Optimisation of red light-emitting diodes irradiance for illuminating mixed microalgal culture to treat municipal wastewater

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Abstract

This paper evaluates the effect of variation in red light-emitting diodes (LEDs) irradiance on the growth rate and biomass productivity of a mixed culture of microalgae grown on synthetic municipal wastewater, with and without CO_2 addition. Red LEDs were used to illuminate microalgal culture from the centre of 21-L stirred-tank photobioreactors made of transparent Plexiglas, each reactor having a working volume of 16 L. The reactors were operated in batch mode with pH control, and under continuous illumination for 30 days at ambient temperature. Mixing was achieved through the use of overhead mechanical stirrers operated at 100±1 and 60±1 revolution per minute, before and after the addition of CO_2, respectively. Three average values of irradiance of 429.9, 582.7 and 730 .8 $\mu mol.s^{-1}.m^{-2}$ were used to illuminate the reactors, with a control reactor operated in the dark. CO_2 addition resulted in about two-fold increase in biomass productivity in all the experimental reactors. The bioreactor with medium irradiance yielded the highest biomass productivity and maximum specific growth rate of 0.034 $g.L^{-1}.d^{-1}$ and 0.109 d^{-1}, respectively. The findings in this study show that both microalgal growth rate and biomass productivity are not always directly proportional to irradiance, despite the influence of process and operational parameters. Furthermore, a medium amount of irradiance resulted in optimum growth and productivity of the mixed microalgal culture.
Keywords: microalgae, biomass productivity, growth rate, optimum irradiance, red light emitting diode, synthetic municipal wastewater.

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1 Introduction

Amount and quality of illumination play a key role in the growth of aquatic photosynthetic organisms such as microalgae. These illumination requirements can influence the ability of microalgae to remove nutrients from wastewater. Conventional light sources such as fluorescent and incandescent lamps are widely used to externally illuminate microalgal photobioreactors (PBRs). However, such light sources have relatively high carbon footprint.

In addition, light attenuation resulting from long path length in PBRs, coupled with high algal concentration, can be one of the shortcomings of illuminating PBRs externally. Furthermore, visible light absorption and scattering by materials suspended in water column result in loss of light photons and consequently reduce the amount of photosynthetic active radiation reaching microalgal cells, leading to light limitation [1].

Internally illuminating microalgal PBRs with red light-emitting diodes can overcome the above shortcomings. LEDs emitting monochromatic light are recently gaining popularity in microalgal cultivation [2, 3]. However, there is a need to optimise the quantity and quality of LED irradiance for illuminating microalgal systems. Nevertheless, optimum quantity of irradiance may differ from one system to another.

Therefore, such optimisation studies are prerequisite to the development of energy-efficient, carbon-neutral microalgal wastewater treatment technologies. LEDs can potentially replace conventional light sources due to the advantages of the former over the latter [4]. Such advantages include lower power consumption; lower input voltage; luminous efficacy [5]; lower start-up time; easy control; monochromatic property; and longer life span [4].

More importantly, LEDs have very low carbon footprint with associated opportunity for carbon credits as well as high potential towards enhancing environmental sustainability; they have lower carbon footprint than fluorescent and incandescent lamps [4]. Interestingly, used LEDs can be recycled easily as they are composed of fairly benign substances compared to incandescent lamps that contain mercury which poses higher pollution risk to the environment [4].

Furthermore, the problems of light limitation associated with PBR scale-up can be overcome through focussed research on the use of LEDs to replace conventional light sources. Due to their small size, many LEDs can be mounted on a narrow strip of matrix board and inserted into PBRs in a water-tight chamber (to prevent short-circuiting when in contact with water) in order to fully utilise their light output and consequently eliminate light limitation.

In this study, red LEDs emitting light at 660 nm were chosen as light source based on the premise that red photons are most efficient in deriving photosynthesis and that they are weakly absorbed by water molecules, leading to minimal loss of light due to absorption [1].

In addition, this wavelength was chosen based on the fact that it falls within the range of maximum photon absorption peaks in chlorophyll *a* and *b* molecules, the main photosynthetic pigments in green algae, i.e. 663 and 645 nm; [1]. This would also facilitate optimal photon energy utilisation.

Therefore, the aim of this study was to determine the optimum irradiance required to illuminate microalgal culture to treat municipal wastewater.

2 Materials and methods

2.1 Bioreactor design

Internally-illuminated stirred-tank type photobioreactor (STPBR) made of transparent Plexiglas was used in this study. Illumination was provided radially from the centre of the STPBRs by LEDs (Kingbright, Taiwan) emitting red light at 660 nm characteristic wavelength. The STPBR was 30 cm deep and 30 cm in diameter, with a total volume of about 21 L. It has a central chamber for housing LEDs soldered onto matrix boards, here referred to as 'LED core'.

The LED core was designed and constructed separate from the central chamber for easy maintenance. The PBR has a light path of 11 cm and a surface-to-volume ratio of about 0.14 cm^{-1}. It was also designed to illuminate the PBR up to a maximum water depth of 28 cm and allow degassing head space of 2 cm. Three STPBRs (R1, R2 and R3) with different irradiance values were used in the study. A similar reactor operated in the dark (R0), served as a control. Power was supplied to the LEDs using AC-DC adaptors (RS Components, UK) while mixing was provided using an overhead mechanical stirrer (IKA, UK).

2.2 Experimental set-up

2.2.1 Irradiance

All the reactors were covered with aluminium foil to concentrate light in the PBRs and to isolate the control reactor maintain it in the dark. Irradiance was measured with LI-192 Underwater Quantum Sensor connected to LI-250 light meter (LI-COR Biosciences, USA). Light measurements were performed in air, distilled water, and microalgal culture, at varying distance beginning from the wall of the LED core to at least the maximum PBR light path length. The average irradiance values used to illuminate the PBRs are presented in Table 1.

Table 1: Average irradiance supplied to the reactors.

Reactor	Irradiance ($\mu mol.s^{-1}.m^{-2}$)	Relative category
R0	0	-
R1	429.9 ± 0.81	Low
R2	582.7 ± 3.16	Medium
R3	730.8 ± 1.52	High

2.2.2 Synthetic municipal wastewater and microalgal inoculum

Synthetic municipal wastewater [6], modified to mimic an anaerobically digested wastewater, was used in the study. It was autoclaved at 120°C for 15 minutes (Rodwell Scientific Instruments, UK) and allowed to cool at room temperature prior to its use in the experiment. The wastewater was inoculated with mixed

microalgal culture. The algal culture was obtained from a previous bench-scale laboratory experiment. It was centrifuged at 1000 g for 20 minutes at 22°C and maintained in 1 L Pyrex beaker under red LED illumination and agitated with a magnetic stirrer (Hanna Instruments, UK) prior to the experiments. The wastewater was prepared and inoculated in a big container from which 16 L was supplied to each reactor.

2.2.3 Reactor operation and monitoring

All the reactors were operated in batch mode with pH control at ambient condition and under continuous illumination [7, 8], for 30 days. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were appropriately used to control pH within the range of 7.0 to 8.5 in all the reactors and overhead mechanical stirrers operated at 60 ± 1 and 100 ± 1 revolution per minute, corresponding to presence and absence of CO_2 addition, respectively, were used to mix the content of the reactors.

Premixed industrial-grade gas composing of 10% CO_2, 6% O_2 and 84% N_2 (BOC Gas, UK) was used in the study. CO_2 addition into the reactors began on the 24th day of the experiment, corresponding to the lowest concentration of inorganic carbon in the mixed liquor. The gas was supplied continuously into each reactor through silicon tubing connected to rotameters (RS Components, UK) which were thence terminally connected to 0.5 mm pore gas spargers (Science Laboratory Supplies, UK). The CO_2 was supplied into each reactor at a flow rate of 100 mL per minute and lasted for 7 d. Samples were collected every 24 h for the first week and subsequently every 48 h for physico-chemical analyses.

2.3 Analytical tests

Samples collected from the reactors were chemically analysed for ammonium (NH_4-N); chemical oxygen demand (COD); total Kjeldahl nitrogen (TKN); anions: nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4^{3-}); and total carbon (TC). In addition, optical density (OD) and cell dry weight (CDW) of the mixed microalgal culture, pH, dissolved oxygen (DO) and temperature were also measured. COD and NH_4-N were, respectively, measured using commercial test kits (Merck, Germany) and a spectrophotometer (spectroquant, Merck, Germany) based on manufacturer's instructions and according to Standard Methods [9].

In addition, anions were determined using ion chromatography with ICS 1000 connected to conductivity detector and anion column (with eluent composing of 1 mM $NaHCO_3$ and 8 mM Na_2CO_3 at a flow rate of 1 mL min^{-1}) whereas TC was determined using automated total organic carbon analyser (Shimadzu, Japan). Dissolved oxygen (DO) and temperature were measured using a potable DO meter (VWR, UK) and pH was monitored using pH meter (Jenway, UK). Furthermore, microalgal CDW and OD were measured using gravimetry and UV-1700 spectrophotometer (Shimadzu, Japan), respectively. All samples for chemical analyses were filtered through 0.2 μm filters (Sartorius, UK) prior to measurements and analyses were performed in replicate except for pH.

3 Results and discussion

3.1 Light measurement

The study began with evaluation of light attenuation which might affect performance of the STPBRs (Fig. 1) since exposure of microalgal cells to sufficient light photons is essential for growth.

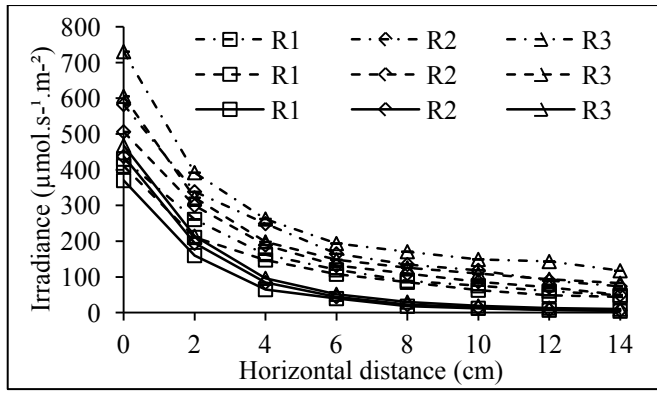

Figure 1: Irradiance versus horizontal distance in the STPBRs (dash dot, in air; dash, distilled water; continuous, 82 mg microalgal CDW L-1).

For all the media used in the light measurement, irradiance exponentially decreased with increasing horizontal distance in the STPBRs (Fig. 1). Interestingly, the irradiance on the wall of the LED core decreased with increasing media densities; having a highest value in air and the lowest in the algal culture. This suggests that the density of a medium primarily promotes the absorption of light photons independent of the distance from the source. The choice of the initial microalgal concentration of the experimental culture was based on the evaluation of the light attenuation with respect to the microalgal CDW shown in Fig. 1 and other CDW values (data not shown).

3.2 Specific growth rate, biomass productivity and optimum irradiance

Microalgal specific growth rates were calculated based on the variation of CDW with time, in natural logarithm, while biomass productivity was computed from CDWs as the amount of microalgal biomass produced daily. Both the growth rate and productivity increased with increasing irradiance up to 582.7 µmol s^{-1} m^{-2}, with subsequent decline up to the maximum irradiance.

Maximum specific growth rate and biomass productivity of 0.109 d^{-1} and 0.034 g L^{-1} d^{-1}, respectively, were achieved at medium irradiance. This suggests that the medium irradiance was optimum for microalgal growth under the experimental conditions. However, the relatively low values in both growth rate and biomass productivity might be due to inorganic carbon limitation observed

in the experimental reactors prior to CO_2 addition. This might have masked the effect of inorganic carbon supplementation on the microalgal culture.

Interestingly, lack of illumination has limited microalgal growth in the control reactor irrespective of the available organic carbon which may favour algal heterotrophic metabolism. This suggests the dominance of obligate photoautotrophs in the microalgal culture [10].

3.3 Wastewater treatment efficiency

Reactor performance with respect to wastewater treatment was evaluated in terms of COD and nutrient removal efficiencies. COD removal efficiency varied with time in all the reactors with maximum of about 82% recorded at high irradiance. This suggests that COD removal does not directly depend on irradiance. Interestingly, removal efficiency of up to 79% was also achieved in the control reactor, suggesting the presence of bacteria in the mixed microalgal culture as algae are not directly responsible for COD removal [11, 12].

Noteworthy, greater COD removal was achieved with CO_2 addition compared to operating the reactors without CO_2 addition. This apparently shows the direct effect of inorganic carbon supplementation on microalgal growth with consequent favourable condition for bacterial degradation of organic matter with the aid of photosynthetic oxygenation.

3.4 Nitrite accumulation

Nitrite concentrations were recorded in the illuminated reactors with maximum of up about 50 mg NO_2-N L^{-1} in the PBR operated at high irradiance. Interestingly, higher concentration of about 88 mg NO_2-N L^{-1} was recorded in the control reactor. The NO_2-N concentration consistently accumulated with time and appeared to be light-dependent as it decreased with increasing irradiance.

This suggests partial nitrification of NH_4-N in the mixed liquor. However, high concentration of nitrite have been reported of possibly posing toxic effects on aquatic organisms, especially under non-steady-state condition [13], which may usually be the case with systems operated in batch mode.

4 Conclusion

CO_2 addition to the mixed microalgal culture treating synthetic municipal wastewater resulted in about two-fold increase in biomass productivity in all the STPBRs with the reactor operated at medium irradiance exhibiting the highest specific growth rate and biomass productivity of 0.109 d^{-1} and 0.034 g L^{-1} d^{-1}, respectively.

However, inorganic carbon limitation observed in the experimental reactors prior to CO_2 addition might have limited the growth and productivity of the mixed microalgal culture which might have consequently affected wastewater treatment efficiency.

In addition, nitrite accumulation might have posed toxic effects on the microbial population and possibly masked the effect of inorganic carbon

supplementation on the microalgal growth. The findings in this study suggest that both microalgal growth rate and biomass productivity are not always directly proportional to irradiance. Furthermore, medium amount of irradiance resulted in optimum growth and productivity of the mixed microalgal culture.

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