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Effect of continuous and daytime mixing on *Nannochloropsis* growth in raceway ponds

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ABSTRACT

Turbulence mixing is critical for generating high microalgal biomass productivity. Mixing also represents a major operational cost in large-scale microalgal production. The integration of photovoltaic cells with microalgae cultivation systems has been shown to eliminate the requirement of conventional grid-supplied electricity in rural areas. However, through such systems, the availability of electricity and the operation of equipment would solely depend on available sunlight and limited to only daytime. In accordance, in this study, we evaluated the effect of continuous paddle wheel mixing (24 h) and daytime mixing (12 h) on the growth, productivity and photosynthesis of *Nannochloropsis* sp. grown in outdoor paddle wheel driven raceway ponds operated at different depths (15 and 25 cm). Specific growth rates, volumetric and aerial biomass productivities were found to be significantly higher in ponds with 24 hour mixing compared to ponds with only 12 hour mixing operated at the same depth. The depth of the cultures did not affect the growth rate and volumetric productivity of cultures in both mixing conditions. Photosynthetic performance of cultures evaluated through chlorophyll *a* fluorescence measurements of photosystem II trended higher in ponds with 24 hour mixing compared to ponds subjected to 12 hour mixing. Our results clearly indicate the importance of continuous mixing to achieve high biomass productivity in cultures of *Nannochloropsis* sp.

1. Introduction

Challenges associated with the rising world population and expanding global economy necessitates the development of alternative food sources and energy production systems that are economical and environmentally tenable [1]. Due to their distinct advantages over terrestrial plants and their inherit potential, microalgal biomass is currently sought after as a sustainable source of multiple commodities such as food, animal feed, high value chemicals and biofuel [2,3].

Nonetheless, the cultivation of microalgae for commodity end-products (e.g. biofuel) is still limited by low biomass productivity and inflated production cost [4]. For a successful large-scale production, various factors must be first taken into consideration in selecting the right cultivation setup and operating conditions for the commercial scale cultivation of microalgal biomass. Among them include: the biology and intrinsic properties of the desired microalgae, climatic conditions of a locality, operating conditions (e.g. mixing and culture depth), desired final product, land and water availability and the utmost important factor which is the cost (capital and operating) [5,6].

Due to their larger capacity, reasonably high production efficacy and significantly lower overall cost, open systems such as paddle wheel driven raceway ponds are currently the preferred option for the mass cultivation of microalgae [7]. In terms of operating culture depth, a lower depth increases the surface-to-volume ratio and improves light penetration into cultures. It has also been shown that depths above 25 cm can limit the availability of light to algal cells at the bottom of the pond [8].

Thus, to improve biomass productivity in open ponds, optimization of turbulent mixing and culture depth are essential to improve light and nutrition availability to algal cells while at the same time reducing cell settling, biofilm formation, thermal stratification and oxygen accumulation from photosynthesis in cultures [9–11]. Common flow rates employed for mixing in raceway ponds range between 20 and 30 cm s^{-1} [12]. Further increase in mixing rates correlate with higher energy requirement while reduction in mixing rates results in laminar flow that can significantly decrease biomass productivity [13–15]. Turbulence mixing in open ponds also represents a major factor in terms of power consumption and operating costs [16]. In general, between 7.5 and 25.7% of annual operating cost of microalgal production is spent on powering paddle wheels in open ponds [14]. Thus, there is significant room for improvement in terms of turbulent mixing in open ponds to reduce energy requirements in commercial scale facilities

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resulting in a less expensive final product. First priority should be focussed on optimizing production efficiency (i.e. mixing and culture depth) while successfully minimizing energy use and associated cost to achieve feasible yields of microalgae. In accordance, innovative prospects such as the integration of photovoltaics cells with microalgae cultivation systems has been shown to not only significantly reduce conventional power requirement but also make the algal system self-sustainable in remote locations away from electrical grids [17,18]. Nonetheless, such PV-microalgae systems away from electrical grid lines would only allow for the operation of paddlewheels during daylight hours [19].

Thus, in order to evaluate the viability and efficacy of such integrated PV-microalgae systems, it is vital to study the feasibility of operating conditions of species of interest. *Nannochloropsis* sp. is widely sought after alga as a raw material for both bioenergy and high value bioactive products [20,21]. Currently, there are many different cultivation facilities around the world working on the commercialization of this particular species [21,22]. However, to date there has been no published data reporting on the effect of mixing duration for the cultivation of this alga. In here, the outdoor growth and photosynthetic response of *Nannochloropsis* sp. (MUR 267) under different mixing regime (12 h and 24 h) and culture depth (15 cm and 25 cm) was evaluated. Four raceway ponds (variants of both mixing durations and culture depth) were employed to evaluate the impact of mixing regime and culture depth on the growth, biomass productivity and photosynthesis of *Nannochloropsis* sp.

2. Materials and methods

2.1. Microalgae culture

The microalgae used in this study were *Nannochloropsis* sp. (MUR 267). *Nannochloropsis* sp. inoculum cultures were initially grown indoor in F/2 Medium at 3.3% (w/v) salinity [23]. Cultures were subsequently scaled up from 1 L conical flasks to 15 L aerated and stirred carboys. When the cultures reached early stationary phase, they were transferred into 2 m^2 paddle wheel driven raceway ponds.

2.2. Experimental setup and cultivation conditions

This study was carried out between 20/09/2016 and 23/10/2016 during the Austral Spring season using four 2 m^2 fibre glass paddle wheel driven raceway ponds at the Algae R&D Centre of Murdoch University (32.0734° S, 115.8392° E). Prior to inoculation, ponds were chemically cleaned using 6% (v/v) sodium hypochlorite for 24 h, followed by multiple washing with tap water before the start of the experiment. The operating condition of each raceway ponds is summarized in Table 1. Pond 1 and 3 were operated at 15 cm depth while Pond 2 and 4 had a depth of 25 cm. (Table 1) Pond 1 and 2 were subjected to 24 hour paddle wheel mixing while Pond 3 and 4 were on 12 h (daytime) mixing regime only. Paddle wheels for Pond 3 and 4 were switched on at 6 am (0.5 h after sunrise) and stopped at 6 pm (0.5 h before sunset) daily (Table 1).

Weather records such as the average daily solar irradiance and maximum air temperature was obtained from the Australian Bureau of Meteorology's Weather Station (www.bom.gov.au, 2016 data from Murdoch station, 009187). Culture temperature and salinity were measured on line and recorded continuously every 30 min, using 600R Series YSI Multiparameter Water Sondes.

Table 1
Operating condition of each pond during the study.

	Mixing duration (hours)	Operating culture depth (cm)	Abbreviation	Pond volume (L)
Pond 1	24	15	24 h/15 cm	300
Pond 2	24	25	24 h/25 cm	500
Pond 3	12	15	12 h/15 cm	300
Pond 4	12	25	12 h/25 cm	500

All ponds were inoculated with the same concentration of *Nannochloropsis* sp. stock culture (2.0×10^7 cells/mL) and were operated at a liquid velocity of 22 cm s^{-1}. The mixing speed in ponds was determined through the tracer method using 1 M HCl [24].

Ponds were operated semi-continuously between 05/10/2016 and 23/10/2016 under the exact outdoor conditions (nutrient replenishment, solar incidence, paddle wheel velocity, etc.) [25]. The semi-continuous operating modes was carried out by harvesting 30% of cultures (4.5 cm for Pond 1and 3 and 7.5 cm for Pond 2 and 4) and replenishing them with fresh f/2 media of 3.3% (w/v) salinity. Average daily evaporative losses was between 8 and 12 L d^{-1} (0.8–1.2 cm) during the experiment period and this loss was compensated using tap water. Harvesting of cultures and growth measurements (i.e. cell counts, biomass analysis and photosynthetic measurements) were carried out every Monday, Wednesday and Friday at 10 am during the semi-continuous culture mode.

2.3. Analytical methods

Culture cell density was measured using an improved Neubauer counting chamber while organic biomass (g L^{-1}) was analysed according to the methods of Moheimani, Borowitzka, Isdepsky and Sing [26].

Chlorophyll fluorescence measurements were performed using a WATER-PAM fluorometer (Walz GmbH, Germany), consisting of a PAM-CONTROL unit and a WATER-ED (emmiter/detector) unit. The WATER-ED unit consisted of light emitting diodes (LED's) which provided the non-actinic measuring light (spectral peak at 650 nm), actinic light/saturation pulse (spectra peak at 660 nm) and far-red light (spectra peak at 730 nm). The effective quantum yield in light (F_q'/F_m') of harvested samples was evaluated using the saturation light method ($\approx$3500 μmol photons $m^{-2} s^{-1}$) [27,28]. Sample collected from each pond was quickly transferred to the ED-unit and measurements were immediately made. A fresh sample was used for each replicate measurement (minimum of 3).

A 34 hour diurnal study was also conducted as part of this study during the semi-continuous cultivation period between 14/10/2016 and 15/10/2016 (Day 25 and Day 26) to evaluate the changes in photosynthetic response of algal cells in all ponds over time. Saturation light pulses (F_q'/F_m') and rapid light curves (RLCs) were performed on samples from each pond at different time intervals over 2 days. Measurements were made at 5 am (before sunrise on 14/10/2016), 7 am (after sunrise), 11 am (midday/afternoon), 2 pm, 6 pm (sunset) and 11 pm for Day 1 (14/10/2016) and at 6 am (before sunrise), 7 am (after sunrise), 11:00 am (midday/afternoon) and 3.00 pm of the subsequent day (15/10/2016). Samples taken were immediately subjected to the photosynthesis measurement. For each sample, at least 3 replicate RLCs were performed with a 10-s actinic exposure duration to each of eight pre-set incremental irradiances (43, 651 96,147, 218, 333, 499 and 709 μmol photons $m^{-2} s^{-1}$) followed by a saturation pulse that lasted for 0.8 s.

Relative electron transport rates (rETR) were calculated based on the equation of rETR = ΦPSII × PAR × ETR factor using the quantum yield values of PSII (ΦPSII) derived from the RLCs, the incident photosynthetic active radiation of the instrument (PAR) (in μmol photons·m^{-2}·s^{-1}) and the ETR factor which is the fraction of light absorbed by the sample and distributed to PSII [29]. For rETR calculations, ETR factor of 0.42 was used arbitrarily since true PSII absorption would require quantitative information on the PSII absorption [29]. These calculated rETR values were then used to plot out the photosynthesis versus irradiance curves (P-I) by plotting rETR values against PAR values [29]. The plotted rETR-PAR curves were subsequently modelled and fitted with a best fit curve through the waiting-in-