.

105 Concentration algae (cell mL^{-1}) = *Average nos. of cell* × *Dilution factor* × 10^4 (1)

106 Algae cell volume (V) (µm^3 cell^{-1}) = $\frac{4}{3}\pi r^3$

107 where, V = Volume of the cell, r = radius of the cell (µm) = $\frac{d}{2}$, where d = cell diameter
108 (µm).

109 Cell volume (L $cell^{-1}$) = $\frac{Cell\ volume\ (\mu m^3\ cell^{-1})}{10^{15}\ (\mu m^3\ L^{-1})}$ (2)

110 Surface area (μm^{-2} $cell^{-1}$) = $4\pi r^2$ (3)

111

112

ii. Experiment - 2

This experiment was conducted under autotrophic conditions, as illustrated in Figure 1. 300 mL of CZ-M1 medium was prepared, based on the methodology previously described in subsection 2.1 above. The medium was autoclaved at 121 °C for 20 minutes using a Sanyo (Panasonic) MCV-B91S autoclave. Initially, 150 mL of *C. zofingiensis* and 100 mL of *H. lacustris* were centrifuged for 15 and 10 minutes, respectively, at 10,000 rpm due to their difference in cell size. The supernatants were removed after centrifugation and 0.05 % NaCl solution was added to the tubes. The algae cells were then centrifuged again for 15 and 10 minutes at 10,000 rpm, and this process was repeated three times. Following the washing procedure, 15 mL of 5 % NaCl was added to the tube in preparation for the experiment. The pH sensor (9680S-10D Horiba pH electrode) was calibrated with pH standard solutions at 4, 6.8, and 9.2. The electrical conductivity (EC) sensor (3552-10D Horiba Conductivity cell) underwent a three-step calibration process involving dry calibration, low and high EC calibrations (1,412 µS cm^{-1} and 12,880 µS cm^{-1}) using 0.01 m and 0.1 m of KCl solutions, respectively, at 25 °C. The dissolved oxygen (DO) sensor (DF Robots, gravity: SKU: SE No. 237) was calibrated using a single-point dry calibration method. Following the calibrations, the probes were sanitized with ethanol before insertion in the reactor.

A total of 15 mL of washed cells of either *C. zofingiensis* or *H. lacustris* were introduced to the reactor, which had the following dimensions: The reactor (height 9.3 cm x diameter 8.9 cm) was filled with the autoclaved CZ-M1 medium with pH of 6.5–6.8. The medium was agitated with a magnetic stirrer set at 250 rpm to initiate the experimental procedure. The stirred tank reactor (STR) was placed in an incubator to maintain a constant temperature at 25 °C. A Python and Arduino program were developed for the purpose of monitoring and controlling the system. Light was provided by using light-emitting diodes (LED) as an energy source. The system was observed in both blue- and red-light environments, with light intensities of 20, 40, 60 and 80 µmol m^{-2} s^{-1}, over a photoperiod of 1:1. Each light intensity was changed at two days interval for algae adaptation and stability in the reactor at each condition. Nitrogen gas (N_2) was typically supplied from a cylinder (0.1 MPa) for the purpose of reducing the dissolved oxygen concentration at the commencement of each cycle, utilizing a solenoid valve (MLV-3-1/8, 200kPa, Takasago, Japan) connected to a relay module. The

devices were connected to an Arduino Uno microcontroller, which was controlled by a Raspberry Pi 3 for real-time data recording and analysis. In each experimental cycle, the algae were exposed to nitrogen gas for 5 minutes, followed by a light environment for 45 minutes and a dark environment for 40 minutes. These conditions corresponded to different light wavelengths and intensities. The data from the proof were recorded at 10-second intervals over a 24-hour period spanning 10 days. While samples were collected twice for analysis during each light intensity.

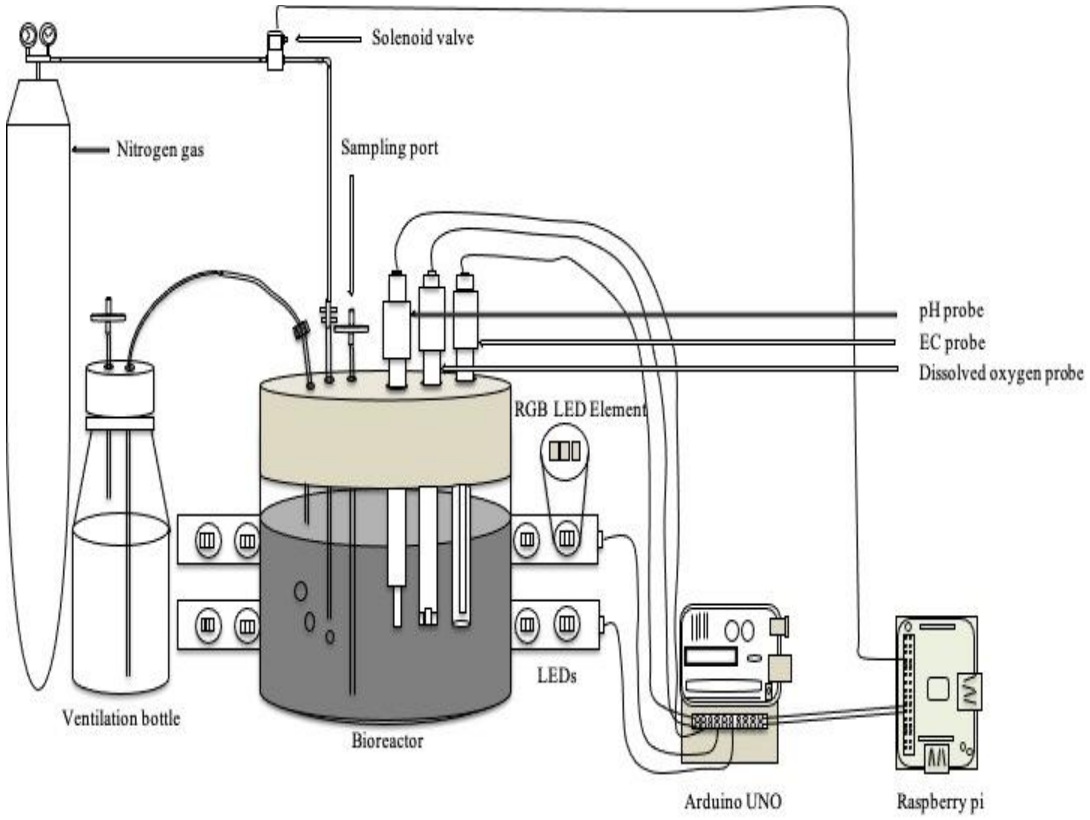


Figure 1: Schematic overview of the stirred tank reactor (STR).

2.3 Determination of Biomass Concentrations

During the incubation period of experiment 2, it was not possible to obtain the requisite volume of liquid for the measurement of suspended solids (SS) at each sampling. Consequently, absorbance was measured using UV-VIS mini-1240, Shimadzu, Japan. While the SS was estimated from the result using the following equation developed from preliminary studies in our laboratory ($R^2 = 0.999$):

$$y = 196.9x^2 + 518.6x \quad (4)$$

where y is estimated SS (mg L^{-1}), x is the absorbance at 660 nm.

a) Oxygen production rate (OPR)

The OPR (µmol O_2 gSS^{-1} min^{-1}) was calculated from the slope of the dissolved oxygen concentration over the last 60 minutes of the light phase $\left(\frac{d[O_2]_L}{dt}\right)$, divided by the biomass concentration (C_b) (Eq.5).

$$OPR = \frac{1}{C_b}\frac{d[O_2]_L}{dt} \quad (5)$$

b) Oxygen consumption rate (OCR)

The OCR (µmol O_2 gSS^{-1} min^{-1}) was calculated from the slope of the dissolved oxygen concentration over the last 30 minutes of the dark phase $\left(\frac{d[O_2]_D}{dt}\right)$, divided by the biomass concentration (C_b) (Eq. 6).

$$OCR = -\frac{1}{C_b}\frac{d[O_2]_D}{dt} \quad (6)$$

c) Microalgae Net Photosynthetic Rate (MNPR)

This was calculated as the difference between *OPR* and *OCR* (µmol O_2 gSS^{-1} min^{-1}).

$$MNPR = OPR - OCR \quad (7)$$

d) Photosynthetic Quotient (PQ)

The PQ was determined as the ratio between the oxygen production rate (Eq. 5) and oxygen consumption rate (Eq. 6).

$$PQ = \frac{OPR}{OCR} \tag{8}$$

3. Results and Discussion

3.1 Algae Cell Characteristics

Table 1: Algae cell characteristics.

Algae	Av. Diameter (μm)	Av. Surface Area (μm^2 $cell^{-1}$)	Av. Volume ($\mu m^3 cell^{-1}$)
C. zofingiensis	5.58 ± 1.35	103.49 ± 49.38	107.14 ± 77.00
H. lacustris	16.56 ± 2.73	885.13 ± 318.03	2,586 ± 1,526

(average ± std)

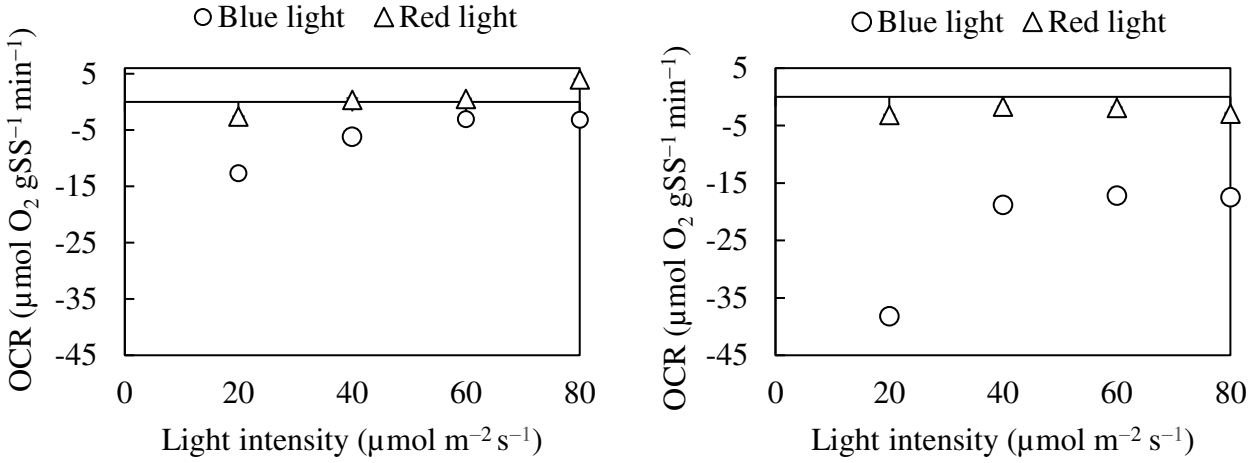
Table 1 presents cell characteristics comparison of *C. zofingiensis and H. lacustris.* The results indicate that the average cell diameter of *H. lacustris* was larger than that of *C. zofingiensis*. During the cell size analysis, the maximum and minimum cell diameters for *C. zofingiensis* were 8.32 μm and 1.99 μm, respectively, while *H. lacustris* had a maximum and minimum cell diameter of 26.86 μm and 11.42 μm, respectively. Additionally, the volume per cell of *H. lacustris* was increased at about 24.1 times as large as *C. zofingiensis* cells.

The smaller cell size of *C. zofingiensis* resulted in a larger surface-to-volume ratio, i.e. specific surface area, of 0.97 ± 0.64 μm^2 μm^{-3} compared to 0.34 ± 0.21 μm^2 μm^{-3} of *H. lacustris*, that may enhance the easier transportation of molecules and ions for metabolism. Additionally, it may had increased the rapid uptake of necessary substances and discharge of unnecessary or harmful compounds including oxygen resulting from photosynthesis. In contrast, the smaller specific surface area of *H. lacustris* may reduce nutrient transport through cell membrane resulting in lower specific growth rate. Furthermore, it also contribute to the less protection of the intracellular environment from changes in the external environment, but conversely, the intracellular environment may be more easily altered by metabolism.

The aforementioned characteristics rendered *C. zofingiensis* a prospective natural source of biomass for livestock feed and a candidate for bioengineering to enhance biomass production.

3.2 Effect of Different LEDs Wavelengths on C. zofingiensis and H. lacustris.

3.2.1 Oxygen Consumption Rates (OCR) per Biomass



a) *C. zofingiensis* b) *H. lacustris*

Figure 2: Oxygen consumption rate (OCR) per biomass in varying light environment.

In the absence of photosynthesis, algae engage in cellular respiration to meet their energy needs. During this process, cellular organic compounds, such as glucose, are broken down in the presence of oxygen to release energy in the form of ATP (adenosine triphosphate). This process consumes oxygen and produces carbon dioxide and water as by-products[14].

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}. \quad (9)$$

During the OCR analysis, an increase in the dissolved oxygen (DO) was observed in the reactor during the dark phase in both algae cultures. It was postulated that this increase in DO concentration may have been due to oxygen excretion from the internal cell space of algae or re-circulation of displaced oxygen during the N_2 gas supply. This resulted in an equilibrium state between the reactor headspace and the medium. The DO increase in the dark phase were in positive values and based on Eq. 6, the negative OCR values recorded

signified oxygen increase. In Figure 2 (a) *C. zofingiensis* showed a negatively large OCR at 20 µmol m^{-2} s^{-1} in blue-light due to the increase supply of oxygen. The oxygen utilization in this environment resulted in specific growth rate of 0.19 d^{-1} as shown in Figure 3. The OCR was observed to vary in response to changes in blue-light intensity. At 80 µmol m^{-2} s^{-1} the OCR was approximately –3.19 µmol O_2 gSS^{-1} min^{-1} which was less than the observations at 20 µmol m^{-2} s^{-1} of light intensity, due to reduced growth rate and lesser negative OCR. The result showed that change in light intensity had an effect on algae growth rate and oxygen consumption. In the presence of red-light, the OCR levels increased in the dark phase to –2.58 µmol O_2 gSS^{-1} min^{-1}, with a specific growth of 0.08 d^{-1} at 40 µmol m^{-2} s^{-1} which was low compared to blue-light at the same light intensity. Furthermore, with changes in light intensity, a less negative OCR was observed, while the algae exhibited lower growth rate to 0.01 d^{-1} at 80 µmol m^{-2} s^{-1}.

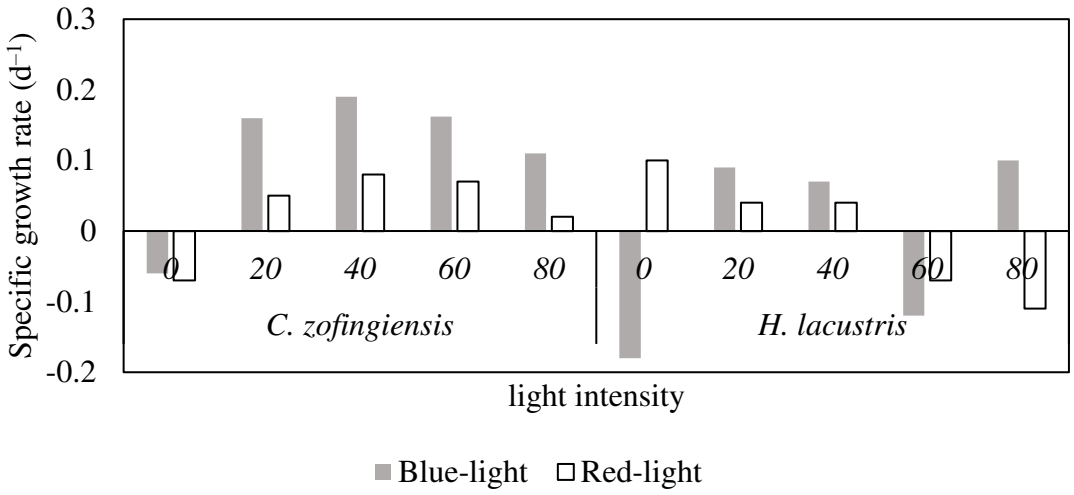
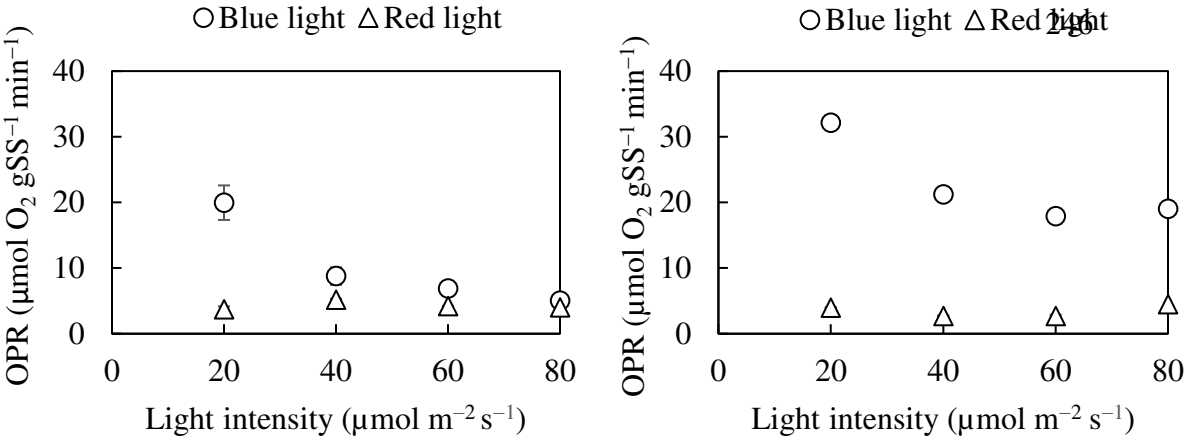


Figure 3: Algae specific growth rate under varying light wavelength and intensities.

In Figure 2 (b) *H. lacustris* exhibited a decreased growth rate and with larger DO rate when subjected to the same light intensity compared to *C. zofingiensis*. In the blue-light environment, *H. lacustris* exhibited OCR of –38.19 µmol O_2 gSS^{-1} min^{-1} due to its increased cell size and high oxygen supply but with a growth rate of 0.089 d^{-1} which was low compared to *C. zofingiensis* growth rate at 20 µmol m^{-2} s^{-1}. There was a reduction in DO rate (reduction in oxygen consumption) with change in light intensity, from 60–80 µmol m^{-2} s^{-1} which might

be due to poor light energy utilization. In contrast, under red-light, at 20 µmol m^{-2} s^{-1}, the DO rate and oxygen supply was less compared to the blue environment at the same intensity. Furthermore, the DO rate was reduced with change in red-light intensity due to decrease in growth rate.

3.2.2 Algae Oxygen Production (OPR) per Biomass



a) *C. zofingiensis* b) *H. lacustris*

Figure 4: Oxygen production rate (OPR) per biomass in different light environment.

Microalgae, like higher plants, can utilize only photosynthetically active radiation (PAR) within the 400–700 nm range for photosynthesis, with blue- and red-light being the most effective[15].

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \quad (10)$$

In Figure 4, the oxygen production rates of *C. zofingiensis and H. lacustris* were evaluated in different light wavelengths and intensities. During the *C. zofingiensis* culture period, the OPR decreased with a change in light intensity in both blue- and red-light environments. During the algae culture in blue-light, the observed OPR was 19.9 ± 14.2 µmol O_2 gSS^{-1} min^{-1} at 20 µmol m^{-2} s^{-1}. A further change in blue-light intensity to 80 µmol m^{-2} s^{-1} led to reduction in OPR to 5.04 ± 0.8 µmol O_2 gSS^{-1} min^{-1}. Furthermore, in the red-light environment, at an irradiance of 20 µmol m^{-2} s^{-1}, the OPR was 1/7.3 times as low as the OPR observed under blue-light at the same intensities. While at 80 µmol m^{-2} s^{-1}, the OPR in the

red-light was 2.1times as low as blue-light at same intensity. *H. lacustris* cells exhibited an increase in OPR in both blue- and red-light environments compared to *C. zofingiensis.* This may be attributed to *H. lacustris* cell size used in the study, in both blue- and red-light environments [Figure 4 (b)], the OPR decreased from 20 – 40 µmol m^{-2} s^{-1} in both algae species, which might be attributed to poor utilization of light energy in this conditions. Conversely, an increase in OPR was observed with change in light intensity at 80 µmol m^{-2} s^{-1} for both blue- and red-light in *H. lacustris* culture due to reduced growth which increased light intensity available in algae biomass.

3.2.3 Microalgae Net Photosynthetic Rate (MNPR) and Photosynthetic Quotient (PQ)

Table 2: Effect of varying light intensity and wavelengths on MNPR and PQ.

(a) MNPR (µmol O_2 gSS^{-1} min^{-1})

light intensity	*C. zofingiensis*		*H. lacustris*	
(µmol m^{-2} s^{-1})	Blue-light	Red-light	Blue-light	Red-light
20	**7.28**	1.18	–6.07	0.85
40	2.56	2.58	**2.38**	0.99
60	3.82	4.55	0.74	0.76
80	1.85	**4.56**	1.53	**1.58**

(b) PQ

light intensity	*C. zofingiensis*		*H. lacustris*	
(µmol m^{-2} s^{-1})	Blue-light	Red-light	Blue-light	Red-light
20	1.6	1.5	–0.8	1.2
40	1.4	**2.0**	**1.1**	**1.6**
60	**2.3**	–11.8	1.0	1.4
80	1.6	–7.2	1.0	1.5

An investigation was conducted to determine the microalgae net photosynthetic rates and the photosynthetic quotient, as defined in Eq. 7 and 8, respectively, of *C. zofingiensis* and *H. lacustris* as detailed in Table 2. The photosynthetic quotient (PQ) is the ratio oxygen production and consumption rates[16]. It is an important parameter in determining the reduction of CO_2 to carbohydrate when all the electrons released from water splitting in PSII are utilized. However, when electrons are drawn from photosynthetic chemical reactions to other reductive sinks, more O_2 will be produced than CO_2 consumed, and PQ will be greater than 1.0[17].

In the presence of blue-light, the net photosynthetic rate of *C. zofingiensis* was found to be highest at 20 μmol m^{-2} s^{-1} with a rate of 7.28 μmol O_2 gSS^{-1} min^{-1}. This rate decreased with an change in light intensity to approximately 2.7 fold at 60 μmol m^{-2} s^{-1}, while the highest PQ of 2.3 was found at 60 μmol μmol m^{-2} s^{-1}.

The higher PQ observed at 60 μmol m–2 s–1 compared to the value reported by[18] at 1500 μmol photons m^{-2} s^{-1} with *Chlorella sorokiniana* suggests that *C. zofingiensis* exhibited an enhanced photosynthetic efficiency under lower light intensities. This indicates a more efficient oxygen evolution process relative to CO_2 uptake, which may contribute to improved biomass productivity under specific light conditions. Furthermore, the observed decline in MNPR and PQ with change in light intensity under blue light suggests possible photoinhibition or altered metabolic activity. In contrast, the increase in MNPR under red light, peaking at 4.5 μmol O_2 gSS^{-1} min^{-1} at 80 μmol m^{-2} s^{-1}, implies that *C. zofingiensis* responds favorably to red-light conditions, likely due to optimized photosystem absorption characteristics. These findings highlight species-specific adaptations to light quality, which could have implications for optimizing cultivation strategies in photobioreactors.

The change in blue-light intensity was conducive to the production and utilization of oxygen by both *C. zofingiensis* and *H. lacustris,* which is utilized in the accumulation of starch, lipids and protein for cell growth and cell division as stated by[19] and[20] . This was higher than in the red-light environment and may be attributed to the lower energy efficiency when utilizing red-light[3]. Moreover, the observed increase in OCR by *C. zofingiensis* in the red-light environment may be a consequence of the consumption of the low volume of oxygen produced in the light phase compared to the blue-light environment. Previous studies on different algae species, including *Chlorella,* have reported variations in cell division under different light conditions, further underscoring the complex relationship between light wavelengths and cellular processes[21–23].

The OCR results indicate that *C. zofingiensis* is capable to accumulate biomass at light intensities between 40–80 μmol m^{-2} s^{-1} in both blue- and red-light environments. Furthermore, the regulation of oxygen in both the light and dark phases may also have contributed to the observed increase in biomass in both environments. *H. lacustris* exhibited

a significant increase in growth at 20, 40 and 80 µmol m^{-2} s^{-1} under the blue-light environment compared to the red-light environment. The alteration in light intensity influenced the algae growth rate and OCR, indicating that light intensities exceeding 40 µmol m^{-2} s^{-1} (red-light) may not be conducive to *H. lacustris* biomass accumulation. Furthermore, the observed decrease in OCR in light intensity from 60–80 µmol m^{-2} s^{-1} may be attributed to sub-optimal utilization of energy from both blue- and red-light to produce sufficient oxygen required for respiration and energy production.

During the OPR analysis, it was found that *C. zofingiensis* demonstrated a reduction in OPR in response to change in light intensity. This phenomenon may be attributed to light stress and mutual shading of algae cells, which occurs as a consequence of an accelerated growth rate [Figure 3]. The OPR exhibited a continued decline and was not affected by change in light intensity, which indicates that the mitochondrial electron-transport chain was capable of functioning during photosynthesis with a primary role in the provision of carbon skeletons for biosynthetic reactions, as suggested by[24] while utilizing oxygen. Furthermore,[20] observed a reduction in photosynthesis in the presence of glucose in light by *C. zofingiensis,* which resulted in the accumulation of high biomass. This finding from previous researchers indicates that the accelerated growth [Figure 3] under the blue-light environment also influenced oxygen production due to elevated carbon fixation.

In the blue-light environment, the algae exhibited an increased OPR compared to the red-light in both algae [Figure 4 (a) and (b)]. This may be attributed to the effect of blue-light photon energy on the algae cells, which provides an energy source through its short wavelengths to excite the P680 chlorophyll dimer via energy transfer from the antenna pigments from the ground state S0 to either S2 or a higher excited state. In contrast, red-light can activate an electron to the S1 singlet state[25]. At 20 µmol m^{-2} s^{-1} in the blue-light environment, *C. zofingiensis* exhibited OPR which was low as observed in *H. lacustris* in the same environment, due to its larger cell size. This finding suggests that small cell size [Table 1] had a lower oxygen production which facilitated faster transport at low light intensity. The OPR and growth exhibited by *C. zofingiensis* in the blue-light environment could be attributed to its adaptation to efficiently utilize light energy from 20–80 µmol m^{-2} s^{-1}. In

contrast, *H. lacustris*, exhibited poor light energy utilization for carbon fixation at low light intensities, while oxygen transport was decreased due to large surface area.

The results from Table 2 indicated the effect of blue-light intensity on *C. zofingiensis* regarding PQ with increased oxygen production which exceeded its consumption ratio. This may be attributed to the synthesis and utilization of difference substrates at different light intensities[18]. The increase in PQ at 60 μmol m^{-2} s^{-1} may indicate the synthesis of carbohydrates and proteins while at 20 μmol m^{-2} s^{-1} these substrates were being utilized for growth. In contrast, *H. lacustris* exhibited a peak in MNPR and PQ under both blue and red-light environment at 80 μmol m^{-2} s^{-1} and were lower than that observed in *C. zofingiensis* under blue-light environment. Notably, *H. lacustris* exhibited increased MNPR and PQ in the red-light compared to blue-light environment at the same light intensity, indicating efficient utilization of red-light.

A comparison of the photosynthetic capacities and responses to different light wavelengths and intensities of *C. zofingiensis* and *H. lacustris* reveals differences in their photosynthetic abilities. The MNPR of *C. zofingiensis* was found to be higher than that of *H. lacustris* under blue-light environment. The highest PQ in the present study was observed in the blue-light environment with *C. zofingiensis*. Since MNPR represents the balance between photosynthesis and respiration[26]. The higher MNPR observed in this study with *C. zofingiensis* in blue-light indicates that the algae produced more oxygen by photosynthesis than it was consumed by respiration, with the excess oxygen being transported outside the cell. This surplus oxygen is often released into the environment, making this microalgae an important contributor to oxygen production in aquatic ecosystems, carbon sequestration, and ecological studies.

3.3 pH

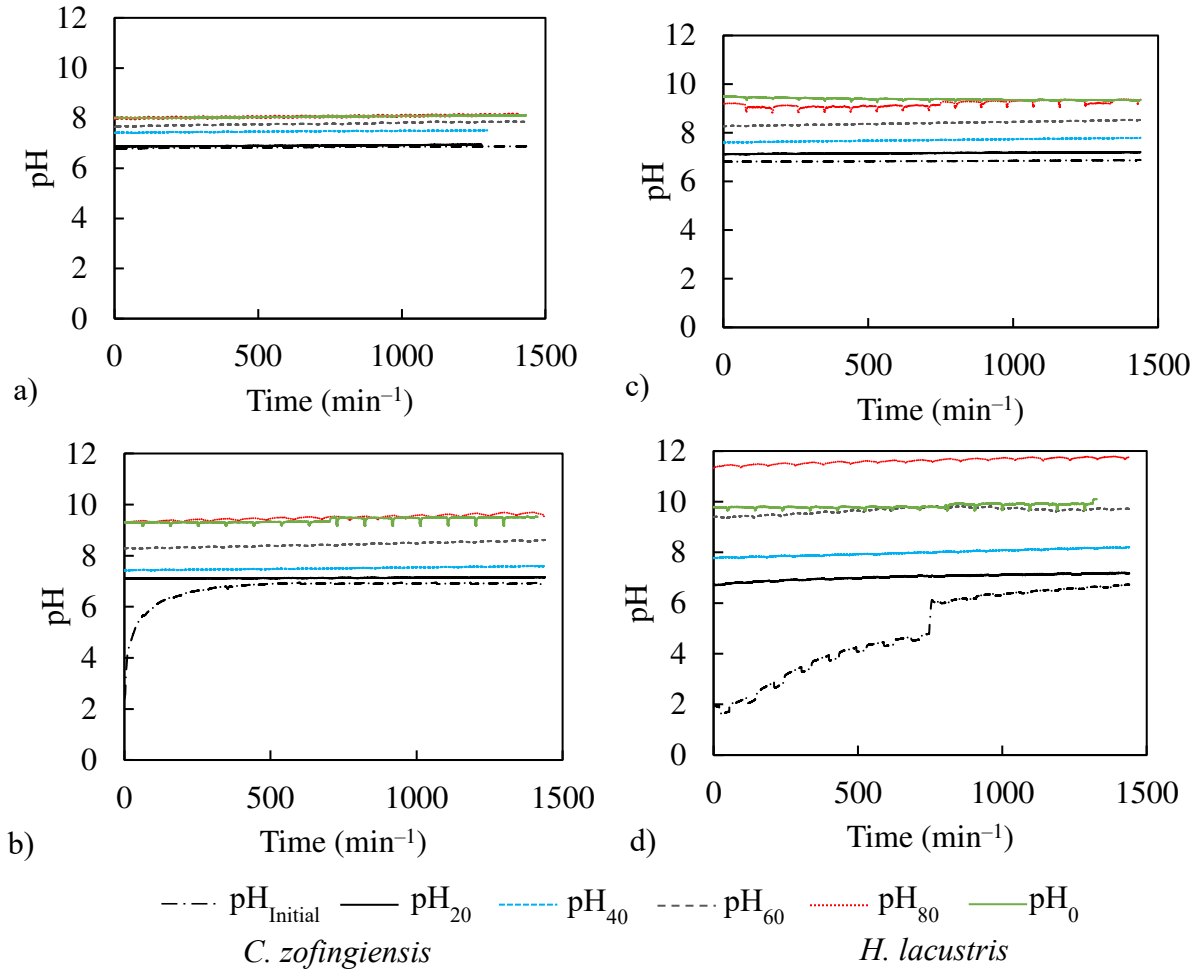


Figure 5: The effect of varying light intensities and wavelengths on pH.

The pH profiles of *C. zofingiensis* and *H. lacustris* were observed under various light wavelengths and intensities, in CZ-M1 medium with an initial pH range of 6.5–6.8 as mentioned in subsection 2.2. In the blue-light environment, the initial pH increased in response to changes in light intensity for *C. zofingiensis* in Figure 5 (a) and (b). The effect of photolysis increased with changes in light intensity, resulting in a subsequent rise in pH values from 6.5 at 20 µmol m^{-2} s^{-1} to 8.1 at 80 µmol m^{-2} s^{-1} in Figure 5 (a). Conversely, in Figure 5 (b), the red-light environment, pH also increased with changes in light intensity, with the highest observed at 9.5–9.6 at 80 µmol m^{-2} s^{-1}. Similar changes in pH were observed

with *H. lacustris* when light intensity was altered as shown in Figure 5 (c) and (d). These pH values were higher than those observed in *C. zofingiensis* under the same light wavelength and intensities. In the blue-light environment, a pH range of 9.3–9.5 was observed at 80 µmol m^{-2} s^{-1}, while in the red-light environment, it was increased to 11.4–11.8 at 80 µmol m^{-2} s^{-1}.

This study demonstrated that variations in light intensity and wavelength resulted in changes in pH. Blue-light resulted in a lower pH compared to red-light in both algae species (Figure 2). This phenomenon may be attributed to the long wavelength of red-light effect on algae cells. The reduction of CO_2 resulted in a shift in chemical equilibrium, leading to a decrease in the concentration of H^+ ions and an increase in pH, thereby rendering the medium more alkaline. Lower pH values were observed in the medium with *C. zofingiensis.* This may be attributed to *C. zofingiensis* increased microalgae net photosynthetic rates above 1.0 µmol O_2 gSS^{-1} min^{-1} in the blue and red-light environment. This suggests that the balance between oxygen production and consumption in the medium were relatively stable. Furthermore,[2] suggested that increase in pH could lead to poor fixation and utilization of absorbed CO_2 as this might be the case of *H. lacustris* in Figure 5 (c) and (d) with increase in pH from 9.3–11.8. The results demonstrated that at pH value of 6.8–6.9 in the blue-light, was favourable for *C. zofingiensis* growth, as previously suggested by[27].

4. Conclusion

The results from this study demonstrated that *H. lacustris* large cells surface area resulted in reduced transportation and diffusion of nutrients within and outside the cell. In contrast, *C. zofingiensis*, with its smaller and non-motile cell size, exhibited enhanced nutrient absorption, utilization, and transportation within and outside the cell. The methodology employed in this study were effective for monitoring and controlling the reactor in both light and dark phases alongside the oxygen production and consumption rates during cultivation. This approach did not disruption the growth and development of the algae. *H. lacustris* exhibited sensitivity to red-light at high light intensity, resulting in high pH which had an effect on its growth rate. Conversely, *C. zofingiensis* demonstrated a superior response to changes in blue-light intensity, resulting in optimized oxygen production and consumption

409 rates. The optimal PQ at 60 μmol m^{-2} s^{-1} indicated a balance between photosynthesis and
410 respiration while maintaining suitable pH levels. This study demonstrates that *C. zofingiensis*
411 has the potential of high oxygen and biomass production, as well as other high-value products,
412 offering a biological solution to environmental issues.
413
414

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Author contribution

Ugwu Chigozie: Performed the experiments, analyzed and interpreted the data, and drafted the paper.

Hideaki Nagare: Conceived and designed the experiments, analyzed and interpreted the data, contributed reagents, and supervised the experiments.

Ayumi Hashiguchi: Reviewed and edited the paper.

Availability of data and materials

All datasets on which the conclusions of the manuscript rely are presented in the main paper.

Conflict of interest statement

The authors affirm that they have no known financial or interpersonal interest that would have appeared to have an impact on this study.

Figure legend

1. Figure 1: Figure 1: Schematic overview of the stirred tank reactor (STR). The cultivation of *C. zofingiensis* and *H. lacustris* was conducted under autotrophic growth conditions in glass flasks. A solenoid valve regulated the supply of nitrogen gas (N_2) in the reactor. Light in the blue and red spectra was provided at different intensities by LEDs. Probes for measuring EC, DO, and pH were used and connected to an Arduino UNO microcontroller, with data recording managed via a Raspberry Pi.

2. Figure 2: Figure 2: Oxygen consumption rate (OCR) per biomass in varying light environment. This shows *C. zofingiensis* (2a) and *H. lacustris* (2b) Oxygen consumption under blue and red light 20 – 80 µmol m^{-2} s^{-1}. Algae cell oxygen consumption was measured for a 10-day period using a dissolved oxygen probe. Data represents means ± SD (n=3). Comparing oxygen consumption rates at different light intensities and wavelengths.

3. Figure 3: Algae specific growth rate under varying light wavelength and intensities. The effect of varying light wavelength (blue and red) at 20–80 µmol m^{-2} s^{-1} on *C. zofingiensis* (left) and *H. lacustris* (right) specific growth rate. The algae growth was measured for a 10-day period using a photospectrometer.

4. Figure 4: Oxygen production rate (OPR) per biomass in different light environment. This shows *C. zofingiensis* (2a) and *H. lacustris* (2b) Oxygen production under blue and red light 20 – 80 µmol m^{-2} s^{-1}. Algae cell oxygen consumption was measured for a 10-day period using a dissolved oxygen probe. Data represents means ± SD (n=3). Comparing oxygen production rates at different light intensities and wavelengths.

5. Figure 5: The effect of varying light intensities and wavelengths on pH. The effect of light intensity (20–80 µmol m^{-2} s^{-1}) and wavelengths on algae culture environment was measured using a pH probe. Comparison of change in pH under varying light

intensities and wavelengths. *C. zofingiensis* a) blue- and b) red-light, *H. lacustris* c) blue- and (d) red-light.

Table legend

1. Table 1: Algae cell characteristics. Algae samples were obtained from the algae growth chamber. 1 mL of algae samples were added to 9 mL of distilled water, to make up 10 ml of working volume for each tube. The samples were vibrated for uniform mixture for algae cell counting and measurement. 10 µl of algae sample was injected into the 0.1 µl JHS standard Thoma hemacytometer and also 10 µl of algae sample was injected into the objective micrometer glass to measure the algae cell size. Both the algae counting and measurement was performed with 40X magnification. (a) cell size, surface area and volume (n = 150) were measured to determine the algae characteristic differences. (b) Data represents means ± SD (n = 5).

2. Table 2: Effect of varying light intensity and wavelengths on MNPR and PQ of *C. zofingiensis* and *H. lacustris* in both blue- and red-light environment. MNPR and OPR comparison of *C. zofingiensis* and *H. lacustris* were carried in both blue and red light environment in 20 – 80 µmol m^{-2} s^{-1} of light intensity for a 10-day period. The MNPR was calculated from the difference between the OPR and OCR while PQ was calculated from the ratio between OPR and OCR.