The effect of light:dark cycles of medium frequency on photosynthesis by *Chlorella vulgaris* and the implications for waste stabilisation pond design and performance

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** Abstract ** The effect of light/dark (L:D) cycle times on the recovery from photoinhibition of green micro-alga *Chlorella vulgaris* (CCAP211/11c) and the cyanobacterium *Synechococcus* (CCAP1479/5) was investigated using an irradiated, temperature controlled oxygen electrode. The onset of photoinhibition in both organisms occurred at irradiances > 300 µmol m\(^{-2}\)s\(^{-1}\) at temperatures >15°C. Light/dark cycle times were controlled independently using a relay timer and shutter placed between the quartz iodide light source and the oxygen electrode chamber. Oxygen evolution decreased rapidly when cells were continuously irradiated at 300, 500 and 750 µmol m\(^{-2}\)s\(^{-1}\). However, *Chlorella* cells irradiated at 300, 500 and 750 µmol m\(^{-2}\)s\(^{-1}\) on a L:D cycle of 60s:20s, 30s:60s and 60s:120s respectively, maintained a constant rate of oxygen evolution over a 24 h incubation period. Exposure time to a given incident irradiance rather than the total light dose received appeared to determine the effect of light/dark cycle times on photosynthesis. A relationship was established between L:D ratio required to maintain constant oxygen production and incident photon flux density. The results suggest that the adverse effects of high irradiances on algae near the surface of a stratified waste stabilisation pond might be ameliorated by controlled mixing of algal cells through the depth of the pond.

** Key terms ** Light-dark cycling photoinhibition; photosynthesis-irradiance response; waste stabilisation ponds

** Introduction ** Algae present in waste stabilisation ponds (WSPs) are exposed to a range of irradiances mediated by attenuation, climate and hydrodynamic conditions. Three physiological regions, all of which influence WSP performance, are recognised in the algal photosynthesis/irradiance (P/I) response curve, firstly, where light limited and secondly, light saturated photosynthesis proceeds and thirdly where photoinhibition occurs. Computer modelling of algal productivity in high rate algal ponds (HRAPs) implies a significant adverse effect of photoinhibition on oxygen production and waste treatment performance (Martin and Fallowfield, 1989; Fallowfield *et al*., 1992). The onset of photoinhibition, for the green algae that predominate in WSPs, may occur at irradiances > 200 µmol m\(^{-2}\).s\(^{-1}\); significantly lower than the incident surface irradiances of 1,500–2,000 µmol m\(^{-2}\).s\(^{-1}\) typical for many WSPs. Photoinhibition is influenced by temperature, previous light history and exposure time (Takahashi *et al*., 1971; Kirk, 1983), however, it may be reversible under certain conditions.

The objective of this study was to determine the effect of light/dark cycle times on the recovery of green micro-algae from photoinhibition. The P/I response of cultures of *Chlorella vulgaris* (CCAP211/11c) and for comparative purposes a cyanobacterium *Synechococcus*, not normally found in waste stabilisation ponds, was determined using an irradiated, temperature controlled oxygen electrode chamber as described by Ratchford and Fallowfield (1992).
Materials and methods

Growth of *Chlorella* and *Synechococcus* cultures

Axenic cultures of *Chlorella vulgaris* (CCAP 211/11c) and the unicellular cyanobacteria *Synechococcus* (CCAP1479/5) were grown in sterile ASM (Gorham *et al.*, 1964) in 250 mL Erlenmeyer flasks on an orbital incubator (100 rpm, 23°C) at a constant irradiance of 70 μmol m⁻².s⁻¹ (40W cool white fluorescent tubes).

Dry matter determination

Triplicate 10 mL samples of culture were filtered through pre-dried and weighed GF/C (2.5 cm) filter pads. The filters were dried overnight at 105°C and the dry matter calculated by difference.

Determination of photosynthesis irradiance (P/I) response curves

The method of Dubinsky *et al.* (1987) modified by Ratchford and Fallowfield (1992) was used. A water jacketed, temperature controlled (23.0 ± 0.1°C) oxygen electrode chamber of cylindrical cross section was constructed from PVC. Algal cells placed in the chamber were maintained in suspension by a magnetic flea. Oxygen concentration was determined using a Clark polarographic electrode (YSI, Ohio). The electrode was calibrated at 0% using a cobalt catalysed solution of sodium sulphite and at 100% using an air saturated solution of distilled water.

Algal cells were diluted with ASM to a constant OD₅₆₀nm of 0.15. The diluted sample was then placed in the oxygen chamber. Prior to P/I determination each sample was incubated, in the chamber, in the dark to permit temperature equilibration and until a constant rate of dark respiration was recorded. To determine the P/I response the cultures were then exposed to irradiances of 4, 8, 12, 16, 20, 30, 50, 100, 200, 300, 500, 750, 1,000 and 2,000 μmol m⁻²s⁻¹ using a quartz-iodide (150 W) projector lamp. The incubation irradiances were set by measuring the light transmitted through the culture in the electrode chamber using a PAR (400–700 nm) cosine corrected quantum sensor (Skye Instruments). The rate of photosynthesis (oxygen evolution) was recorded for 6 minutes at each of the above irradiances.

The effect of light/dark cycle times on the recovery from photoinhibition of *C. vulgaris* and *Synechococcus* was determined using a shutter controlled by a relay timer placed between the quartz iodide light source and the oxygen electrode chamber. The timer/shutter permitted independent control of both light and dark (L:D) exposure times. Previous studies had shown that the oxygen electrode had a response time of 7–10 s to attain 90–95% of the final measured value. Consequently, measurements of the response of photosynthesis or respiration were made on light or dark periods of > 10 s. The maximum incubation time in the light or dark was 280 s. To avoid nutrient limitation over the extended incubation time 400 mgC L⁻¹ (NaHCO₃, pH 7.0) in ASM was injected into the culture in the chamber. Oxygen saturation was between 20–50% at the start of the experiments and never exceeded 85% throughout the 24 h incubation. The effect of various L:D ratios upon the rate of oxygen evolution and the maximum rate of photosynthesis (Pₘₐₓ) was determined at irradiances of 300, 500 and 750 μmol m⁻²s⁻¹ which had previously been shown to be photoinhibitory.

Results

Typical photosynthesis/irradiance response curves for *C. vulgaris* and *Synechococcus* cultured at 23°C/70 μmol m⁻²s⁻¹ and determined at 15, 23, 30 and 35°C are shown in Figures 1 and 2 respectively. The maximum rate of photosynthesis and the rate of photoinhibition were clearly temperature dependent, with little photoinhibition occurring at 15°C.
Notwithstanding, the onset of photoinhibition in both organisms occurred at irradiances >300 µmol m⁻²s⁻¹ at temperatures >15°C.

Figure 3 shows the effect of dark periods of 10, 15 and 20 s following irradiance at 300 µmol m⁻²s⁻¹ for 60 s over a 200 minute incubation. The oxygen evolution decreased rapidly when cells were continuously irradiated. However, at L:D cycles of 60:10 s and 60:15 s the decrease in O₂ evolution occurred over a prolonged period. At a L:D cycle of 60:20 s oxygen evolution not only remained constant over 200 minutes but also over a 24 h incubation period, the respiration rate also remained constant (Figure 4).

The results of light:dark cycling experiments conducted at incident irradiances of 500 and 750 µmol m⁻²s⁻¹ are shown in Figures 5 and 6 respectively. The rate of photosynthesis declined rapidly when cells were continuously exposed to these irradiances. Increasing the period the cells remained in the dark following a period of light exposure increased the time before the onset of photoinhibition caused a decrease in photosynthetic rate. At an incident irradiance of 500 µmol m⁻²s⁻¹ the time to onset of photoinhibition extended from – 50 min to 100 min as L:D cycle time increased from 30:15 s to 30:45 s until, at 30:60 s, a constant rate of photosynthesis was maintained. Similarly, at 750 µmol m⁻²s⁻¹ the time to onset of photoinhibition increased from 25 min to 250 min at L:D cycles of 60:30 s and 60:90 s respectively. A constant rate of photosynthesis, unaffected by photoinhibition, was achieved at an L:D cycle of 60:120 s at 750 µmol m⁻²s⁻¹. The maximum constant rate of photosynthesis, achieved on the L:D cycles used in this study, decreased with increasing incident irradiance from – 50 mg O₂ g DW⁻¹ hr⁻¹ at 300 µmol m⁻²s⁻¹ (L:D 60:20 s) to 23 mg O₂ g DW⁻¹ hr⁻¹ at 750 µmol m⁻²s⁻¹ (L:D 60:120 s).

Cells of Synechococcus were irradiated at 300 µmol m⁻²s⁻¹ on light dark cycles of 60:15 s and 30:45 s which resulted in both cultures receiving the same total light dose of 18,000 µmol m⁻² in 75 s. The cultures on the 30:45 s L:D cycle maintained a constant rate of photosynthesis, however, for those on 60:15 s L:D cycle the rate was initially lower and declined over 100 minutes. These results suggest that exposure time to a given incident irradiance rather than the total light dose received determines the effect of light dark cycle times on photosynthesis.

The relationship between irradiance and the ratio of L:D cycle time required to maintain a constant P_max is shown in Figure 7 and described by Eq. (1):

\[ R_t = 40588.4 \times \text{PFD}^{-1.73} \]  

where PFD is the incident irradiance (µmol s⁻¹ m⁻²) and \( R_t \) is the ratio of L:D exposure time (seconds) required to maintain a constant \( P_{max} \) (mg O₂ g DW⁻¹ hr⁻¹). This relationship was determined from 112 experiments on cycle times between 10–280 s. Interpolation indicates that at an incident irradiance of 470 µmol s⁻¹ m⁻² a light:dark cycle ratio of 1:1 is required to maintain a constant rate of photosynthesis.

**Discussion**

Light:dark cycles similar to those used in this work are known to occur in ponds and lake systems (Gallegos and Platt, 1981; Prezelin _et al._, 1991). Light:dark cycles of the order of seconds to minutes can be caused by cloud passage, surface waves, floating macrophytes and edge shadows (Walsh and Legendre, 1983); internal breaking waves and Langmuir circulation (Denman and Gargett, 1983). Richmond _et al._ (1980) suggested that light:dark cycles ranging from seconds to minutes might enhance productivity of algal cultures. Overall phytoplankton productivity affects many of the biochemical processes and cycling of matter that occurs in the natural environment (Kirk, 1983). Small changes in productivity can effect important changes in this complex chain of events which leads to...
Figure 1 Photosynthesis irradiance curves for C. vulgaris cultured at 23°C/70 µmol m⁻²s⁻¹ and measured at 15°C (○), 23°C (●), 30°C (■) and 35°C (▲).

Figure 2 Photosynthesis irradiance curves for Synechococcus cultured at 23°C/70 µmol m⁻²s⁻¹ and measured at 15°C (○), 23°C (●), 30°C (■) and 35°C (▲).

Figure 3 The effect of light:dark (L:D) cycles on the maximum rate of photosynthesis (mg O₂ g DW⁻¹ hr⁻¹) of C. vulgaris at an incident irradiance of 300 µmol m⁻² s⁻¹, L:D cycle of 60:10 s (■), L:D of 60:15 s (●), L:D cycle of 60:20 s (○) and continuously irradiated (□).

Figure 4 The effect of light:dark cycles of 60:20 s at an incident irradiance of 300 µmol m⁻² s⁻¹ on photosynthesis and respiration of C. vulgaris, maximum rate of photosynthesis (■), respiration rate (●).

Figure 5 The effect of light:dark (L:D) cycles on the maximum rate of photosynthesis (mg O₂ g DW⁻¹ hr⁻¹) of C. vulgaris at an incident irradiance of 500 µmol m⁻² s⁻¹, continuous light (x), L:D cycle of 30:15 s (■), L:D of 30:30 s (●), L:D cycle of 30:45 s (○) and a L:D cycle of 30:60 s (□).

Figure 6 The effect of light:dark (L:D) cycles on the maximum rate of photosynthesis (mg O₂ g DW⁻¹ hr⁻¹) of C. vulgaris at an incident irradiance of 750 µmol m⁻² s⁻¹, continuous light (x), L:D cycle of 60:30 s (■), L:D of 60:60 s (●), L:D cycle of 60:90 s (○) and a L:D cycle of 60:120 s (□).

Figure 7 Light:dark ratio which maintained constant photosynthetic oxygen production rate (mg O₂ DM⁻¹ hr⁻¹) at various incident photo flux densities (PFD µmol s⁻¹ m⁻²), Chlorella vulgaris (○), Synechococcus (■).
problems in long term predictions and modelling (Soeder et al., 1985; Sager and Richman, 1991; Talbot et al., 1991).

The results presented here suggest that light/dark cycling is a means by which algal cells can recover from the effects of photoinhibitory irradiances. The mechanism of recovery is unclear and may be via cellular maintenance and increased protein synthesis or through energy transfer preventing saturation of the photo-reaction centres. It is now generally accepted that light:dark cycles of medium frequency can affect the productivity and photosynthesis of micro-algal cells (Grobbelaar et al., 1992; Kroon et al., 1992). Evidence is growing which suggests that the light harvesting complexes and photoreaction centres are to a greater degree affected by the light regime within the light/dark cycle (Kroon et al., 1992). Reducing the adverse impact of photoinhibition may permit increased organic loading or reduced area requirements gained by an improvement in treatment performance. This might be achieved, firstly via a better understanding of fluid dynamics within WSPs and integrating this understanding with algal ecophysiology, and secondly by modification of pond configurations to achieved the desired outcomes such as using baffles described by Mihalyfalvy et al. (1998) for high rate algal ponds.

Conclusions

Light attenuation influences photosynthetic activity throughout the pond depth. High surface irradiances and temperatures may inhibit photosynthesis and thereby oxygen production. The results of this work suggest that the adverse effects of high irradiances on algae (and ultimately photosynthetic oxygen production) near the surface of a stratified waste stabilisation pond might be ameliorated by controlled mixing of algal cells through the depth of the pond.

References


