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Action spectra of oxygen production and chlorophyll *a* fluorescence in the green microalga *Nannochloropsis oculata*

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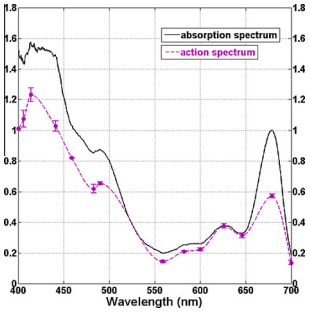
HIGHLIGHTS

- First action spectrum of O_2 evolution and chlorophyll fluorescence in *N. oculata*.
- Novel procedure to generate representative and reproducible action spectra.
- Economic implications of light quality upon the photosynthesis response.
- More efficient growth under blue light than red light at sub-saturating irradiance.
- Optimum light-use efficiency at 625 nm, but lowest PSII quantum yield at 646 nm.

GRAPHICAL ABSTRACT



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ABSTRACT

The first complete action spectrum of oxygen evolution and chlorophyll *a* fluorescence was measured for the biofuel candidate alga *Nannochloropsis oculata*. A novel analytical procedure was used to generate a representative and reproducible action spectrum for microalgal cultures. The action spectrum was measured at 14 discrete wavelengths across the visible spectrum, at an equivalent photon flux density of 60 μmol photons m^{-2} s^{-1}. Blue light (~414 nm) was absorbed more efficiently and directed to photosystem II more effectively than red light (~679 nm) at light intensities below the photosaturation limit. Conversion of absorbed photons into photosynthetic oxygen evolution was maximised at 625 nm; however, this maximum is unstable since neighbouring wavelengths (646 nm) resulted in the lowest photosystem II operating efficiency. Identifying the wavelength-dependence of photosynthesis has clear implications to optimising growth efficiency and hence important economic implications to the algal biofuels and bioproducts industries.

Abbreviations: α, rate constant of gross photosynthesis; *E*, irradiance (W m^{-2}); E_k, minimum saturating irradiance; F_0, minimal fluorescence yield in the dark-acclimated state; F_m, maximal fluorescence yield in the dark-acclimated state; F', minimal fluorescence yield in the light-acclimated state; F_m', maximal fluorescence yield in the light-acclimated state; fAQ_{PSII}, proportion of light absorption directed towards PSII; MC-PAM, multi-colour pulse-amplitude-modulated fluorometer; NPQ, non-photochemical quenching; *P*, gross photosynthesis; P_{max}, maximum gross photosynthesis; PAR, photosynthetically active radiation (400–700 nm); PFD, photon flux density (μmol photons m^{-2} s^{-1}); PI curve, photosynthesis versus irradiance curve; PSI, photosystem I; PSII, photosystem II; Φ_{PSII}, operating efficiency of PSII.

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1. Introduction

Sustainable transport fuels of the future may be produced using microalgae. Algal biofuel technology exploits algal photosynthesis and biosynthesis processes to produce oils using only sunlight, carbon dioxide, water and limited nutrients. Oil production capacity from microalgae far exceeds that from any higher plant, including traditional biofuel crops such as corn, sugarcane and palm (Georgianna and Mayfield, 2012; Larkum et al., 2012). Consequently, research and pilot projects are being carried out worldwide to expand this technology to support major industrial process scaling (Wijffels and Barbosa, 2010).

The green microalga of the genus *Nannochloropsis* (class Eustigmatophyceae) is a leading candidate for biofuel production due to its ability to accumulate high oil content (28.7% of cellular ash-free dry weight) with reported oil productivities of ~25.8 mg L^{-1} day^{-1} (Gouveia and Oliveira, 2009); thus, research has recently focussed on better understanding the fundamental attributes that regulate biomass productivity, and ultimately oil production, for species from this genus. The photosynthetic apparatus of *Nannochloropsis* is unusual in that the only chlorophyll pigment it contains is chlorophyll *a*; as such, light absorption properties are significantly different compared to other common, commercially-relevant, green microalgae such as *Spirulina*, *Chlorella* or *Dunaliella*, which also contain chlorophyll *b* (Kandilian et al., 2013). Furthermore, the species *Nannochloropsis oculata* has the capacity to grow in saline, brackish and hypersaline water, which ensures that it will never compete with food crops for fresh water. Since production facilities can be constructed on arid or marginal land, biofuels derived from *N. oculata* do not compete with food crops or animals for arable land (Bartley et al., 2013; Borowitzka and Moheimani, 2013). The biofuel productivity of *N. oculata* is now known to be affected by a number of environmental parameters, including light and temperature regimes (Sukenik et al., 2009; Tamburic et al., 2014).

Algal growth is impossible without illumination, so it is not surprising that *N. oculata* oil productivity is principally determined by its light environment, in terms of both the quantity and quality of available light (Simionato et al., 2011). Providing appropriate illumination requires an understanding of all its constituent factors: wavelength, irradiance, photo-saturation, light attenuation and light history. The photosystems of *N. oculata* are only capable of absorbing light within the 400–700 nm wavelength range (Haxo and Blinks, 1950), i.e. photosynthetically active radiation (PAR); this absorption is governed by the presence and concentration of photosynthetic pigments, the composition of the photosynthetic pigment-protein complexes, and the constituents of the photosynthetic electron transport chain;. As such, wavelength-specific absorption is highly variable amongst taxa with different pigment arrays (Millie et al., 2002). In *N. oculata*, carotenoids and chlorophyll *a* are responsible for absorption of blue light, while chlorophyll *a* also absorbs red light (Kandilian et al., 2013).

Irradiance is a measure of light pour incident on a surface. As a general rule, the response of photosynthesis to irradiance follows a classical and highly conserved pattern (MacIntyre et al., 2002), the photosynthesis–irradiance curve (PI curve): under relatively low irradiances, algal growth rate and irradiance increase in proportion since more photons become available for photosynthesis; however, under relatively high irradiances, photosynthesis remains constant (or declines) with increasing irradiance as the photosynthetic electron transport chain becomes saturated (and ultimately photoinhibited). Raising irradiance above the photosaturation limit should be avoided because it reduces the photosynthetic efficiency of cells (Sukenik et al., 2009), which results in energy loss through heat dissipation (such as non-photochemical quenching) and fluorescence, as well as the energy costs associated with repairing damaged photosynthetic apparatus (Raven, 2011). However, algal cells in culture rarely receive the same number of photons constantly as a result of light attenuation, in particular where cell densities are high and cultures optically thick (Lehr and Posten, 2009). Flexibility of the photosynthetic apparatus for light harvesting and light utilisation via photo-acclimation, as observed in *Nannochloropsis* (Sforza et al., 2012), is thus a key attribute for large scale culturing.

An action spectrum measures the rate of photosynthesis across different PAR wavelengths. It can be measured in terms of a proxy for photosynthetic efficiency, such as chlorophyll *a* fluorescence (Emerson and Arnold, 1942), or in terms of oxygen evolution, the tangible result of photosynthesis (Haxo and Blinks, 1950). Importantly, an action spectrum is different to an absorption spectrum since not all absorbed photons lead to photosynthetic oxygen production, which occurs at photosystem II (PSII). For example, photons may be absorbed by photosystem I (PSI), their excitation energy may be dissipated as heat, or emitted as fluorescence that can be quenched by various photochemical and non-photochemical processes (Baker, 2008; Suggett et al., 2003). Furthermore, absorbed energy may lead to oxygen evolution that is internally recycled (and hence not detected by conventional oxygen sensors) via a number of alternative photochemical reactions, such as the Mehler reaction or photorespiration (Cardol et al., 2011).

Measuring the action spectrum is important because it provides the best description of the wavelength-specific response of that algae's photosynthesis and importantly, it can be used to identify which wavelengths are utilised most efficiently. In terms of *N. oculata* biofuel production, the impacts are significant since the spectral composition of artificial illumination in small-scale laboratory systems could be optimised to enhance photosynthetic efficiency. Specifically, it may be possible to determine the wavelength ranges that drive photosynthetic primary production with higher efficiency and reduce the energetic costs of maintaining redundant photoprotective processes (Raven, 2011). Such 'tuning' of the spectral nature of illumination to increase absorption efficiency could result in two beneficial effects: (i) enhanced algal growth rate, or (ii) reduced power consumption to achieve the same growth rate. Large-scale outdoor demonstration facilities could also be retrofitted with inexpensive light filters to modulate the solar spectrum incident on *N. oculata* cultures in order to enhance growth and oil productivity.

The aim of this study is to develop and measure the first complete action spectrum for oxygen evolution and chlorophyll *a* fluorescence in *N. oculata*. The objective is to better understand photosynthetic responses at different wavelengths and develop a more effective basis for optimising the light delivery to *N. oculata* in both artificial and natural environments.

2. Methods

2.1. Nannochloropsis strain and stock cultures

N. oculata (Droop) Green (Australian National Algae Culture Collection; strain CS-179) was grown in three separate 250 mL cultures using f/2 seawater medium at 25 °C (Labec Temperature Cycling Chamber incubator, Labec Pty Ltd, Australia). Stock cultures were subjected to a 12 h/12 h light/dark cycle under fluorescent illumination with a photon flux density (PFD) of 50 ± 5 μmol photon m^{-2} s^{-1} PAR. Cultures were diluted (1/20 v/v) with fresh media 1 week prior to experimentation; consequently, all cultures were in exponential growth phase (as verified by cell counts using a haemocytometer) and 7–12 days old at the time of experiment.