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Influence of light regime on the performance of an immobilised microalgae reactor for wastewater nutrient removal

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Abstract

Microalgae immobilised within a resin shaped into beads have demonstrated the ability to remediate nutrients from wastewater effluents within hydraulic retention times as low as 3 hours. Methods to further optimise performance consider parameters relating to the bead with the impact of external conditions seldom investigated. Light is an essential parameter for microalgal growth with its effect on suspended cultures well documented. This work explores the influence of light on nutrient remediation by immobilised microalgae in order to recommend an optimal lighting solution for an immobilised microalgae technology based on *Scenedesmus obliquus* encapsulated within calcium-alginate beads. White light (400 – 700 nm) at a photon flux density (PFD) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was determined optimal when illuminating a packed bed configuration. When considering phosphate, these conditions supported a remediation rate of 10.7 (± 0.01) $mgP \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ in comparison to 10.2 (± 0.01) and 10.1 (± 0.01) $mgP \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ for the blue (465 nm) and red (660 nm) spectra respectively. Although similar performance was demonstrated, light transmission trials determined white light to

penetrate to greater bed depths resulting in a larger photoactive zone. A PFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was regarded as optimal when considering performance, attenuation depth and effective use of total supplied light. In addition, photoperiods trials determined lighting periods <12 h extended the overall treatment time.

Keywords: Nitrogen, phosphorus, photon flux density, photoperiod, wavelength

1. Introduction

Microalgae consume several macronutrients, including ammonium (NH_4^+) and phosphate (PO_4^{3-}), at relatively constant stoichiometric ratios approximately equal to that of the stoichiometry of the microalgal biomass [1]. This consumption results in an increase in biomass density and subsequent nutrient removal from the surrounding medium. Accordingly, when sourced from wastewater effluents, microalgae based technologies can be an effective approach for nutrient removal. As algae are photosynthetic organisms, reactor designs must ensure effective delivery of light to the algal biomass. The most common embodiment for wastewater treatment is a high rate algal pond (HRAP). A HRAP enables solar light penetration through the use of raceway ponds with water depths of 20 – 60 cm [2]. These ponds contain a relatively dilute biomass concentration of approximately 0.2 $gDW \cdot L^{-1}$ [3] characterised by a microalgal and bacterial community [4]. As such, HRAPs are implemented in locations with a suitable annual climate enabling irradiation through solar radiation. However, this results in a variation of photoperiod lengths (summer vs winter daylight hours) and light photon flux densities (PFDs, $\mu mol \cdot m^{-2} \cdot s^{-1}$) with the equivalent of approximately 700 to 1,200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ reported [2,5] with less than 50% of sunlight within the range of photosynthetically active radiation (PAR) [6]. The need for shallow depths and dilute biomass concentrations in HRAPs results in the ponds needing to be operated at long

hydraulic retention times (HRT) of 4 – 10 days [2,7] with large associated footprints [8] which are unsuitable for implementation in locations of limited land availability, such as the United Kingdom (UK).

Intensification of a microalgal based reactor, coupled with a reduction in reactor footprint, can be achieved through immobilising algal populations within resins shaped into beads [9]. Immobilisation enables hyperconcentration of algal biomass up to 3.3 $g(DW)\cdot L^{-1}$ [10], with treatment efficiencies >70% for NH_4^+-N and >80% PO_4^{3-}-P when treating tertiary wastewater effluent at hydraulic retention times (HRT) as low as 3 hours [11].

The PFD and wavelength are essential parameters for microalgal growth and nutrient remediation [12,13] and as such are recognised as key design features for microalgal bioreactors [14,15]. For instance, PFDs between 150 – 400 $\mu mol\cdot m^{-2}\cdot s^{-1}$ are reported for optimal growth of suspended *Scenedesmus obliquus* [16,17], with higher PFDs resulting in photoinhibition. PFDs below this range (10 – 150 $\mu mol\cdot m^{-2}\cdot s^{-1}$) exhibit a reduction in biomass productivity [16] and nutrient removal due to light limitation.

Furthermore, microalgal chlorophyll molecules absorb light within the blue (450 – 495 nm) and red (650 – 700 nm) region of the spectrum most efficiently [18]. Suspended algae grown within single wavelength blue light are associated with increased phosphorus remediation [19], and improved nitrogen uptake through the activation of protein synthesis [20] and gene expression [21], whereas red light is associated with increased specific growth rate [22,23].

In addition to PFD and wavelength, the antenna structure of the light harvesting complex of microalgae are unable to use all the photons absorbed in constant light [15]

with photons either utilised in the production of ATP or lost as refracted light or heat [24,25]. Light:dark (L:D) periods of 14:10 h and 12:12 h are commonly chosen for photo-autotrophic cultures with dark periods allowing recovery of the photosynthetic apparatus from photoinhibitive stress [26]. However, recent studies have determined the optimal light:dark regime is further related to light intensity and wavelength [27,28], with a 16:8 h L:D photoperiod necessary for a suspended culture of *Chlorella vulgaris* when illuminated at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by white florescent light in comparison 12:12 h for a blue LED light [28], with the translation of these findings to an immobilised system unknown.

Whilst work has been undertaken to optimise an immobilisation system in terms of cell stocking ($cells \cdot bead^{-1}$), bead concentration ($beads \cdot mL^{-1}$) and bead size [29,30] the influence of lighting design and operation have not been as extensively evaluated nor optimised. Key aspects include (1) selecting the most appropriate wavelength thereby eliminating energy use on unwanted wavelengths [18]; and (2) optimising the total delivered photon flux [31] through determination of optimal light intensity and lighting regime. Accordingly, the current study aims to determine the influence of the lighting regime on wastewater nutrient remediation by immobilised microalgae in order to propose recommendations for lighting strategies for the immobilised microalgae technology.

2. Materials and methods

2.1 Microalgal cultivation and immobilisation procedure

The freshwater species *S. obliquus* (276/42) was obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK) and cultured in 50 L of Jarwoski Medium. Cultures were grown under a 24 hour light regime of approximately 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at

the culture surface which reduced to light limited conditions of < 50 μmol·m^{-2}·s^{-1} for the lower half of the tank. As such, the culture was circulated under constant mixing by a circulation pump (900 $L.h^{-1}$) (Hydor Koralia Nano 900) to enable biomass exposure to light:limited light (dark) conditions of approximately 12:12 h. Biomass was harvested during the latter stages of the exponential growth phase (approximately 10 days) to enable maximum biomass recovery and stored overnight at 4ºC prior to immobilisation.

Beads were produced following previously described methods [11,32]. To summarise, a sodium alginate (Na-alg) solution was mixed with concentrated algal biomass for a final Na-alg concentration of 2% and a final biomass concentration of approximately 10^5 cells·$bead^{-1}$ following bead production. The volume of algal concentrate required was calculated upon determination of the harvested cell concentration using a haemocytometer and light microscope (Olympus, BH Series) and the ability to produce approximately 4,000 beads (3 mm diameter) per 100 mL of algae-resin (estimated during preliminary trials).

The algae-resin solution was pumped by a peristaltic pump through tubing with a 4 mm internal diameter, capped with a 1 mL pipette tip and dripped into a 2% $CaCl_2$ solution from a height of 30 cm. The beads were left to solidify within the solution overnight and then washed several times with DI water to remove any surplus $CaCl_2$ prior to use.

The initial cell·$bead^{-1}$ concentration was confirmed by dissolving a sample of 10 beads within a known volume of 2% sodium citrate and determining the cell concentration (cells.mL^{-1}) using a haemocytometer and light microscope (Olympus, BH Series) in triplicate. The cell concentration was then used to back-calculate and confirm

approximately 10^5 cells·bead^{-1} during production. Blank beads were produced using the same method with no addition of algal biomass.

2.2 Wastewater

Secondary wastewater effluent was delivered weekly from a wastewater treatment plant (WWTP) located in the Midlands, UK with population equivalence (PE) of 32,000 which utilises an oxidation ditch for the main biological process. The effluent was supplemented with KH_2PO_4 and NH_4Cl to maintain consistent concentrations throughout the trials of approximately 1 mg·L^{-1} PO_4^{3-}-P and 2.5 mg·L^{-1} NH_4^+-N (N:P 5.5:1). Supplementation of the source wastewater was necessary, as the treated effluent from this WWTP is discharged into a catchment designated as a Special Area of Conservation (SAC) under the EC Habitats Directive (92/42/EEC) and a Site of Special Scientific Interest (SSSI). In order to meet the tighter consent limits, enhance upstream treatment is undertaken and as such, effluent from this site does not represent the characteristics of an average secondarily treatment effluent in the UK without this supplementation. Wastewater was used upon delivery with the remainder stored at 4ºC until use.

2.3 Experimental set up and light regime

Trials were run in batch over 24 hours within an Algem™ Labscale Photobioreactor (Stewartby, UK) (Figure 1) maintained at a constant temperature of 20ºC. Conical flasks of 1 L were filled with 600 mL of wastewater effluent and a bead concentration of 10 beads.mL^{-1} based on previous experiments that demonstrated effective treatment with HRTs below 12 hours [11]. Reactors were mixed via a gimbal system at 120 rpm (Figure 1), with fluidisation of the beads confined to the lower third of the vessel simulating a packed bed configuration. Reactors were illuminated by an LED panel at

the base of the reactor over a surface area of 133 cm^2 and operated within the white (400 – 700 nm), blue (465 nm) and red (660 nm) spectra at PFDs from 50 to 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, representing a range of intensities considered as light limited to light saturated for suspended cultures.

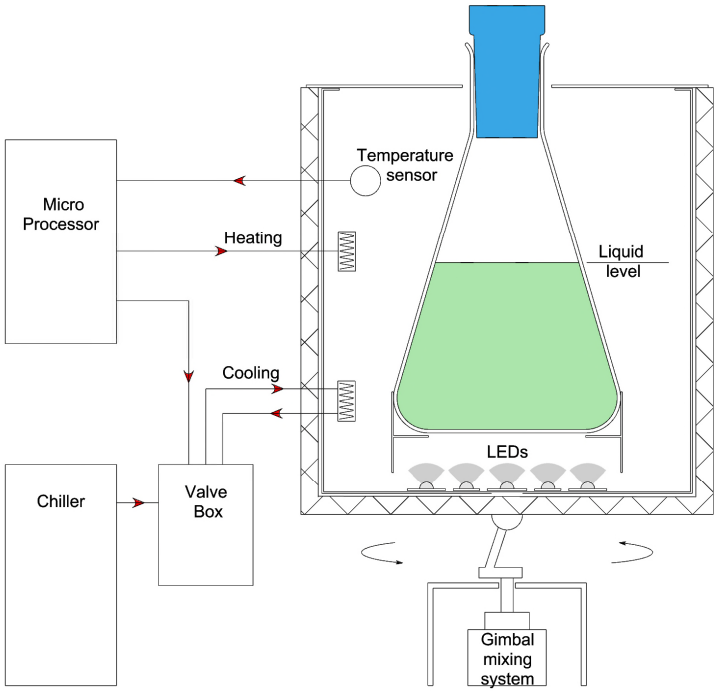


Figure 1: Schematic of Algem™ Labscale Photobioreactor

A series of experiments were undertaken within the Algem™ to determine the optimal lighting parameters for nutrient remediation (Table 1). Those parameters which demonstrated enhanced performance were then further analysed to determine light transmittance (2.3.1) and attenuation depth within a bed of immobilised *S.obliquus* beads, with results informing the design requirements of an immobilised microalgal reactor (Table 1).

Table 1 Summary of experimental conditions

	Wavelength*	Intensity (µmol·m^{-2}·s^{-1})	Photoperiod L:D (h)	Technical replicates
Optimal wavelength and PFD for nutrient remediation	W, B, R	50, 200, 500, 1,000	24:0	2
Light transmittance	W, B, R	1,000	-	2
Attenuation depth	W	200, 400, 600, 800, 1,000	-	-
Photoperiods	W	200	12:12, 6:6, 3:3, 1.5:1.5	2

*W = white (400 – 700nm), B = blue (465 nm) and R = red (660 nm)

Subsequent trials to analyse the impact of reduced lighting periods through photoperiods were completed by programming the Algem™ to turn the LEDs on and off as necessary. Photoperiod lengths of light:dark (L:D) of 12:12 h were initially selected as the baseline photoperiod commonly applied to photo-autotrophic cultures [26], then decreased to photoperiods of 6:6, 3:3 and 1.5:1.5 h which were run continuously over the 24 h experimental period, with each variation in regime delivering the same total light over the experimental period.

Quantification of the total photons (µmols) applied during the trial was calculated through Equation 1, to enable comparisons to light delivery through continually illuminated regimes; where PFD = µmol·m^{-2}·s^{-1}, A = illuminated surface area (0.0133 m^2) and t_l = length of the light period (seconds).

$$\mu mols\ photons\ applied\ = PFD\ \times A\ \times\ t_l \qquad \text{(Equation 1)}$$

2.3.1 Light transmittance

Light transmittance through the microalgal beads and blank beads was completed to compare light attenuation and transmittance depth. Such information is important when designing and sizing a reactor to ensure sufficient light delivery to the algal biomass. Beads were illuminated within the Algem™ at PFDs between 200 - 1,000 µmol·m^{-2}·s^{-1} under the white, red and blue spectra. Light transmittance was measured in duplicate at four bead bed depths with a light meter (Apogee Quantum MQ-200 PAR Meter). The variation of light transmission (T) as a function of distance (l) in mm was calculated according to the Beer-Lambert law (Equation 2) to determine the attenuation coefficient (a).

$$a = \frac{-logT}{l} \qquad \text{(Equation 2)}$$

2.3.2 Light energy yield

The light utilisation efficiency in relation to biomass yield and nutrient remediation was calculated using (Equation 3), where Y = biomass yield or nutrient remediation yield (g·mol^{-1} photons), C = biomass concentration (g·L^{-1}), µ = specific growth date (d^{-1}), V = reactor volume (0.6 L), PFD = µmol·m^{-2}·s^{-1}, and A = illuminated surface area (0.0133 m^2). Biomass concentration (g·L^{-1}) was determined upon dissolving the beads to determine the cell·mL^{-1} concentration then estimating the biomass concentration from previous growth trials enabling a correlation with cell·mL^{-1}.

$$Y = \frac{C \times \mu \times V}{PFD \times A \times 86400 \times 10^{-6}} \qquad \text{(Equation 3) [33]}$$

2.4 Batch nutrient remediation sample analysis and biomass growth

Samples of the treated effluent containing no microalgal beads were collected over a 24 h period. Analysis included pH, NH_4^+-N and PO_4^{3-}-P with phosphate analysis including

the total (tPO_4^{3-}-P) and dissolved (dPO_4^{3-}-P) fractions. Insoluble phosphorus, characterising phosphate precipitation, was determined through deducting the concentration of the dissolved fraction from the total concentration. Dissolved PO_4^{3-}-P was analysed following syringe filtration at 0.45µm (Millipore, DE), whereas total phosphate (tPO_4^{3-}-P) was analysed using unfiltered samples. Residual N and P concentrations were analysed in duplicate using Spectroquant test kits (Merck Millipore) and read via a Spectroquant Nova 60 spectrophotometer and reported as the mean ± standard error. Remediation performance was quantified as the reduction in the residual nutrient concentration, with removal associated to either direct uptake by the immobilised microalgae or precipitation/volatilisation facilitated by alkalisation of the local environment through biological activities of the microalgae. Statistical analyses on transformed data were undertaken by one-way ANOVA to identify statistically significant differences ($p < 0.05$).

Biomass growth within the beads was evaluated upon dissolving a sample of 10 beads within 2% sodium citrate and back-calculating the biomass concentration as previously described. Specific growth rate of the immobilised microalgae was then determined using (Equation 4), where µ = specific growth rate (d^{-1}), $x1$ and $x2$ the cell·$bead^{-1}$ concentration at time $t1$ and $t2$, reported as the mean ± standard error.

$$\mu = \frac{\ln(\frac{x1}{x2})}{t2 - t1} \qquad \text{(Equation 4)}$$

During preliminary experiments lasting 24 hours, a suspended biomass concentration of approximately 0.4 mg·L^{-1} was released from the beads, representing approximately 0.07% of the total microalgal biomass within the reactor. As such, the overall contribution to nutrient remediation by the suspended biomass is considered negligible.

3. Results and discussion

3.1 Wavelength and PFD

The remediation rate for PO_4^{3-}-P under white light increased from 10.7 (± 0.01) $mgP \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ at 50 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 12.2 (± 0.02) $mgP \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ at PFDs ≥ 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equivalent of 2.2 to 2.5 $mgP \cdot L \cdot d^{-1}$) corresponding to near complete exhaustion of the available phosphate (Figure 2a) to a residual concentration of 0.1 $mgP \cdot L^{-1}$ within 12 hours at PFDs > 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Although an improved remediation rate was demonstrated with increasing PFD, the light utilisation efficiency associated to nutrient remediation was found to decrease from 38.6 $\mu gP \cdot mol^{-1}$ photon at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 2.2 $\mu gP \cdot mol^{-1}$ photon at 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

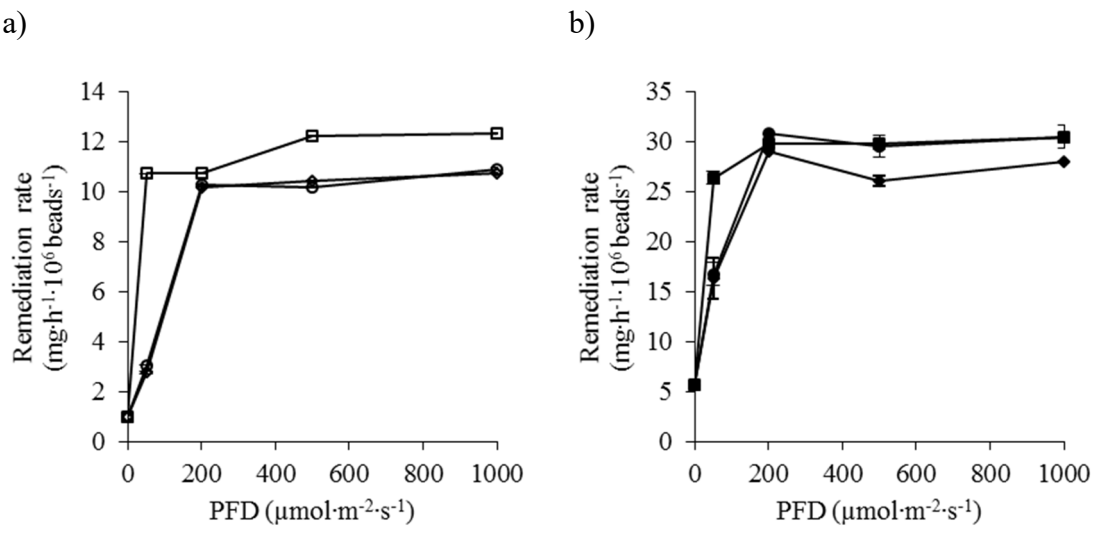


Figure 2 a) Nutrient removal rate for a) PO_4^{3-}-P remediation under white (□), red (◊) and blue (○), and b) NH_4^+-N removal under white (■), red (♦) and blue (●) (mean ± standard error of n = 2 technical replicates).

Remediation of nitrogen followed a similar profile with the rate increasing from 26.4 (± 0.6) to 30.5 (± 0.11) $mgN \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ (equivalent of 8.4 to 9.7 $mgN \cdot L \cdot d^{-1}$) as the PFD increased from 50 to 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ respectively (Figure 2b). The equivalent

photon efficiency decreased from 146.8 μgN·mol^{-1} photon at 50 μmol·m^{-2}·s^{-1} to 8.5 μgN·mol^{-1} photon at 1,000 μmol·m^{-2}·s^{-1}.

Comparison to equivalent experiments conducted under blue and red light revealed that white light demonstrated a marginally improved remediation rate for PO_4^{3-}-P at all PFDs, however the difference between all wavelengths was not found to be statistically significant ($p > 0.05$). Blue and white light performed similarly for NH_4^+-N at 200, 500 and 1,000 μmol·m^{-2}·s^{-1} (Figure 2b). The difference was greatest under low lighting conditions (50 μmol·m^{-2}·s^{-1}) where the remediation rate for blue light was 16.8 (±1.11) mgN·h^{-1}·10^6 beads^{-1} and 3.1 (±0.01) mgP·h^{-1}·10^6 beads^{-1} (equivalent to 3.6 mgN·L·d^{-1} and 0.7 mgP·L·d^{-1}), and for red light 16.3 (±2.11) mgN·h^{-1}·10^6 beads^{-1} and 2.8 (±0.05) mgP·h^{-1}·10^6 beads^{-1} (equivalent to 3.5 mgN·L·d^{-1} and 0.6 mgP·L·d^{-1}). This equates to a reduction in the efficiency of utilisation of the available photons at 50 μmol·m^{-2}·s^{-1} of 28.7% and 41.8% of that observed under white light for PO_4^{3-}-P and NH_4^+-N for red light and 31.3% and 42.9% for blue light respectively. When comparing white, red and blue light at the higher PFD of 1,000 μmol·m^{-2}·s^{-1}, the difference became much less significant where the photon utilisation efficiency was the same in regards to PO_4^{3-}-P and slightly lower for NH_4^+-N.

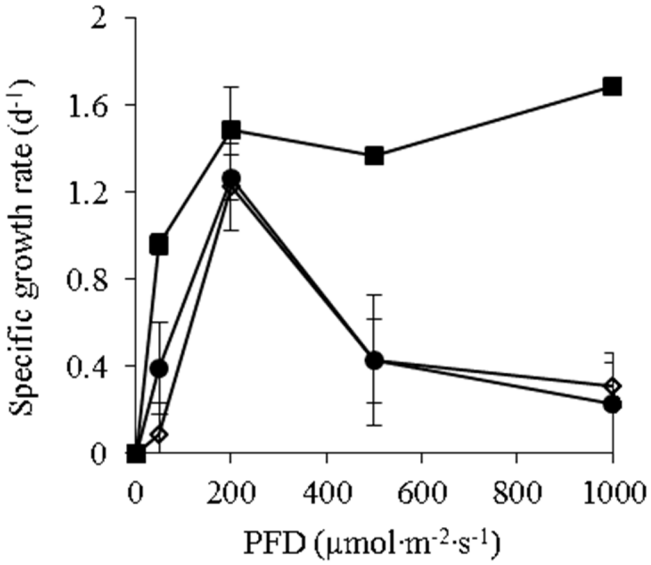


Figure 3 Specific growth rate of immobilised microalgae under white (400 – 700 nm) (■), red (660 nm) (◊), blue (465 nm) (●) (mean ± standard error of n = 3 technical replicates)

Microalgal growth was most consistently enhanced when irradiating the immobilised algae with white light compared to red or blue light across all tested PFDs, illustrated by specific growth rates of 0.96 (±0.05), 0.39 (±0.21) and 0.09 (±0.04) for white, blue and red light respectively at an PFD of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 3). The results are congruent with previous studies concerning a suspended culture of *S.obliquus* [19] and reflect that whilst chlorophyll molecules absorb light within the blue and red region of the spectrum most efficiently [18], increased growth is expected under white light through the availability of both blue and red wavelengths [31]. However, studies using immobilised *Chlorella vulgaris* reported enhanced growth from 0.38 d^{-1} to 0.81 d^{-1} upon switching from white to red light at an approximate PFD of 33 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 12 h HRT [34] suggesting the impact may be species specific. However in this case, the nutrient remediation performance was similarly enhanced under white light with ~80% NH_4^+-N removal and 100% PO_4^{3-}-P [34].

Across all wavelengths optimum growth occurred at a PFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ which resulted in specific growth rates of 1.49 (±0.2), 1.27 (±0.11) and 1.23 (±0.11) for white, blue and red light with biomass yields 0.72, 0.53 and 0.52 $g \cdot mol^{-1}$ photons (Figure 3). Irradiating the immobilised algae at greater PFDs reduced the specific growth rate in the case of blue and red light (Figure 3) with reduced biomass yields of 0.04 and 0.03 $g \cdot mol^{-1}$ photons at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 0.01 and 0.01 $g \cdot mol^{-1}$ photons at 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for red and blue light respectively, consistent with a reduction in the conversion of light energy to biomass growth and associated nutrient uptake. In contrast, the specific growth remained stable when irradiating at higher PFDs with white light such that the immobilised beads demonstrated no photo saturated inhibition which has been reported in the case of suspended cultures at PFDs > 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [16]. For instance, a reduction in biomass concentration of suspended *S.obliquus* from 1.2 x 10^8 to <8 x 10^7 $cells \cdot mL^{-1}$ was observed when cultivated under white light at PFDs increasing from 150 to > 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [16].

Remediation rates averaged 29.7 (± 0.3), 29.2 (±0.3) and 30.8 (±0.3) $mgN \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ for white, red and blue light respectively at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equivalent to 7.2, 7.0 and 7.4 $mgN \cdot L \cdot d^{-1}$) (Figure 2a). The marginally greater remediation rate under blue light reflects similar total removal but reduced growth compared to white light congruent with its association with the activation of protein synthesis [20] and gene expression [21], in addition to the activation of the enzyme nitrate reductase [35]. These similarities in nitrogen removal within white and blue light have been previously reported for a suspended culture of *S.obliquus*, despite a 45% increase in production rate within the white light regime [19].

A pH increase of the localised environment was evident in all batch trials, from approximately 7.8 to 11.4. Red and blue light at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ achieved a slightly reduced pH of 10.4. Alkalisation of the localised environment is a known by-product of microalgal photosynthesis and as such, an indirect removal mechanism is likely to have contributed to NH_4^+-N remediation through volatilisation. Although not directly measured ammonia speciation pKa estimates volatilisation of free ammonia of between 2 and 99% at pH values of 7.4 and 11.4 at 20ºC. In contrast, the indirect removal of PO_4^{3-}-P through precipitation was not found to take place as residual concentrations of tPO_4^{3-}-P was similar to dPO_4^{3-}-P throughout the trials.

3.2 Light transmittance

Subsequent trials were undertaken to analyse light transmittance for white, blue and red light at a fixed intensity of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for blank beads and beads containing *S.*obliquus. As anticipated light absorption by the beads containing algae was greater than the blank beads for all wavelengths, attenuating approximately 15, 14 and 48% more light than the blank beads at bed depths <20 mm for white, blue and red light respectively (Figure 4).

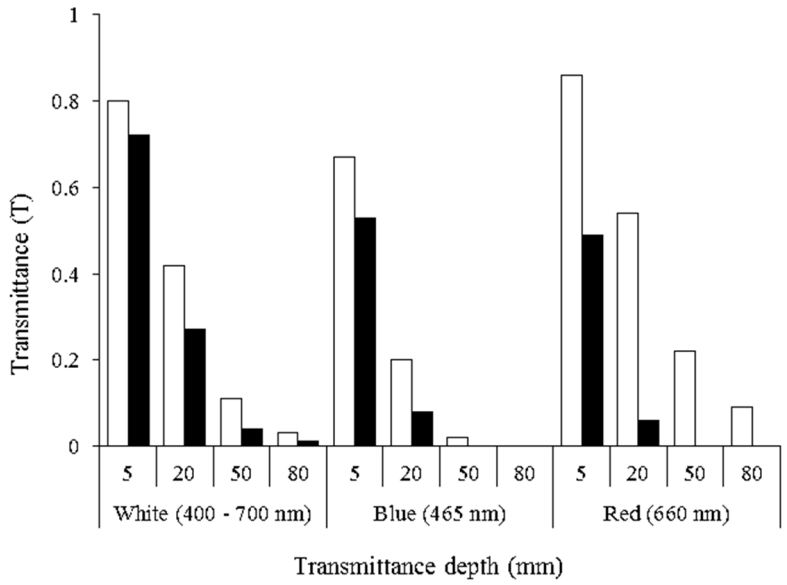


Figure 4 Light transmittance at 1,000 µmol·m^{-2}·s^{-1} for white, blue and red wavelengths, where (□) blank beads and (■) algal beads

The reduction in transmittance of red light in comparison to white and blue for the algal beads, may be contributed to the higher quantum efficiency by *S.obliquus* for photons within the red wavelength [21] thereby preventing transmission depths seen within white and blue light and a reduction in specific growth rate as previously observed when compared to white and blue. The red and blue wavelengths were absorbed within shorter distances by the immobilised biomass and reduced by almost 50% within 5 mm with no light beyond 50 mm. Transmission was better achieved by white light attaining a light limited PFD of 50 µmol·m^{-2}·s^{-1} at a maximum bed depth of approximately 43 mm for the algal beads, in comparison to 39 and 24 mm for red and blue light at an initial PFD of 1,000 µmol·m^{-2}·s^{-1} (data not shown). White light would therefore be considered the preferable lighting option, allowing the photoactive zone within an immobilised reactor to be larger in size when applied to full scale treatment in comparison to a reactor lit solely by a blue or red light regime.

3.3 Attenuation depth

The implications of light attenuation was further analysed for an immobilised system to profile the reduction in white light transmittance of varying PFDs (200 – 1,000 µmol·m^{-2}·s^{-1}) within a packed bed of algal beads. A similar pattern of attenuation was observed for all PFDs with an initial reduction of approximately 20% within the first 10 mm, increasing to > 98% at depths > 50 mm (Figure 5).

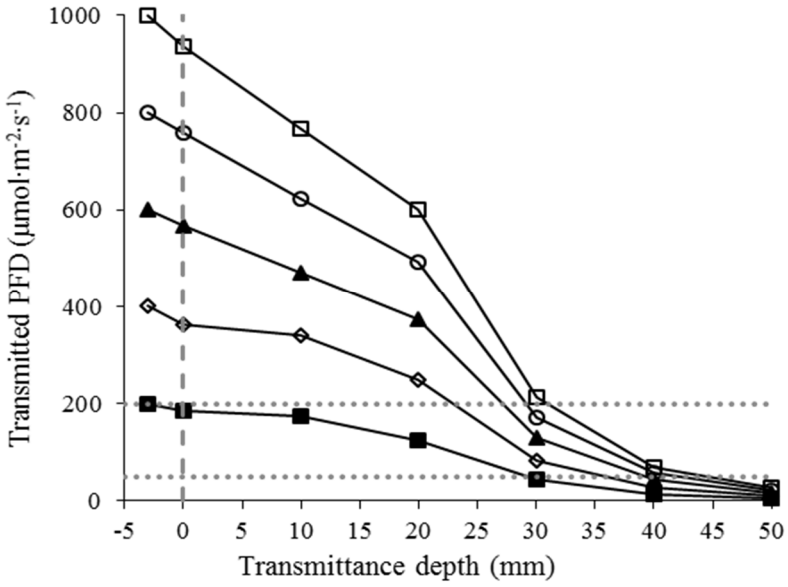


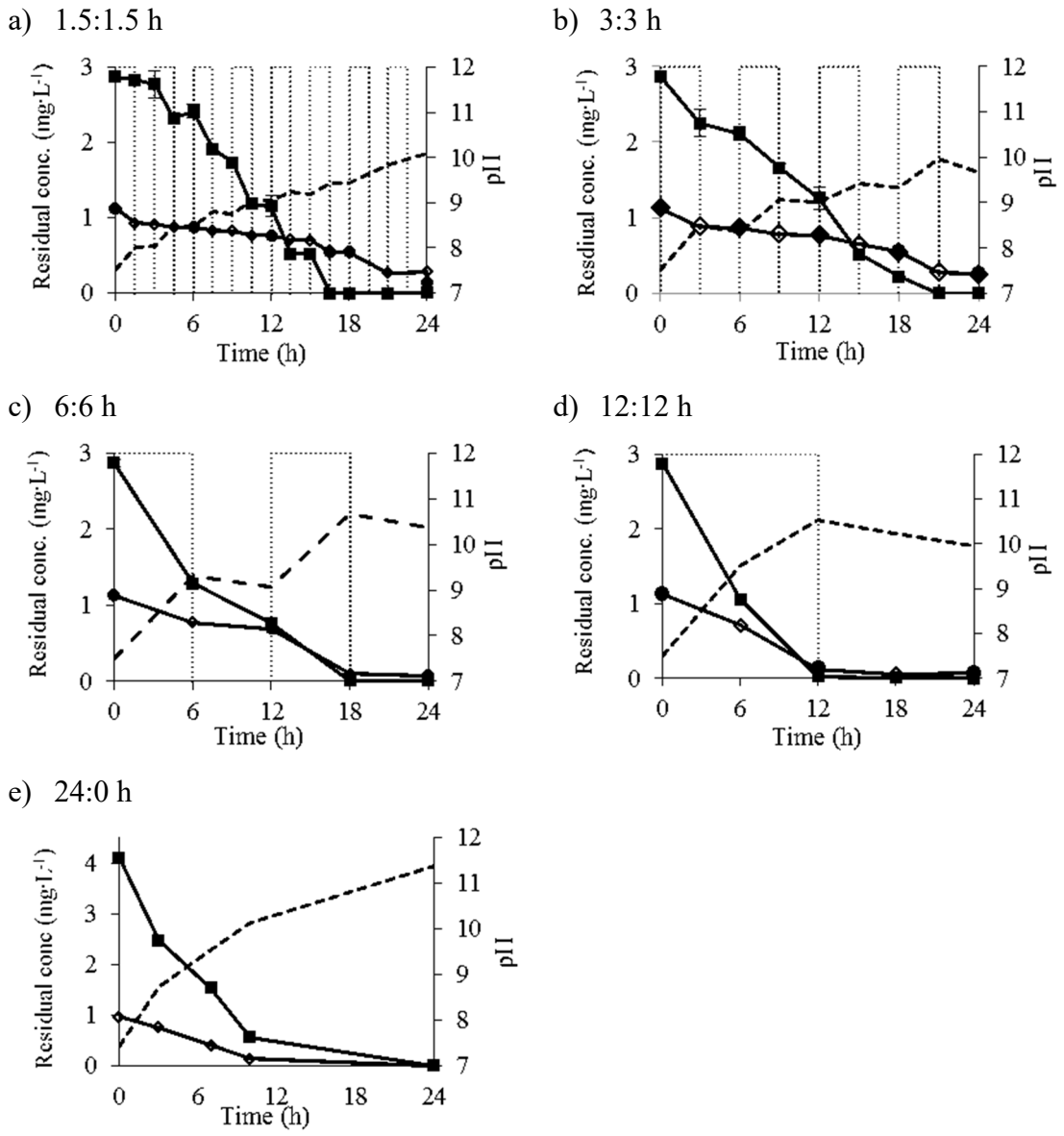
Figure 5 White light transmittance depth through a bed of beads in relation to PFD with (--) denoting the base of the Pyrex conical flask and initial reduction in PFD and (..) the photoactive zone for effective activity and (■) 200, (◊) 400, (▲) 600, (○) 800, (□) 1,000 µmol·m^{-2}·s^{-1}.

Operation at different PFDs altered the depth the light was able to reach and thus controlled the size of the photoactive volume within the reactor. A target level of 50 µmol·m^{-2}·s^{-1} is known to maintain effective activity [16,17] which was delivered to bed depths of 20.9, 32.0, 38.5, 42.9 and 46.2 mm for PFDs of 200, 400, 600, 800 and 1,000 µmol·m^{-2}·s^{-1} respectively. Irradiating the beads at PFDs beyond 200 µmol·m^{-2}·s^{-1} was not previously observed to increase growth or substantially enhance nutrient

remediation (Figure 2 and 3). In comparison, all the light supplied at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ could be utilised by the microalgae as the light supplied was within the PFD band of 50 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the maintenance of effective activity. The total bed depth within the PFD band of 50 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is estimated as 20.9, 21.3, 21.5, 21.4 and 21.3 mm for starting PFDs of 200, 400, 600, 800 and 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ respectively (Figure 5). An initial PFD of between 200 - 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is therefore recommended for a packed bed configuration to enable effective use of the total supplied light with a greater conversion of the provided light energy to nutrient remediation (demonstrated at lower PFD).

3.4 Photoperiods (light:dark cycle)

Subsequent trials using white light at a PFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were undertaken to analyse the impact of reduced lighting periods through photoperiods of 1.5:1.5, 3:3, 6:6 12:12 h L:D in comparison to constant light (24:0). The residual ammonia concentration reduced to below 0.1 $mg \cdot L^{-1}$ within 16.5, 21, 18, 12 and 12 hours for 1.5, 3, 6, 12 and 24 h L:D respectively (Figure 6). With regards to PO_4^{3-}-P, the overall rates were slower than for NH_4^+-N and the impact of the higher PFD was more pronounced such that removal to <0.1 $mgP.L^{-1}$ occurred only at 6:6, 12:12 and 24:0 following 18 h of treatment (6:6), equivalent to 12 h illumination, and 12 h for 12:12 and 24:0 respectively.



(■)NH_4^+-N, (◊) dPO_4^{3-}-P (▲) tPO_4^{3-}-P and (- -) pH.

Figure 6 Photoperiod trials for light:dark regimes (mean ± standard error). Dashed profile denotes photoperiod, sections with top border representing the light period (mean ± standard error).

The remediation rate within the light episodes of the photoperiods for 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased from 5.4 (± 1.9) to 8.3 (± 1.4) $mgP \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ for 1.5 to 12 h L:D regime (equivalent of 1.3 to 2.0 $mgP \cdot L \cdot d^{-1}$) and 16.9 (± 3.8) to 23.7 (± 6.4) $mgN \cdot h^{-1} \cdot 10^6$ $beads^{-1}$ for NH_4^+-N (equivalent of 4.1 to 5.7 $mgN \cdot L \cdot d^{-1}$). Likewise, remediation during the

corresponding dark episodes was found to increase with increasing length of dark period from 0.3 (± 0.2) to 1.0 (± 0.4) mg PO_4^{3-}-P·h^{-1}·10^6 $beads^{-1}$ for 1.5 to 6 h (equivalent of 0.1 to 0.2 mgP·L·d^{-1}), corresponding to 6 – 13% of the remediation rate during the light episodes. Minimal uptake was observed within the dark episode of the 12:12 regime as remediation occurred to approximately 0.1 mg·L^{-1} within the initial 12 hours of light.

The observed results during the dark period are congruent with the storage of chemical energy ATP (adenosine triphosphate) during the light dependant reactions of photosynthesis [25]. The ATP produced during the light period is subsequently utilised during the light independent reaction (Calvin Cycle) [24] in the conversion of CO_2 to glucose and the anabolism of amino acids and nucleotides from the assimilated NH_4^+-N and PO_4^{3-}-P into proteins and nucleic acids for growth. Accordingly the duration of the light and dark cycles influences the efficiency of the treatment as sufficient ATP must be produced during the light phase for use during the dark phase. However, when comparing the performance of constant lighting and photoperiods, a total light period (treatment time excluding dark periods) of approximately 12 h was required for the 24:0, 12:12 and 6:6 h lighting regimes to reach a residual concentration of 0.1 mgP·L^{-1} (3:3 and 1.5:1.5 h were found not to reach this residual concentration during the experimental period (Figure 6a and b)). As such, when considering the light energy all scenarios applied the equivalent of 114.9 mols of photon. In these circumstances, when irradiating immobilised microalgae with white light at 200 µmol·m^{-2}·s^{-1}, the application of photoperiods of <12 h L:D are not found to benefit performance nor reduce the total delivered photon flux. In addition, wastewater treatment works operate 24 hours a day and are expected to consistently meet a discharge consent, even during light limited

periods i.e. overnight. As such, the reduction in treatment performance during these periods would be avoided through constant illumination.

Overall, the effect of the studied light regimes and the outcomes on remediation performance by immobilised microalgae and the proposed recommendations for the lighting regime are summarised in Table 2.

Table 2 Recommended lighting regime for an immobilised microalgae reactor for wastewater nutrient remediation.

Lighting regime	Figure	Outcome
Wavelength (white, blue or red); PFD (50, 200, 500 or 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$)	2a, 2b	White, blue or red , >200 $\mu mol \cdot m^{-2} \cdot s^{-1}$
Light transmittance (white, blue or red) Fixed at 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$	4	White
Transmittance depth (white) 200, 400, 600, 800 or 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$	5	200 – 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$
Photoperiod (12:12, 6:6, 3:3 or 1.5:1.5) using white at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$	6	>12 h

4. Conclusions

The remediation performance of immobilised *S.obliquus* was found to perform similarly under a white, blue and red wavelengths for PO_4^{3-}-P and NH_4^+-N at light intensities from 200 to 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with maximum specific growth observed at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for all lighting regimes. However, when considering light attenuation within the bead bed, white light transmitted further thereby providing a larger photoactive zone in comparison to a reactor lit solely by a blue or red light regime. Increasing the white light PFD beyond 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ altered the depth the light was able to reach but was not found to increase growth or enhance remediation performance.

As such, white light at a PFD of 200 – 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is recommended for a packed bed configuration to enable effective use of the total supplied light.

The potential to reduce lighting through photoperiods found light periods <12 h extended the overall treatment time, with 12 h of light necessary to achieve residual concentrations of < 0.1 $mg \cdot L^{-1}$ for PO_4^{3-}-P and NH_4^+-N.

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