Biological and Environmental Sciences Journal for the Tropics 19(3) December, 2022 ISSN 0794 – 9057; eISSN 2645 – 3142

BEST JOURNAL 19(3): 83 - 93
Date received: 07/01/2023
Date accepted: 14/01/2023
Printed in Nigeria

EFFECTS OF CO_2 ADDITION ON RED LED-ILLUMINATED MICROALGAL BIOREACTORS TREATING MUNICIPAL WASTEWATER

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ABSTRACT
Use of red light coupled with carbon dioxide (CO_2) addition can potentially enhance wastewater treatment efficiency in microalgal photobioreactors (PBRs). This study investigated the effects of red light-emitting diodes (LEDs) irradiance and CO_2 addition on mixed microalgal culture, treating municipal wastewater. Batch operation of CO_2-enriched bench-scale microalgal PBRs resulted in high treatment efficiency of municipal wastewater. The PBRs were operated at irradiance ranging from 25 to 234 µmol/s.m^2 at 12:12 light-dark cycles, treating municipal wastewater containing 83, 50 and 13 mg/L of soluble chemical oxygen demand (SCOD), ammonia, and phosphate, respectively, for 19 d. Up to 75% SCOD removal was achieved in a PBR operated at 182 µmol/s.m^2, with ammonia removal greater than 90%. However, high nitrite concentration in the bioreactors might have limited microbial growth, and affected the PBR performance. This study demonstrated the feasibility for achieving effective municipal wastewater treatment and carbon sequestration, at bench-scale.

Keywords*:* Bench-scale; Carbon capture; Light-emitting diodes; Mixed microalgal culture; Photobioreactors; Real municipal wastewater; Wastewater treatment efficiency

INTRODUCTION

Municipal wastewater has traditionally been treated using waste stabilisation ponds, activated sludge systems, trickling filters, etc. Some of these systems require energy which is usually generated through the combustion of fossil fuels. This consequently emits CO_2 into the atmosphere. However, stringent regulations on reducing carbon emissions (Department of Energy and Climate Change, 2009; Environment Agency, 2009; Xiao *et al*., 2022) coupled with escalating energy prices call for the need to develop energy-efficient wastewater treatment technologies.

Any treatment process that couples carbon capture with wastewater treatment can be considered as a sustainable option. Wastewater treatment using microalgae is gaining popularity because of its energy efficiency and higher carbon capture ability (Mohammed *et al.*, 2014; Abdelfattah *et al*., 2023). Microalgae use light, CO_2, nutrients and water to produce biomass through photosynthesis (Ludwig *et al.*, 1951; Oswald *et al.*, 1953; Humenik and Hanna-Jr, 1971; Masojidek *et al.*, 2004; Hsueh *et al.*, 2009; Aditya *et al*., 2022). However, insufficient carbon and illumination are the main parameters limiting microalgal photosynthesis and wastewater treatment efficiency.

In view of the above, this study investigated two key limiting parameters in microalgal cultivation, i.e., carbon and illumination, with a view to reduce carbon limitation through CO_2 addition, and minimise light limitation by using red LED as light source, and consequently exploring the effect of these factors on bacterial SCOD removal. Nutrients were not considered to be limiting parameters in this study since they are readily available in municipal wastewater (Humenik and Hanna-Jr, 1971; Yun *et al.*, 1997).

LED can potentially replace conventional light sources due to their inherent advantages (Mehta *et al.*, 2008). These include low power consumption, higher luminous output (Matthews *et al.*, 2009), low start-up time, easy control, monochromatic property, and long serviceability of up to 10 years (Mehta *et al.*, 2008).

Therefore, batch laboratory experiments were conducted in red LED-illuminated microalgal bioreactors at bench-scale to evaluate the effects of CO_2 addition and variation of irradiance on microbial growth rate, biomass productivity, and the efficiency of municipal wastewater treatment. The results of the study provided critical information for pilot-scale studies on municipal wastewater treatment using microalgal photobioreactors (Mohammed, 2013).

MATERIALS AND METHODS

Light Source and Light Measurement

Red LEDs with characteristic wavelength of 660 nm (Maplin Electronics, UK) were used as light source to illuminate bench-scale microalgal bioreactors. The LEDs were soldered onto Vero boards and powered by a variable AC-DC power supply (Maplin Electronics, UK), set at 9 V. The power supply to the LED light source was controlled by a mains timer, operated at 12:12 light-dark cycle (Lee and Lee, 2001; Jacob-Lopes *et al.*, 2009).

Four LEDs were connected in series, forming four arrays for each illuminated bioreactor except for R6/R12 with six arrays each (Table 1). The values of the irradiance, number of LED and clamping heights for all the bioreactors are shown in Table 1. Bioreactors R1 and R7 were operated in the dark, and served as control.

Table 1: Average values of irradiance measured at the culture surface of the bioreactors

Reactor	Clamping height (cm)	Number of LED	Irradiance (µmol/s.m^2)
R1; R7	n/a	0	0
R2; R8	30.6	16	25.29
R3; R9	28.0	16	64.47
R4; R10	23.0	16	113.87
R5; R11	15.5	16	181.19
R6; R12	16.0	24	234.30

n/a = not applicable

The Vero boards were clamped horizontally to illuminate the microalgal bioreactors at different clamping heights (Table 1) for the experimental bioreactors in order to achieve five different values of irradiance.

Microalgal Inoculum

Mixed microalgal culture of unidentified species was used to inoculate the bioreactors. The original microalgal culture was a mixture of algal strains collected from Cramlington Sewage Treatment Works, UK (55^0 5' 16" N, 1^0 35' 55" W). The mixed microalgal culture was grown in batch mode on Bold's Basal medium (Culture Collection of Algae and Protozoa, 2010) in a 20-L transparent polythene jars, prior to its use in this study.

The culture was illuminated with cool white fluorescent lamps (in a separate experiment from another study) at an average light intensity of 71 µmol/s.m^2, and aerated with compressed air at flow rate of 2 L/min, to serve as carbon source. After 7 d, the resulting microalgal culture was centrifuged at 1000 × g for 20 min at 22 °C using a centrifuge (Thermo-Fisher, Germany) to concentrate the algal biomass and separate it from the medium according to Standard Methods (APHA, 2005). The microalgal biomass was then re-suspended in filtered municipal wastewater whose characteristics are shown in Table 2.

Wastewater

Settled domestic wastewater was collected from the effluent line of the primary settling tanks at Sedgeletch Sewage Treatment Works, Houghton-le-Spring, UK (54^0 51' 4" N, 1^0 29' 43" W) in October and used as substrate in the experiments, without addition of any nutrient media. Prior to the experiments, the wastewater was centrifuged at 5300 × g, for 30 min, at 22 °C. The supernatant was filtered through 0.45 µm glass fibre filters (VWR, UK) to remove all suspended solids. The filtered wastewater (characteristics shown in Table 2) was used as growth medium with its natural ammonium content as sole source of nitrogen to the microalgae. It is notable that no nitrite was detected in the wastewater.

Table 2: Characteristics of filtered wastewater before and after inoculation with microalgal culture

	NH_4-N	COD	IC	OC	NO_3^-	PO_4^{3-}	DO	pH
					(mg/L)*			
a	45 (0.0)	83 (1.0)	60.0 (1.9)	52.4 (1.0)	0.27 (0.01)	11.8 (0.3)	4.22 (0.01)	7.76
b	49 (2.1)	83 (3.0)	59.6 (0.3)	54.0 (0.5)	0.32 (0.01)	12.6 (0.2)	4.22 (0.01)	7.50

* Except for pH; a: before inoculation; b: after inoculation with microalgal culture.
Key: Dissolved oxygen (DO), organic carbon (OC), inorganic carbon (IC) and chemical oxygen demand (COD)

Bioreactor set-up and operation

Two sets of microalgal bioreactors were set-up and operated with and without CO_2 addition, respectively. The experiments were conducted in bioreactors consisting of 1-L Pyrex beakers illuminated from the top with red LEDs. A total of 12 bioreactors (Table 1) were set up and monitored for 19 d.

Six bioreactors were supplied with premixed industrial-grade gas composing 10% CO_2, 6% O_2 and 84% N_2 and the other 6 were operated without CO_2 addition, but under natural supply of CO_2 from ambient air. Ten of the bioreactors were illuminated with red LEDs, with every pair of bioreactors having the same amount of irradiance, except for R1 and R7 which were operated in the dark.

All of the bioreactors were set up and operated concurrently in batch mode, with neither pH nor temperature control (at mean temperature of 22 ± 3 ºC), but having same initial biomass and substrate concentration. Aluminium foil was used to shield the bioreactors from external light, reflect stray LED irradiance back to the bioreactors.

The gas was supplied at constant flow rate via 6-outlet brass manifold connected to silicon tubes. The silicon tubes connected the manifold to 0.5 mm pore gas spargers (SUPA Aquatic Supplies Ltd, UK) inside the bioreactors. A control valve at each of the manifold outlets and a rotameter (Key Instruments, Trevose, PA) were used to regulate and set the gas flow rate at 40 $mL.min^{-1}$.

In addition, each bioreactor was equipped with a magnetic stirrer (Hanna Instruments, UK) set at approximately 100 rpm to continuously agitate the culture. The gas was supplied to the bioreactors concomitant with illumination.

Analytical tests

About 60 mL of mixed liquor was withdrawn after every 48 hours from each of the bioreactors for routine analyses. The wastewater was filtered through a 0.2 µm filter (Sartorius, UK) prior to chemical analyses, and all measurements were carried out in triplicate. The wastewater was not supplemented with any nutrient media in order to test the treatability of the wastewater in its natural state. In addition, the culture was not adapted to high CO_2 concentration prior to these experiments (Yun *et al.*, 1997).

Samples collected from the bioreactors were analysed for the following parameters: NH_4-N, SCOD, DO; anions: nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}). In addition, total suspended solids (expressed as cell dry weight, CDW); optical density (OD, measured at 560 nm wavelength), pH and temperature were also monitored.

SCOD and ammonia were measured with commercial test kits (Merck, Germany) according to phenate method (APHA, 2005), based on the manufacturer's instructions. Both SCOD and NH_4-N removal efficiencies were calculated using Equation 1.

$$\eta_r = \left(\frac{(C_0 - C_t)}{C_0}\right) * 100 \qquad (1)$$

Where: η_r is the percentage removal efficiency; C_0 and C_t are initial and final values of SCOD and NH_4-N concentrations, respectively.

Anions were measured using ion chromatography system with chemical suppression of eluent conductivity (APHA, 2005) by ICS-1000 Ion Chromatography System (Dionex, USA) equipped with AS40 Automated Sampler and an Ionpac AS14A, 4 x 250mm analytical column (Dionex, USA). The eluent containing 1 mM $NaHCO_3$ and 8 mM Na_2CO_3 solution was injected at a flow rate of 1 mL/min, with sample injection loop of 25 µL. DO and temperature were measured with a multi parameter probe connected to a potable DO meter (DO200; VWR International, UK) whereas pH was measured using a pH meter (Jenway, England).

Microbial growth rates were determined using Equation 2 from the line of best fit at the exponential growth phase of the plot of natural logarithm of CDW values against time of cultivation. CDW was determined from the results of total suspended solids and volatile suspended solids (APHA, 2005). The CDW values were correlated with corresponding OD values in Equations 3 and 4, for bioreactors with and without CO_2 addition, respectively (with Pearson's correlation coefficient shown in parentheses).

$$\mu_{max} = \frac{1}{t_2 - t_1} \ln\left(\frac{CDW_{t2}}{CDW_{t1}}\right) \qquad (2)$$

$$CDW_{CO_2} = 0.5535 OD_{560;\, CO_2} - 0.0469 \text{ (R = 0.99)} \qquad (3)$$

$$CDW_n = 0.9982 OD_{560;\, n} - 0.0017 \text{ (R = 1.00)} \qquad (4)$$

In Equations 2, 3 and 4, μ_{max} is the maximum specific growth rate; CDW_{t1} and CDW_{t2} are the cell dry weights in g/L corresponding to the initial and final times of cultivation; t_1 and t_2, respectively. The subscripts CO_2 denotes added CO_2, n denote natural surface aeration with ambient air, and *560* the wavelength at which the OD was measured.

The OD of the algal culture was measured with UV-1700 Phamarspec spectrophotometer (Shimadzu, Japan) at 560 nm wavelength according to Wang *et al.* (2007) with distilled water as reference, using transparent plastic cuvettes (10 mm path length; VWR, UK). Whenever the OD reading on the spectrophotometer exceeded 0.5, a dilution with distilled water was applied to the sample and the result multiplied with the appropriate dilution factor.

Light Measurement

Irradiance was measured using LI-192 underwater quantum sensor connected to LI-250 light meter (LI-COR Biosciences, USA). Light measurements were performed with the light sensor placed internally at the centre of the bioreactor (for the culture and distilled water media) with the sensor placed in the direction of illumination (Figure 1).

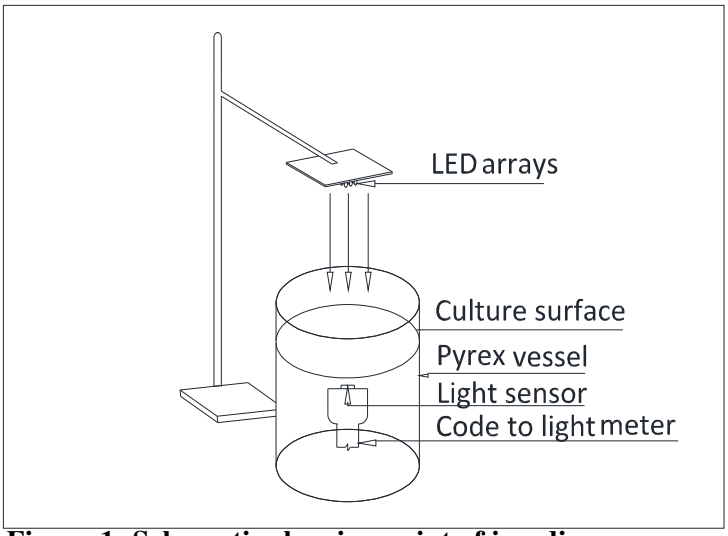

Figure 1: Schematic showing point of irradiance measurement

RESULTS AND DISCUSSION

Light Attenuation

Figure 2 shows the results of the irradiance measurements carried out in bioreactors with and without aluminium foil cover: in air; filled with either deionised water or with mixed microalgal culture. Higher irradiance values were obtained when the bioreactors were covered with aluminium foil than when they were uncovered due to reflection of stray LED light back into the culture (Figure 2).

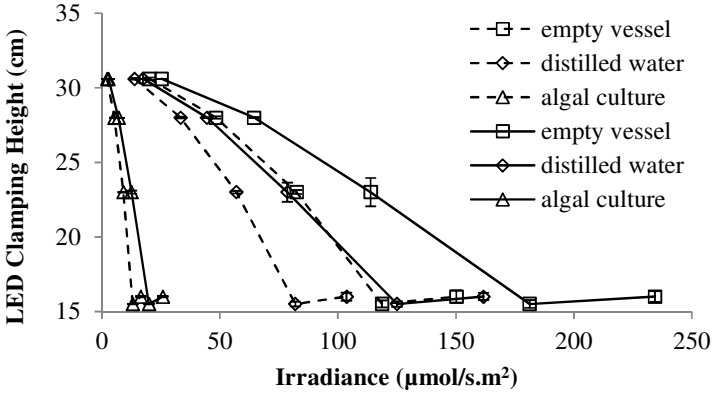

Figure 2: Irradiance versus height of LED clamping (solid lines: reactors wrapped with aluminium foil; dashed lines: reactors without aluminium foil)

As expected, the sensor recorded the least amounts of irradiance when the bioreactor vessels were filled with microalgal culture, without foil cover (i.e., 2.78 µmol/s.m^2 at 30.6 cm; Figure 2), suggesting light attenuation by the suspended microalgal biomass; the amount of irradiance decreasing with increase in distance from the light source (i.e., LED clamping height. On the one hand, an amount of irradiance of about 25.8 µmol/s.m^2 was recorded in the algal culture at 16 cm clamping height when the bioreactors were covered with aluminium foil (Figure 2), and the corresponding irradiance recorded in the bioreactors without aluminium foil cover was 16.52 µmol/s.m^2.

This suggests that to achieve a desired minimum irradiance of at least 25 µmol/s.m^2 (which was used in the pair of bioreactors with the least amount of irradiance in the main experiments; Table 1), the LED had to be clamped no higher than 16 cm above the culture and the bioreactors had to be operated with aluminium foil cover. This light measurement experiment provided data for selecting the LED clamping heights to achieve the desired irradiance values required for the main experiments. Furthermore, the maximum achievable irradiance was identified for the lowest practical height above the bioreactors.

It is important to note that the two points that appear horizontal (Figure 2) are for 15.5 and 16.0 cm clamping heights, with the latter height having greater irradiance value of 234.3 µmol/s.m^2 than the former with irradiance a value of 181.2 µmol/s.m^2. This is because 24 LEDs were clamped at 16.0 cm, compared to 15.5 cm and other clamping heights (i.e., 23 and 30.6 cm) which had 16 LEDs each.

Biomass productivity and maximum specific growth rates

Figure 3 shows variation of microbial cell dry weight (CDW) with time. Highest CDW values were obtained in the bioreactors illuminated with highest amount of irradiance.

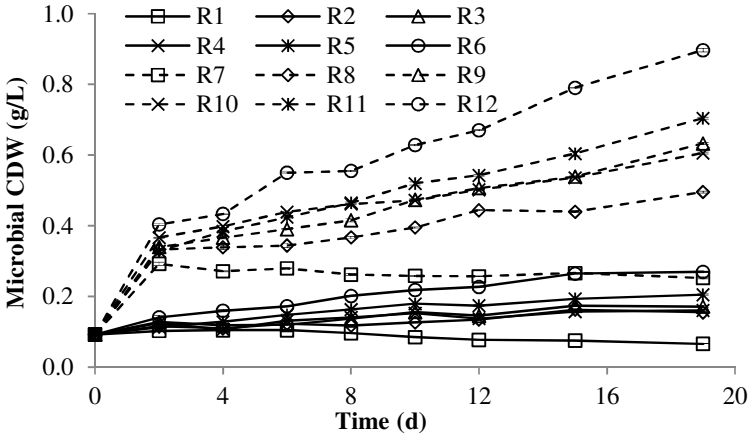

Figure 3: Time courses of microbial CDW (continuous lines, with CO_2 addition; dashed lines, without CO_2 addition)

The cell density of the mixed microbial culture increased with the time of cultivation, and the increase in the CDW was greater in bioreactors without CO_2 addition than in those with CO_2 addition (Figure 3). This was unexpected as CO_2 is known to be a growth-limiting substrate for phototrophic organisms. Surprisingly, the CDW value on day 15 for the control reactor with neither CO_2 addition nor illumination was almost the same as that for CO_2-enriched bioreactor with the highest irradiance. This suggests that bacteria (growing at faster rate than microalgae) have contributed in microbial biomass production in the control bioreactors.

The growth trend in the CO_2-enriched bioreactors may possibly be explained based on the presence of microalgal species that were intolerant to high CO_2 concentration considering that the 10% CO_2 concentration used in the experimental bioreactors is 250-fold higher than that in ambient air (i.e., 0.04% CO_2).

This might have stressed the microalgae, leading to prolonged acclimation period in the bioreactors with CO_2 addition. Conversely, low CO_2 concentration in the other bioreactors might have favoured growth of microalgal strains adapted to natural levels of CO_2, resulting in higher biomass productivity than in the bioreactors with CO_2 addition.

Additionally, the culture in the bioreactors without CO_2 addition did not appear to require any acclimation period, as the cultivation condition is similar to the conditions of the inoculum before the start of these experiments. The rapid increase in CDW within the first 2 d (Figure 3) in the bioreactors without CO_2 addition supports this argument, or the rapid increase in the CDW possibly resulted from increase in bacterial population. The absence of an acclimation period clearly favoured these bioreactors over those with CO_2 addition, such that early stress caused by high CO_2 concentration probably reduced the microalgal growth in the CO_2-enriched bioreactors. Moreover, the low biomass productivity recorded in the CO_2-enriched reactors might be due to the low cell density of the starting biomass of the culture (Perez-Garcia *et al.*, 2011), coupled with intolerance of high CO_2 concentration (Hanagata *et al.* 1992; Sung *et al.*, 1999).

The microbial growth rates in the experimental bioreactors generally increased with increasing irradiance up to irradiance values of 114 (R4 and R10) to 181 µmol/s.m^2 (R5 and R11; Figure 4). Interestingly, the growth rates corresponding to such irradiance values are higher in the CO_2-enriched bioreactors than in those without CO_2 addition, indicating bacterial growth. However, the maximum value obtained in this study (i.e. 0.06 per day; Figure 4) is much lower than the value of 0.39 per day reported by Termini *et al.* (2011), achieved in microalgal culture predominated by *Scenedesmus* sp., treating settled municipal wastewater. Nevertheless, bacteria might have dominated microalgae in the mixed culture used in the current study.

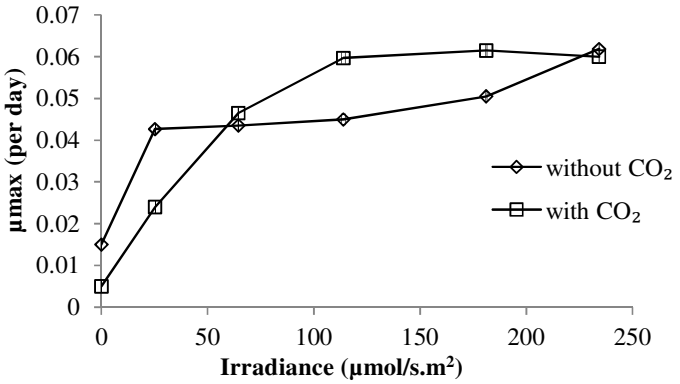

Figure 4: Variation of maximum specific growth rates with red LED irradiance for all of the bioreactors

Conversely, the maximum CDW values corresponding to maximum irradiance obtained in this study (i.e. ≈ 0.3 and 0.9 g/L for bioreactors with and without CO_2 addition, respectively) are much higher than the CDW values reported in another study (Wang *et al.* (2007) cultivated under red LED irradiance of 300 µmol/s.m^2. Interestingly, the maximum values obtained here for the CO_2-enriched bioreactors and those without CO_2 addition are, respectively, about 8- and 23-fold higher than the CDW reported in Wang *et al.* (2007) for almost similar amounts of irradiance.

However, the value of red LED irradiance corresponding to the maximum growth rate reported by Wang *et al.* (2007) was 3000 µmol/s.m^2, which is about 13-fold higher than the maximum irradiance used in the current study. Therefore, it may be concluded that even the highest amount of irradiance used in this study might still have limited the productivity of the mixed microbial culture, thereby resulting in very low growth rates. However, Wang *et al.* (2007) and Termini *et al.* (2011) used isolated microalgal strains which would have had, if any, minimal competition between microbial species in their cultivation systems. In addition, Wang *et al.* (2007) cultivated their alga solely on Zarrouk (nutrient) medium containing, among other chemical constituents, about 583 mg/L of HCO_3^- and 729 mg/L of NO_3^-. Therefore, direct comparisons of growth rates are difficult to make.

Wastewater Treatment Efficiency

The wastewater treatment efficiency in the current study was evaluated in terms of ammonium and SCOD removal obtained in reactors operated at different conditions.

Ammonium removal

Figure 5 shows the variation of the maximum ammonium removal efficiency with irradiance in all of the bioreactors. Each point in Figure 5 represents the highest removal efficiency achieved in each bioreactor.

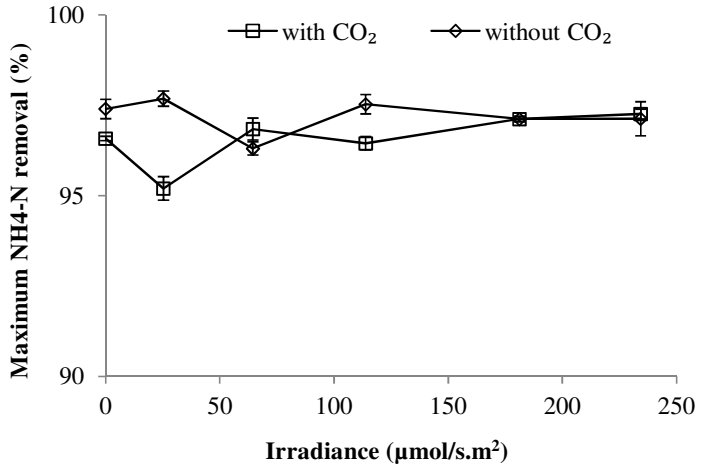

Figure 5: Variation of ammonium removal efficiency with red LED irradiance

Ammonium removal efficiencies greater than 90% were achieved in all of the experimental bioreactors. Up to an irradiance value of 50 µmol/s.m^2, CO_2 addition did not appear to have enhanced the ammonium removal efficiency (Figure 5). The ammonium removal efficiency achieved in the CO_2-enriched bioreactors exceeded that of the bioreactors without CO_2 addition only at irradiance value of 64.5 µmol/s.m^2. From 181.2 µmol/s.m^2 onwards, ammonium removal appears to be independent of both CO_2 addition and illumination. The ammonia removal efficiencies found in this study are in tandem with values reported in the literature in both photobioreactors and conventional algal ponds (Park and Craggs, 2010; Silva-Benavides and Torzillo, 2012).

SCOD removal

In contrast to NH_4-N removal, the SCOD removal efficiency increased with increasing irradiance irrespective of CO_2 addition (Figure 6). Similarly, each point in Figure 6 represents the highest SCOD removal achieved in each bioreactor.

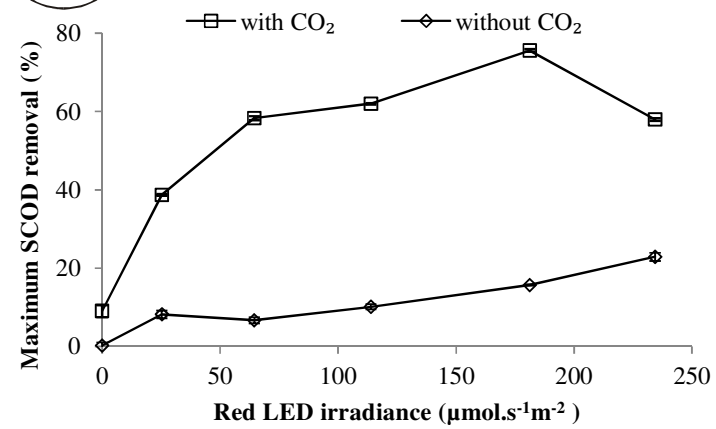

Figure 6: SCOD removal efficiency versus red LED irradiance in the bioreactors

Maximum removal efficiency greater than 75% was achieved in the bioreactors with CO_2 addition. This value is not far from 89% SCOD removal in other studies (Hammouda *et al.,* 1995; Nguyen *et al*., 2020) in a domestic wastewater treatment system dominated by *Chlorella vulgaris* and *Scenedesmus* sp. operated in batch mode, illuminated with fluorescent lamps and supplemented with nutrients. Much higher SCOD removal was observed in the bioreactors with CO_2 addition than in those without CO_2 addition; however, it is not clear whether this was due to the inorganic carbon supplementation. Furthermore, it may be concluded that 'optimum' SCOD removal was achieved at an irradiance value of 181.2 µmol/s.m^2 which is lower than the maximum value used in this study.

Interestingly, SCOD removal efficiency of about 10% and less than 1% were recorded in the control bioreactors with and without CO_2 addition, (in R1 and R7, respectively). This observation contrasts the ammonium removal results in which almost identical values were obtained in the control bioreactors. The low level of SCOD removal observed in the control bioreactors may be due to presence of microalgal species such as *C. vulgaris*, (and some bacteria), that can grow heterotrophically in the dark (Ogbonna *et al.*, 2001; Perez-Garcia *et al.*, 2011) and consume SCOD substrates.

CONCLUSION

This study demonstrated the feasibility of using red LED to treat municipal wastewater under low light intensity, at bench-scale. In addition, coupling carbon sequestration with wastewater treatment resulted in enhanced microbial growth rates, high NH_4-N and SCOD removal efficiencies. Maximum ammonia removal and specific growth rate were achieved at an *optimum* low irradiance value of 181.2 µmol/s.m^2. The need for upscaling warranted further investigation at pilot-scale, under high light intensity.

ACKNOWLEDGEMENT

The authors are grateful to the Petroleum Technology Development Fund, Abuja, Nigeria for awarding PhD Scholarship to the corresponding author, and to Mr Rob Hunter of the School of Engineering, Newcastle University, UK, for constructing the LED arrays.

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