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Optimization of the outdoor raceway cultivation to improve microalgae biomass production in *Parachlorella kessleri* wild type and starch-deficiency mutants

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ABSTRACT: This research focuses on the optimization of microalgal biomass production in outdoor raceway cultivation. Microalgae pose many advantages over higher plants in biofuel production such as higher photosynthetic efficiency, higher biomass productivity, and higher growth rate. To achieve high outdoor algal productivity, algal culture should be provided with appropriate light intensity. Excessive light intensity causes photoinhibition, whereas low light levels become a growth-limiting factor. Cultivation depth is a key factor for outdoor raceway cultivation. Maximum biomass was achieved at 0.163 g/l at 10 cm depth. Light shading alleviated photoinhibition, resulting in a higher growth rate than the control culture with no light shading. An increase in shading levels at low cell density led to higher photosynthetic efficiency (Fv/Fm). To examine whether the outdoor cultivation conditions were more critical for microalgae species with low tolerance to high light stress, an experiment was conducted in the lab-scale raceway ponds using *Parachlorella kessleri* wild type and two starch-deficient mutants (ST05 and ST01). Final biomass obtained from the experiment showed the highest biomass productivity in the wild-type strain, followed by the ST05 and ST01 mutant strains, respectively.

KEYWORDS: *Parachlorella kessleri*, starch-deficiency mutants, biomass production optimization, outdoor raceway cultivation, improving microalgae biomass

INTRODUCTION

Microalgae have been recognized as the sources of energy-rich feedstock and value-added products. Algae are regarded as sustainable sources of fuel because they can easily grow in a wide range of cultivation conditions—including wetlands, wastelands, wastewater, and even seawater—while sequestering carbon dioxide for photosynthesis and promoting rapid growth. Hence, it can be used for a greenhouse gas sequestration strategy. Other advantages of using microalgae as an energy feedstock are that microalgae do not require large area for cultivations like other oil crops, and their cultivations do not compete with the food crops which may lead to controversy of 'Food vs. Fuel'. Microalgae also have a short doubling time. They can double their biomass within 24 h. Oil fraction of algal biomass ranges from 20 to 60% of dry biomass depending on the species and culture conditions [1].

Mass cultivation is needed to provide enough biomass of microalgae as a source of feedstock for replacing fuels. Open pond cultivation is known for cheaper construction cost and easier operation and scale-up than closed system. The general depth that is used in the raceway pond system is about 20–30 cm, in which biomass was mixed by paddle or left unstirred. Some disadvantages of using this system are the difficulty of maintaining constant growth parameters such as temperature, pH, and light [2].

Large-scale algal cultivation can be achieved only by optimizing growth conditions through research on algal physiology and genetics. To maximize the efficiency of large biomass production and achieve higher lipid yield, the genetic manipulation of algal strains and suitable open pond cultivations must be studied together. Most of the strains with higher lipid content are difficult to cultivate under outdoor cultivations, leading to low biomass and lipid productivity. Several algal strains were studied for biodiesel production, including *Chlorella*, *Botryococcus*, *Scenedesmus*, *Nannochloropsis*, *Dunaliella*, *Chlorococcum*, and *Tetraselmis* [3–5]. However, limited studies have been conducted using *Parachlorella kessleri*. In plants and algae, starch and TAG are the main storage compounds, synthesized from shared precursors in algal cells. Blocking starch synthesis has helped improve TAG yield [6]. Starch-deficient mutants generated by UV mutagenesis showed a significant decrease in starch content and an increase in the lipid content of 24.19% and 25.22%, respectively, in *P. kessleri* mutant strains, compared with 15.56% in the wild-type (WT) strain [6].

Algal biomass productivity is the net result of photosynthesis and endogenous respiration. Assessing the rate of these mechanisms during outdoor cultiva-

tion is challenging because algal activity is influenced by numerous factors such as light intensity, temperature, pH, dissolved oxygen concentration, and nutrient availability [7]. Several green algae can acclimate to lower light under light/dark fluctuations better than under constant light of the same irradiance [8]. It was observed that inoculum cultures acclimated under high light intensity tolerated excess light under outdoor conditions better than those acclimated under low light [9]. The light regime seems to be the most critical one since other factors can be manageable [10]. In order to maximize the algal productivity per unit of area, cultivation systems should ideally be limited by no factors other than the amount of light energy reaching the algae.

This study focuses on optimizing the suitable outdoor cultivation conditions involving light and its effects such as the culture depth, initial cell density, and light shading control. When the suitable conditions were met, they were used to further explore the performance of wild type versus starch-deficient strains in outdoor cultivations.

MATERIALS AND METHODS

Algae strains

Microalgae used in this research, *P. kessleri* (wild type) was previously isolated from Bueng Borraped, Nakonsawan province, Thailand [11]. The mutant strains ST01 and ST05 were generated and characterized in our laboratory by Darunsart [12]. Briefly, *P. kessleri* (2×10^5 cells/ml) were exposed to UV irradiation from a 30 W germicidal lamp (GE Energy, Japan) at a distance of 40 cm for 3 min 30 s with regular stirring at 50 rpm using a magnetic stirrer. The exposure dosage was preliminarily determined to achieve a 10% survival rate. Then, the cells were immediately incubated overnight in the dark before being plated on Tris-acetate-phosphate (TAP) agar medium. All wild type and mutant strains were cultivated in modified BG-11 culture medium (Table S1) at all growth stages throughout the experiments.

Chlorophyll concentration measurement

Chlorophyll concentration was determined spectrophotometrically, following extraction with 80% acetone, according to the method described earlier [13]. Briefly, a 1 ml sample of algal culture was centrifuged at 8,000 rpm for 10 min. The supernatant was decanted, and the resulting cell pellet was resuspended in 1 ml of 80% acetone. The mixture was then incubated in the dark for 30 min to facilitate pigment extraction. Following a second centrifugation step (8,000 rpm, 10 min), the absorbance of the supernatant was measured at 645 nm and 663 nm. The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Total Chl) were calculated in μg/ml using the following equations:

$$\text{Chl a} = (12.7 \cdot A663) - (2.69 \cdot A645)$$
$$\text{Chl b} = (22.9 \cdot A645) - (4.68 \cdot A663)$$
$$\text{Total Chl} = (20.2 \cdot A645) + (8.02 \cdot A663)$$

Growth under different depths of cultivation

The algae stock was cultured by using $30 \times 30 \times 50$ cm (L × W × H) plastic containers under outdoor conditions with different light path lengths of 10, 20, and 30 cm for 5 days under the ambient temperature. Large water chambers were used as natural cooling chambers to prevent the algal culture from heat stress (Fig. S1A).

Light shading and initial cell density experiment

To prevent the photoinhibition from high light intensity, shading equipment were applied. Cultured chamber with selected depth from former experiment which was 10 cm was used in this study. The microalgae were cultivated under 3 different levels of shading roofs: no shading, 20% shading, and 50% shading. The roof was made of blue nylon net fabric. Microalgae were cultivated in plastic boxes with dimensions of 18.5×25.5 cm, with a working volume of 4.7 l. Microalgae were cultivated in 4 different initial cell densities (0.0175, 0.035, 0.105, and 0.140 g/l) for 2 days. Growth rate, biomass, and Fv/Fm were collected every day.

Lab-scale raceway pond cultivation

This study hypothesizes that wild type and starch-deficient mutant strains can be cultured under outdoor conditions. Experiments were performed in 2 identical oblong-shaped raceway ponds under the outdoor conditions. The lab-scale raceway pond was made of plastic, with overall dimensions of $60 \times 40 \times 25$ cm (L × W × H) (Fig. S1B). A clear acrylic plate was used as a middle-wall partition (24 cm in length) to divide the tub into a raceway with 18-cm lane width. The experiments were conducted at 25 l working volume of cultivation media with a 15 l/min flow rate controlled by a circulation pump. The experiment was done in the greenhouse equipped with evaporative cooling system at Mahidol University, Salaya campus.

Acclimatized stock culture experiment

P. kessleri was prepared as stock culture with 2 different methods. The first one was cultured under normal laboratory conditions using an LED lamp with a light intensity of approximately 400 μmol photons/m^2/s. The second stock culture was prepared under outdoor conditions with uncontrollable light intensity and temperature. The inoculum cultures were cultivated for 5 days. Then, the stock cultures prepared from both laboratory and outdoor conditions were cultured in the raceway cultivation pond for 5 days. Growth

rate (OD_{750}), biomass, pH, and Fv/Fm were measured every day.

Potential growth under outdoor cultivation of starch-deficient mutant strains

P. kessleri wild type and 2 starch-deficient strains (ST01 and ST05) were cultivated under outdoor conditions in raceway ponds with 3 different initial cell densities and cultured at different periods (up to 7 days) under the outdoor conditions in a lab-scale raceway system. The 3 different initial cell densities were studied (0.005, 0.05, and 0.1 g/l). Growth rate (OD750), biomass, pH, and Fv/Fm were measured every day.

Biomass concentration measurement

Algal biomass was measured as dry cell weight. Ten milliliters of cultures were filtered through 47-mm glass microfiber filters, pre-dried at 60 °C, and pre-weighed before use. Then, culture filters were washed twice with distilled water and dried at 60 °C until completely dried and weighed. The biomass concentration was calculated as follows:

Biomass concentration (g/l) = [Final weight of filter paper (g) − Initial weight of filter paper (g)] / Volume of sample (l).

Specific growth rate measurement

Every 2 days of cultivation at different light intensities, cell density was measured using a spectrophotometer at 680 nm. The data obtained were used to determine specific growth rate (μ) as follows:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

where μ is the growth rate (day-1); t_1 and t_2 are the initial and final times; N_1 and N_2 are the initial and final cell densities, respectively.

RESULTS

Growth under different depths of cultivation

On the first day of cultivation, the culture in all reactors, regardless of their depths, provided the same growth rate. However, only culture in 10-cm reactor continuously grew at constant rate throughout the experiment (Fig. 1A). Maximum biomass was achieved at 0.163 g/l from the 10-cm reactor and decreased by 37% and 61% in 20-cm and 30-cm reactors, respectively (Fig. 1B). Fv/Fm values ranged from 0.2 to 0.6 in all cultures, with the average light intensity of 1,100 µmol photons/m^2/s (Fig. 1C) and an average temperature of 38 °C.

Effects of Light shading and initial cell density

When cells were cultivated at low initial cell densities (0.0175 and 0.035 g/l), they grew better under 20% shading than under no shading control (Fig. 2), whereas at a higher initial cell density (0.105 g/l), the highest final biomass was achieved in the culture without shading. There was no difference between shading levels in cultures with a very high initial cell density (0.140 g/l).

Lab-scale raceway pond cultivation of *P. kessleri*

Effect of acclimatized stock culture

The outdoor-acclimated inoculum culture was compared with the non-acclimatized one (cultivated under normal laboratory conditions) as a feedstock for a lab-scale raceway pond cultivation under outdoor conditions. The algal strain used was the *P. kessleri* wild-type strain, which has lower light-intensity tolerance at about 800 µmol photons/m^2/s [12]. After the algal stocks were cultivated for 7 days, the growth rate was slightly higher in the non-acclimatized culture than in the acclimatized one. However, there is no significant difference in specific growth rate and in final biomass concentration (Fig. 3). Both types of stock cultures were able to proliferate up to 0.24 g/l of cell dry weight after 7 days of cultivation (Fig. 3B). The relationship between Fv/Fm and light intensity was as expected. When cultures were exposed to high light intensity, they exhibited lower Fv/Fm (Fig. 3C). The Fv/Fm in both non-acclimatized and acclimatized cultures drastically dropped on day 2 of cultivation but remained in the range of 0.2 and 0.3 for the rest of the experiment. Total chlorophyll contents of the non-acclimatized culture were higher than those of the acclimatized culture with no significant difference ($p > 0.05$) (Fig. 3D). The results in this experiment also showed that higher chlorophyll concentrations were found in the high initial cell density cultures (0.05 and 0.1 g/l) compared with the 0.005 g/l culture, which had the lowest concentration. The overall pH range was similar between the 2 groups: acclimatized and non-acclimatized. The starting pH was 8.5 and increased slightly to approximately 9.0 in both groups.

Potential growth of starch-deficient mutant strains under outdoor cultivation

To test how the 2 starch-deficient mutant strains (ST01 and ST05) grew under outdoor conditions compared with the WT strain, the experiment was conducted by cultivating the *P. kessleri* WT and the 2 mutant strains in lab-scale raceway ponds with 3 different initial cell densities (0.005, 0.05 and 0.1 g/l) under outdoor conditions. The growth rates of *P. kessleri* cultivated with different initial cell densities were analyzed and presented in Fig. 4A,D; Fig. 4B,E; and Fig. 4C,F. The culture with the lowest initial cell density (0.005 g/l) exhibited the lowest growth during 7 days of cultivation compared with the other 2 higher initial cell densities. However, all 3 strains showed similar growth rates. The WT strain grew better than both starch-deficient strains when cultivated initially at 0.05 g/l,

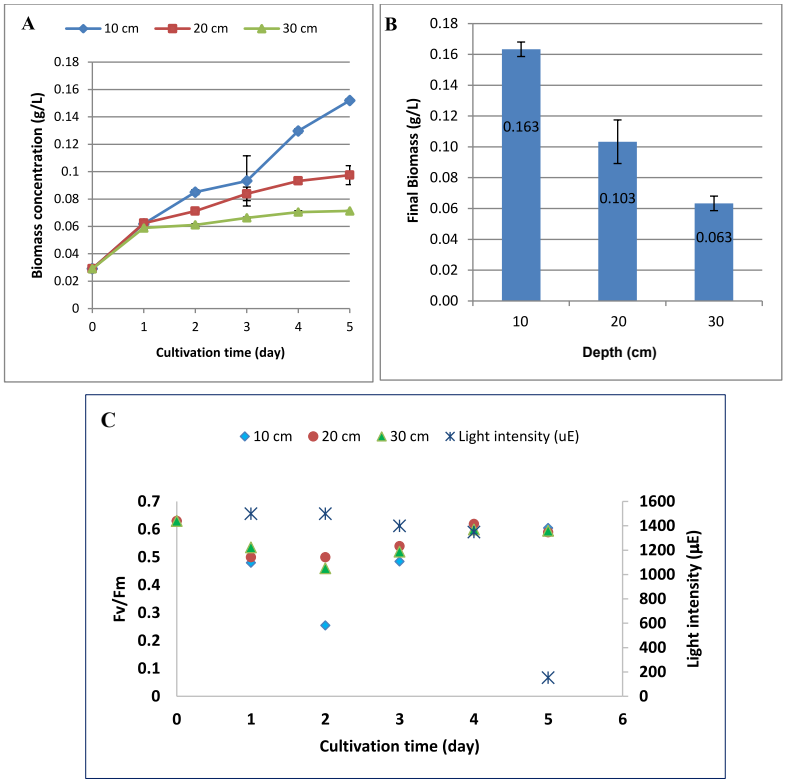

Fig. 1 Growth and photosynthetic performance of *P. kessleri* cultivated outdoors at different culture depths (10, 20, and 30 cm). (A) Growth rates, (B) final biomass concentrations, and (C) maximum photochemical efficiency (Fv/Fm) with corresponding daily light intensities. Data are presented as mean ± standard deviation (SD).

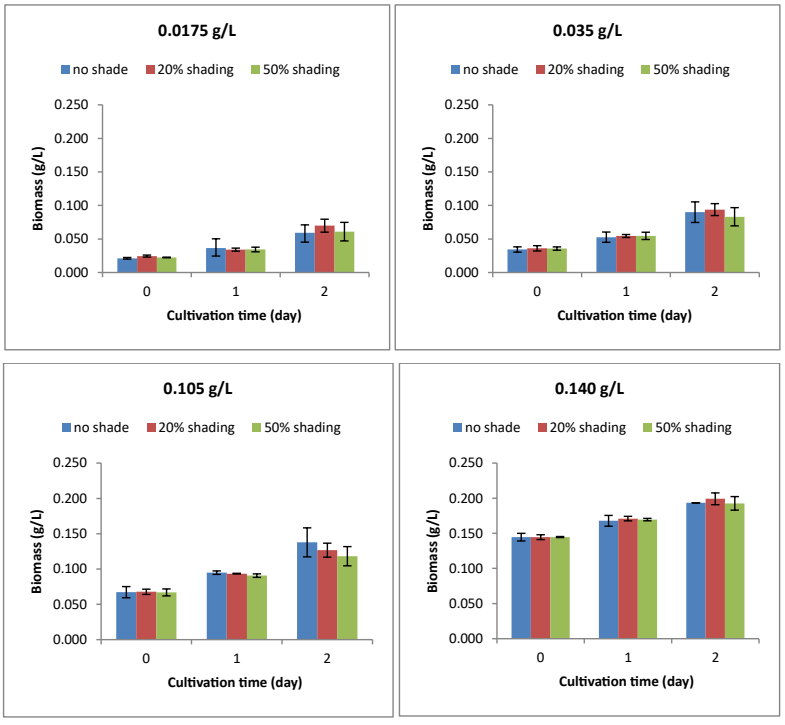

Fig. 2 Final biomass of *P. kessleri* cultivated under 3 different shading conditions: no shade, 20% shade, and 50% shade. For each condition, the algae were grown at 4 different initial cell densities: 0.0175, 0.035, 0.105, and 0.140 g/l. Data are presented as mean ± SD.

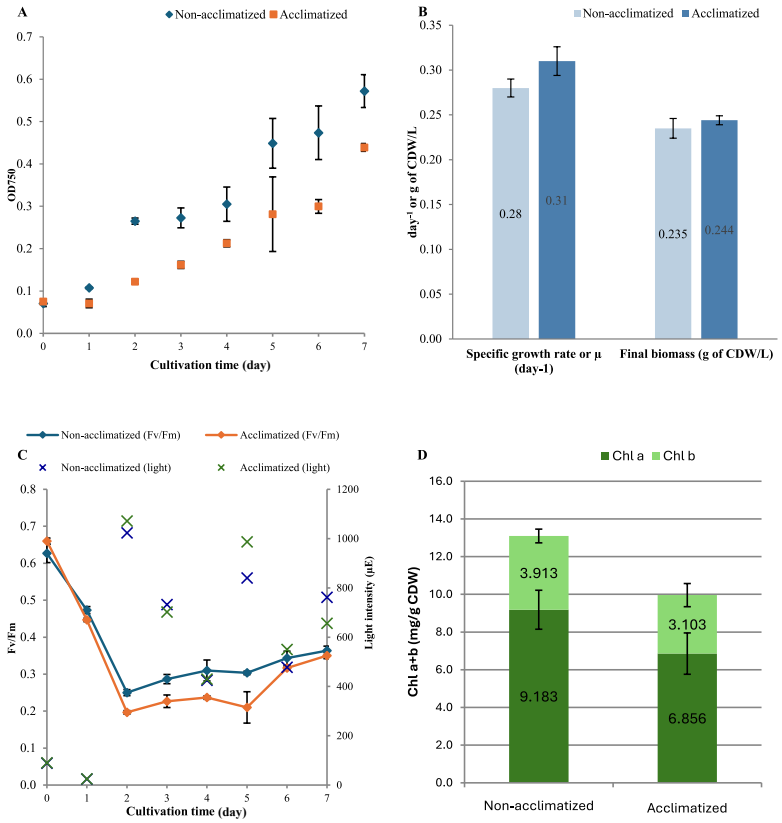

Fig. 3 Comparison of non-acclimatized and acclimatized stock cultures of *P. kessleri* grown in a 25-l outdoor raceway pond. (A) growth curve; (B) specific growth rate and final biomass (measured as cell dry weight, CDW); (C) maximum photochemical efficiency (Fv/Fm) in relation to daily light intensity; and (D) total chlorophyll content. Data are presented as mean ± SD.

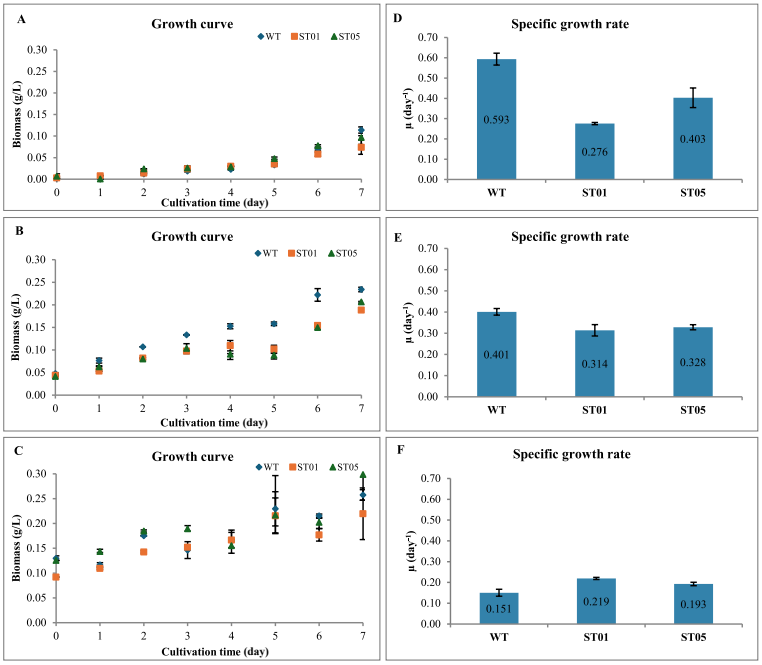

Fig. 4 Growth performance of *P. kessleri* wild-type (WT) and mutant strains (ST01 and ST05) cultivated outdoors. Growth curves shown for initial cell densities of (A) 0.005, (B) 0.05, and (C) 0.1 g/l. The corresponding specific growth rates for each density shown in panels (D), (E), and (F), respectively. Data are presented as mean ± SD.

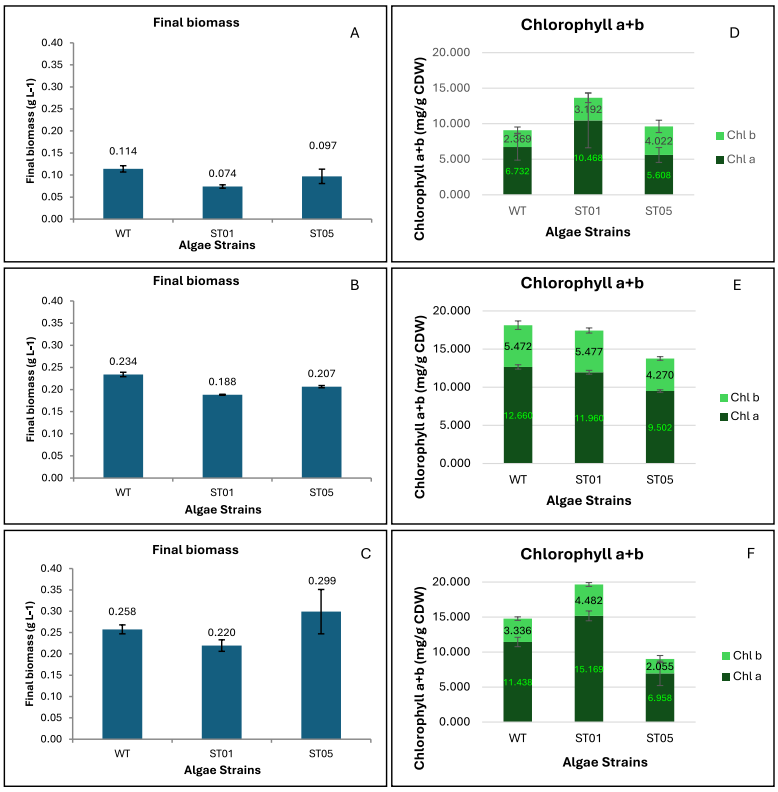

Fig. 5 Final biomass and chlorophyll a+b concentration of *P. kessleri* wild-type (WT) and mutant strains (ST01 and ST05) cultivated under outdoor conditions at 3 different initial cell densities: 0.005 g/l (A, D), 0.05 g/l (B, E), and 0.1 g/l (C, F). CDW = cell dry weight; values are expressed as mean ± SD.

whereas ST05 showed the highest growth at an initial concentration of 0.1 g/l. The results showed that the specific growth rate decreased when initial cell density increased. Cells cultivated in the highest initial cell density (0.1 g/l) yielded the lowest specific growth rate in all types of strains. The highest specific growth rate was obtained in WT under the lowest initial cell density cultivation. The growth rate was lowest in ST01 strain and slightly higher in ST05 followed by WT in 0.005 and 0.05 g/l initial cell density cultivations. After 7 days of cultivation, the final biomass was highest in ST05 (0.299 g/l) at the 0.1 g/l initial cell density and lowest in ST01 at 0.005 g/l. When comparing the strains, WT grew best at every culture density, followed by ST05 and ST01.

On the other hand, although the cells cultivated with low initial cell density reached lower growth, they achieved the highest specific growth rate. The highest specific growth rate at 0.593 day^{-1} was observed from the WT strain cultivated at 0.005 g/l. The WT strain also exhibited the highest growth rate at the initial cell density of 0.05 g/l compared with the 2 starch-deficient strains.

In contrast to the results obtained from 0.005 and 0.05 g/l, both ST01 and ST05 strains showed a higher specific growth rate than the WT strain in 0.1 g/l initial cell density cultivation (Fig. 4F). The highest final biomass was from WT, followed by ST05 and ST01 at 0.114, 0.097, and 0.074 g/l, respectively (0.005 g/l initial cell density, Fig. 4A). The highest final biomass was also observed in the WT strain, followed by ST05 and ST01 with 0.234, 0.207, and 0.188 g/l, respectively (0.05 g/l initial cell density, Fig. 4B). However, the ST05 strain showed the highest final biomass in 0.1 g/l initial cell density at 0.299 g/l, followed by the WT and ST01 at 0.258 and 0.220 g/l, respectively (Fig. 4C). The chlorophyll concentration was very low in 0.005 g/l cultivation compared with the other 2 initial cell density cultures (Fig. 5). The total chlorophyll content of ST05 was significantly lower than those of the WT and ST01 strains in both 0.05 and 0.1 g/l cultures. Chlorophyll a/b ratios obtained from the experiment were similar in all strains. The chlorophyll a/b ratios obtained from 0.1 g/l initial cell density cultivation ranged from 3.3 to 3.4 which were higher than those measured in 0.05 g/l culture (2.1–2.3). Photosynthetic efficiency (Fv/Fm) of PSII in all microalgae strains remained in the range of 0.1–0.6 (Fig. 6). On sunny days of cultivation, the photosynthetic efficiency was slightly lower than that

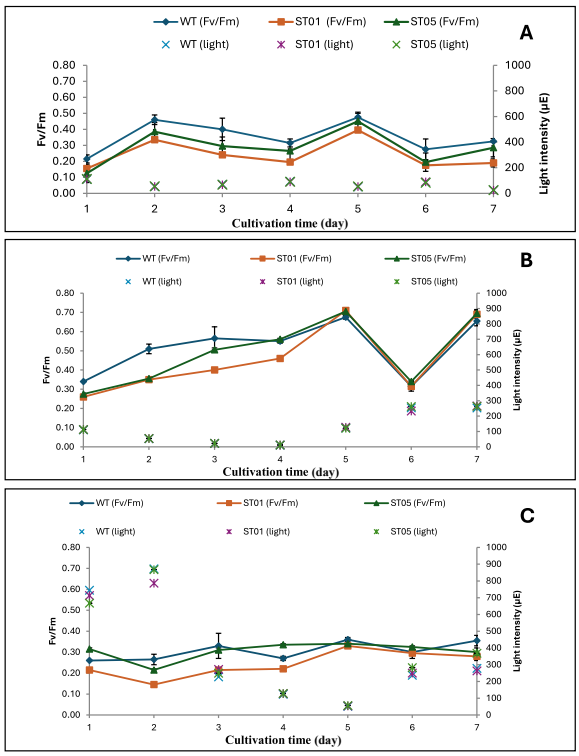

Fig. 6 The maximum quantum yield of photosystem II (Fv/Fm) in *P. kessleri* wild-type (WT) and mutant strains (ST01 and ST05). Outdoor cultures at 3 different initial cell densities: (A) 0.005 g/l, (B) 0.05 g/l, and (C) 0.1 g/l. All data are presented as mean ± SD.

on cloudy days. Fv/Fm of the WT strain was the highest among all strains on almost every day during cultivation. The starting pH was 8.5 and increased slightly to 8.7 ± 0.3 by the end of cultivation.

DISCUSSION

Effects of light shading and initial cell density

In commercial algal photobioreactors, light intensity is typically about 200–400 µmol/m^2/s (µE), whereas peak natural solar intensity is about 2,000 µmol photons/m^2/s of photosynthetically active radiation (PAR) [14]. Light itself cannot be controlled under outdoor environment, so there is a possibility that natural light can harm algal culture, since high light intensity can cause photoinhibition in algal cells. The variation of initial cell densities together with light shading controls has been studied to examine whether both factors can alleviate the photoinhibition effects caused by high light intensity. The results revealed that when cells were cultivated at low initial cell density (0.0175 and 0.035 g/l), they grew better under 20% shading than under no shading. This indicates that physical shading is needed for low initial cell density cultures, whereas in higher initial cell density culture (0.105 g/l), the highest final biomass was achieved in the culture with no shading control, since algal cells themselves provided self-shading. At low culture densities, high light intensity caused photoinhibition, so the culture needs external shading. However, in dense cultures, light penetration was limited due to algal cell density, so shading control is no longer needed [15]. The high initial cell densities can alleviate the photoinhibition effect, and photosynthetic efficiency can confirm this. At low initial density, the Fv/Fm of no light shading was lower than that measured from the other 2 levels of shading control. The difference in Fv/Fm values among the shading levels decreased as the initial cell density increased.

Raceway pond cultivation

Effects of acclimatized stock cultures

The growth rate of the non-acclimatized inoculum culture was higher than that of the acclimatized one, but there was no significant difference in either specific growth rate or final biomass concentration. This is indicated by the slightly lower photosynthetic efficiency (Fv/Fm) and total chlorophyll content of the acclimatized inoculum culture compared with the non-acclimatized inoculum culture throughout the cultivation period. The lower chlorophyll content found in the acclimatized culture may be due to cell adaptation after being exposed to high light intensity prior to cultivation.

Growth of starch-deficient mutant strains under outdoor cultivation

Not surprisingly, the WT strain grew best at all culture densities, followed by ST05 and ST01. Under outdoor conditions, algal cultures were exposed to solar radiation where the light intensity continuously changes during the day; this strongly affects cell physiology, growth, and biochemical composition [16]. *P. kessleri* cellular metabolism must be significantly affected by the natural day/night illumination cycles.

On sunny days, the high light intensity causes stress in algae, leading to a decrease in Fv/Fm. It was found in this study that Fv/Fm under the high light conditions was lower than on cloudy days, when cells were exposed to lower light intensity. This can be explained by the actual irradiance decreased by both natural shading and mutual shading of the increasing cell density [17]. This was confirmed by the higher total chlorophyll a+b in the low light compared with the high light culture and by its increase with increasing cell density. The chlorophyll a/b ratios obtained from all initial cell density cultivations ranged from 2.1 to 3.4, which are typical of those found in algae [18]. There is no significant difference in chlorophyll a/b ratios in all types of strains.

It can be concluded that shading, either by mechanical or mutual cell shading, can alleviate photoin-

hibition, resulting in a higher growth rate than the control culture with no light shading. An increase in the level of shading at low cell density led to higher photosynthetic efficiency (Fv/Fm). This can be applied to an actual algal culture, which requires physical shading at the beginning of cultivation to avoid light stress. The outdoor culture conditions for biodiesel production were more vital to starch-deficient mutant microalgae strains with low tolerance to high light stress (strains ST05 and ST01). Final biomass obtained from the experiment showed the highest biomass in ST05 (0.299 g/l) from the 0.1 g/l initial cell density cultivation. This shows a promising result for future studies.

Since starch biosynthesis acts as an "electron and carbon sink", the starch-deficient mutants lack this crucial metabolic pressure-relief mechanism. Their photosynthetic system is more susceptible to damage under high light; therefore, shading is crucial for their outdoor cultivation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.078.

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REFERENCES

1. Chisti Y (2007) Biodiesel from microalgae. *Biotechnol Adv* **25**, 294–306.
2. Benedetti M, Vecchi V, Barera S, Dall'Osto L (2018) Biomass from microalgae: the potential of domestication towards sustainable biofactories. *Microb Cell Fac* **17**, 1–18.
3. Mathimani T, Mallick N (2020) A comprehensive review on harvesting of microalgae for biodiesel — Key challenges and future directions. *Renew Sustain Energy Rev* **91**, 1103–1120.
4. Oni BA, Sanni SE, Ibegbu AJ, Aduojo AA (2021) Experimental optimization of engine performance of a dual-fuel compression-ignition engine operating on hydrogen-compressed natural gas and Moringa biodiesel. *Energy Rep* **7**, 607–619.
5. Pugkaew W, Meetam M, Yokthongwattana K, Leeratsuwan N, Pokethitiyook P (2019) Effects of salinity changes on growth, photosynthetic activity, biochemical composition, and lipid productivity of marine microalga *Tetraselmis suecica*. *J Appl Phycol* **31**, 969–979.
6. Sirikhachornkit A, Vuttipongchaikij S, Suttangkakul A, Yokthongwattana K, Juntawong P, Pokethitiyook P, Kangvansaichol K, Meetam M (2016) Increasing the triacylglycerol content in *Dunaliella tertiolecta* through isolation of starch-deficient mutants. *J Microbiol Biotechn* **26**, 854–866.
7. Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: A review. *Renew Sust Energ Rev* **14**, 217–232.
8. Yarnold J, Ross IL, Hankamer B (2016) Photoacclimation and productivity of *Chlamydomonas reinhardtii* grown in fluctuating light regimes which simulate outdoor algal culture conditions. *Algal Res* **13**, 182–194.
9. Carvalho AP, Silva SO, Baptista JM, Malcata FX (2011) Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Appl Microbiol Biot* **89**, 1275–1288.
10. Zhang G, Li F, Luo J, Qin Y, He Z, Ding F, Zeng Y (2025) Non-motile cell development and astaxanthin accumulation in *Haematococcus pluvialis* under different light spectra. *ScienceAsia* **51**, ID 2025056.
11. Chaichalerm S, Pokethitiyook P, Yuan W, Meetam M, Sritong K, Pugkaew W, Kungvansaichol K, Kruatrachue M, et al (2012) Culture of microalgal strains isolated from natural habitats in Thailand in various enriched media. *Appl Energ* **89**, 296–302.
12. Darunsart P, Rattanapongchai W, Laitummanoon J, Pokethitiyook P, Kruatrachue M, Sirikhachornkit A, Meetam M (2014) Isolation of microalgal mutants with reduced starch accumulation and increased lipid production. In: *Proc of the 2nd ASEAN Plus Three Graduate Research Congress (2nd AGRC)*, Bangkok.
13. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24**, 1–15.
14. Gordon JM, Polle JE (2007) Ultrahigh bioproductivity from algae. *Appl Microbiol Biot* **76**, 969–975.
15. Wahal S, Viamajala S (2010) Maximizing algal growth in batch reactors using sequential change in light intensity. *Appl Biochem Biotech* **161**, 511–522.
16. Taleb A, Legrand J, Takache H, Taha S, Pruvost J (2018) Investigation of lipid production by nitrogen-starved *Parachlorella kessleri* under continuous illumination and day/night cycles for biodiesel application. *J Appl Phycol* **30**, 761–772.
17. Beneragama C, Goto K (2010) Chlorophyll a:b ratio increases under low-light in shade-tolerant *Euglena gracilis*. *Trop Agric Res* **22**, 12–25.
18. Humbeck K, Hoffmann B, Senger H (1988) Influence of energy flux and quality of light on the molecular organization of the photosynthetic apparatus in *Scenedesmus*. *Planta* **173**, 205–212.

Appendix A. Supplementary data

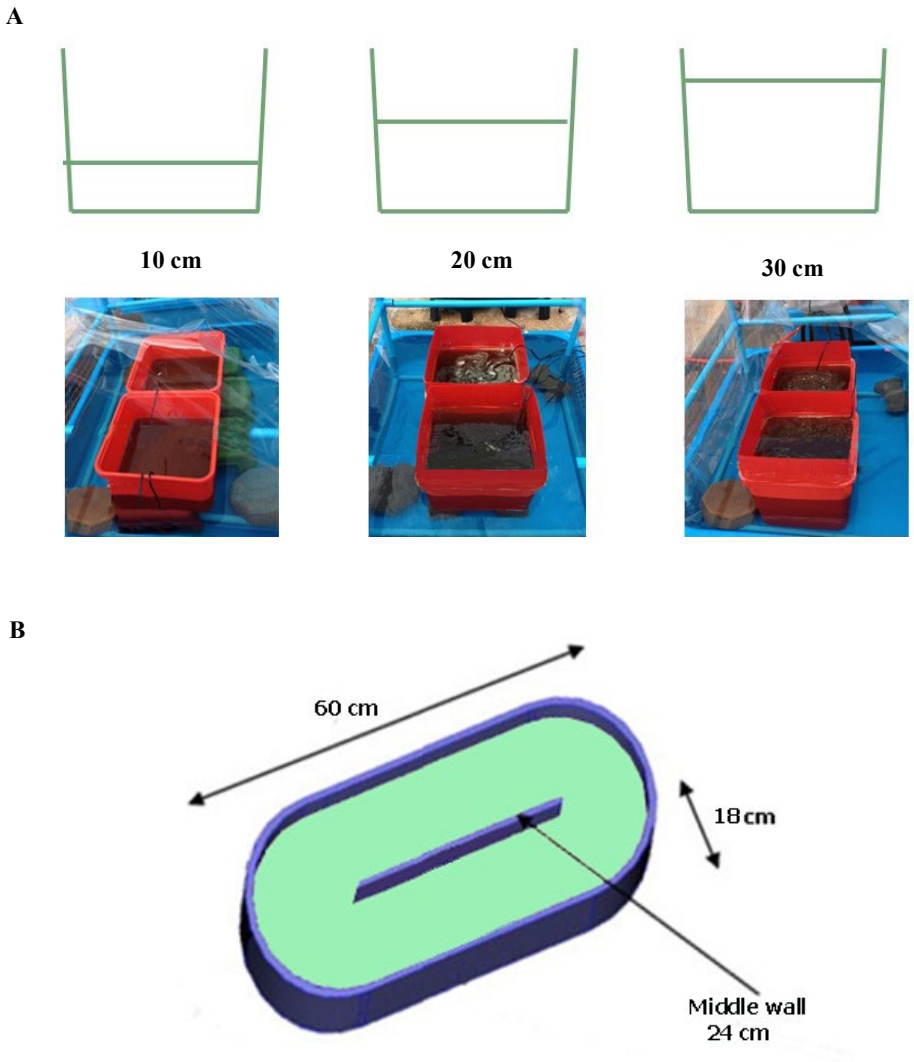

Fig. S1 The lab-scale raceway cultivation pond. (A) Effects of depth (light path lengths of 10, 20, and 30 cm) on algal cultivation under outdoor conditions. (B) Dimensions of the lab-scale raceway cultivation pond.

Table S1 Preparation of modified BG-11. The Fe citrate stock solution was prepared by dissolving citric acid and ferric ammonium citrate in 1 l of deionized water (dH_2O). To prepare BG-11 medium, 900 ml of dH_2O was mixed with 1 ml of Fe citrate solution, and then the remaining components were added. The volume was adjusted to 1 l with dH_2O, and the medium was autoclaved. The final pH was 8.5.

Component	Stock solution	Quantity	Molar concentration
Fe citrate solution		1 ml	
Citric acid	6.0 g/l dH_2O	–	3.12×10^{-5} M
Ferric ammonium citrate	6.0 g/l dH_2O		3.00×10^{-5} M
Macronutrient			
$NaNO_3$	–	1.5 g	1.76×10^{-2} M
$K_2HPO_4\cdot3\,H_2O$	40.0 g/l dH_2O	1 ml	1.75×10^{-4} M
$MgSO_4\cdot7\,H_2O$	75.0 g/l dH_2O	1 ml	3.04×10^{-4} M
$CaCl_2\cdot2\,H_2O$	36.0 g/l dH_2O	1 ml	2.45×10^{-4} M
Na_2CO_3	20.0 g/l dH_2O	1 ml	1.89×10^{-4} M