The impact of light intensity and wavelength on the performance of algal-bacterial culture treating domestic wastewater

Keerthi Katam[1]*, *Rishika* Ananthula[2], *Sushmitha* Anumala[2], *Malinee* Sriariyanun[3], *and Debraj* Bhattacharyya[2]

[1]Department of Civil Engineering, École Centrale School of Engineering, Mahindra University, Telangana, 500043, India
[2]Department of Civil Engineering, Indian Institute of Technology Hyderabad, Kandi, Telangana, 502285, India
[3]Biorefinery and Process Automation Engineering Center, Chemical and Process Engineering, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, 10800, Thailand

Abstract. Light is the main energy source for microalgae, and the intensity and wavelength of light influence cell metabolism and biomass composition, which, in turn, affects wastewater treatment. The objective of this study is to examine how different light intensities and light wavelengths affect the growth of mixed algal-bacterial culture while treating sewage. Three different light intensities (100, 200, and 300 µmol/m2-s) of four different light wavelengths (blue, red, white, and yellow) were selected for this study. The dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved phosphorus (DP) in influent and effluent samples were measured, along with chlorophyll content in the biomass. The highest chlorophyll concentration of 3.5 mg/L was observed at 100 µmol/m2-s intensity of red light. The concentration of chlorophyll decreased as light intensity increased, with exception of white light. The highest DOC removal of 84% was observed at 300 µmol/m2-s intensity of blue light whereas the highest DN (51%) and DP (80%) removal was observed with a red light intensity of 100 µmol/m2-s. Overall, blue light with an intensity of 300 µmol/m2-s and red light with an intensity of 100 µmol/m2-s were found to be the most efficient at removing carbon and nutrients. The results suggested that the color and intensity of light influence algal-bacterial growth and wastewater treatment efficiency.

Keyword. Artificial light, Carbon removal, Chlorophyll, Nutrient removal

1 Introduction

The application of algae in wastewater treatment has been recognized as a sustainable approach for simultaneous treatment and useful biomass production in the last couple of decades [1,2]. Despite the known benefits, algae have only been employed in wastewater treatment as a polishing unit in algal ponds. One of the most challenging aspects of using microalgae for commercial applications is determining the best conditions for large-scale biomass production. The design of the cultivation system has a significant effect on microalgal growth in wastewater treatment. The large-scale algal reactor configurations mainly include open suspended-growth bioreactors, closed suspended-growth photobioreactors, and biofilm or attached growth photobioreactors. Open algal systems use solar energy to grow and have a low energy cost but it has drawbacks in regulating operating parameters, such as light intensity or temperature, thereby, impacting efficient algal cultivation [3–6]. Due to a lack of natural light on rainy days and too much light in the summer, open algal wastewater treatment systems are inefficient in nutrient conversion. Light is one of the important factors for microalgal photosynthesis, both light wavelength and intensity have effect on algal growth and lipid accumulation.

Microalgae require light to synthesize essential molecules, including Adenosine triphosphate and Nicotinamide Adenine Dinucleotide Phosphate Hydrogen [7]. These reactions take place in photosystems in microalgae, and light energy is absorbed by chlorophyll pigments and carotenoids that make up a photosystem antenna complex. The light energy is absorbed and delivered to the reaction center, where it is transformed into chemical energy for photosynthesis [8]. Chlorophylls are magnesium-tetrapyrrole molecules that are crucial to the charge-separation processes that take place in reaction centers and in the light-harvesting complexes, which capture light energy [9,10]. The light reaction and the dark reaction are the two processes of photosynthesis. The two photoreactions in the light reaction are photosystem I and photosystem II, which utilize photon energy associated with two different wavelengths (700 nm and 680 nm) [11]. Chlorophyll-a is the major pigment in algal cells, while chlorophylls b, c, d, and f are minor pigments. The primary electron donor in the reaction centers of photosystems I and II is chlorophyll-a [9]. The Calvin cycle, also known as the dark reaction, is the component of the light reaction in which the energy acquired is used to produce glucose.

* Corresponding author: keerthi.katam@mahindrauniversity.edu.in

Microalgae use light with wavelengths ranging from 400 to 700 nm for photosynthesis. Depending on the species, microalgae absorb different wavelengths. In green microalgae, for example, chlorophylls, a primary pigment that absorbs light in the 450–475 nm and 630–675 nm wavelength ranges, and carotenoids, an accessory pigment that absorbs light in the 400–550 nm wavelength range [12,13]. Red light (600–700 nm) and blue light (400–500 nm) stimulate microalgae; however, their growth rates and lipid content fluctuate with light intensity [14]. The growth is accelerated by increasing light intensity up to a point, which varies based on the microalgal species [15]. Photo-inhibition can be caused by high light intensity beyond the saturation point [16].

Therefore, closed systems with artificial lights are preferred for optimizing algae growing conditions for biofuel production and nitrogen removal in wastewater treatment facilities because operating variables, such as light conditions and intensities can be controlled [17–20]. In a closed suspended or attached cultivation system, artificial lights are used to provide constant light intensity for the algal culture. Artificial light, especially light-emitting diodes (LEDs), can be an attractive way for effective algal cultivation. LEDs are now the most efficient artificial light sources, with a photosynthetically active radiation (PAR) efficiency of 80-100 percent. LEDs provide color combinations with wavelengths that correspond to blue, green, yellow, orange, red, and other light, as well as a high intensity that closely resembles the spectrum of sunlight [15,21,22]. The advantages include low power consumption, a wide range of intensity and a small size. Considering the high costs of fluorescent lights, LED light sources are a more cost-effective choice because of their lower operating costs, longer lifespan, and spectrum benefits.

The light intensity ranging between 26 - 400 µmol/m^2-s is the ideal light intensity for microalgae growth. The activation of lipid synthesis is triggered by an increase in light intensity. For maximum lipid productivity, microalgal species and strains require flux between 60 and 700 µmol/m^2-s [14]. The effect of light intensity and photoperiod on the algal activity is species-dependent; the optimal light intensity for photosynthesis is 200 µmol/m^2-s (~ 8700 lux). Some algal species like *Selenastrum minutum* and *Scenedesmus obliquus* can grow at light intensities of 400–500 µmol/m2-s [7,23]. In real wastewater treatment plants, most of the algal species interact with the bacterial culture present in the wastewater. In mixed algal and bacterial culture systems, ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are more sensitive to light than algae [24,25]. The exposure to 75 µmol/m^2-s under 12:12 h light/dark conditions inhibited both AOB and NOB [24]. The photo-inhibition of AOB and NOB can affect N removal during wastewater treatment in algal-bacterial systems, especially under high light conditions. Although several studies have examined the effect of distinct wavelengths of light (blue, green, red, and white) and their effect on algae growth, lipid accumulation and oxygen production [15,26–28], limited research has been done on understanding the effects of light on algal-bacterial co-culture systems [29,30]. The objective of this study is to examine the response of algal-bacterial consortium to different LED light wavelengths and intensities in terms of chlorophyll and the removal of carbon and nutrients from wastewater.

2 Materials and methods2.1 Materials and Methodology

2.1 Collection of seed culture and wastewater

The algal culture was collected from Mehboob Sagar lake, Hyderabad, Telangana, India. The obtained culture was centrifuged to remove the debris and was cultivated in the algal medium for about 15 days. The culture was examined under a microscope after 15 days and it was observed that *Chlorella sp*., *Scenedesmus sp*., and pennate diatoms were the dominant species in the biomass. *Chlorella sp*. and *Scenedesmus sp*., are green algal species.

The aerobic bacterial culture was collected from a decentralized domestic wastewater treatment plant in Hyderabad, Telangana, India. The obtained biomass was kept in continuous aeration until further use. The wastewater was collected on a daily basis from the student dorms (about 3000 students) and dining hall disposal point at the Indian Institute of Technology Hyderabad, Telangana, India. The sterilization of wastewater was not performed in order to use real wastewater for the study.

The characteristics of the domestic wastewater are; pH of about 7.0 ± 0.4 The dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved phosphorus (DP) concentrations were 65.8 ± 8.09, 25.7 ± 5.2, and 4.3 ± 0.6 mg/L respectively. The carbon and nutrient values were observed to be on the lower side of typical domestic wastewater [31]. The C: N: P of the wastewater is 15:6:1 which is less than the optimal C: N: P molar ratio (56:9:1) suitable for algal growth [32]. The optimum N/P value for significant nutrient removal from domestic wastewater ranges from 5:1 to 30:1 [33,34].

2.2 Experimental design

Four different light wavelengths were used in this experiment as an illumination source. These were blue (450 – 460 nm), red (640 – 670 nm), yellow (570 – 590 nm), and white (400 – 700 nm). Three different levels of intensities (100, 200, and 300 µmol/m2-s) for each wavelength were tested. Artificial lighting using LED light strips was provided. The transparent glass bottles with a wide opening of 500 mL volume capacity were used as test reactors. LED light strips were installed around the outer perimeter of the reactor. To avoid the intrusion of external light, the test reactors were covered with aluminium foil and the opening was closed with cotton. In total, twelve reactors were set up as shown in Figure 1 (a). The experimental design and naming for each test reactor have been given in Table 1. The three light intensities of 100, 200, and 300 µmol/m^2-s were named as I1, I2, and I3, respectively. The test reactors that were illuminated to blue wavelengths with

intensities of I1, I2, and I3 were given the names B1, B2, and B3, respectively. Similarly, the red wavelengths I1, I2, and I3 were given the names R1, R2, and R3, respectively. Yellow wavelengths with intensity I1, I2 and I3 are represented by Y1, Y2 and Y3, whereas white wavelengths with intensities I1, I2 and I3 are represented by W1, W2 and W3 respectively. Figure 1 (b) shows the light intensity of 300 µmol/m^2-s for all wavelengths in empty test reactors. The reactors were filled with an equal proportion of algae and bacteria at the beginning of the experiment. The initial cell density in all of the test reactors was the same (volatile suspended solids = 1500 mg/L). The test reactors were kept on a magnetic stirrer and were continuously mixed at 100 rpm for 7 days with a 12/12 h light-dark cycle. The experiment started with the light phase and then continued with the dark and light cycles. 2-mL mixed culture samples were collected from the test reactors every four hours for the first 24 hours and then for every 12 hours for the next six days. The supernatant was collected after centrifuging the samples for 20 minutes at 5,000 rpm. The chlorophyll concentration of the separated biomass was determined, and the supernatant was filtered through 0.45-m membrane filter paper and examined for dissolved carbon and nutrient concentrations.

Figure 1. Experimental set-up

Table 1. Experiment design of the study

Light color	Light intensity (µmol/m^2-s)			Temperature (°C)	
	I1 (100)	I2 (200)	I3 (300)	Light period	Dark period
Blue	B1	B2	B3	34 – 35	26 – 28
Red	R1	R2	R3	38 – 39	26 – 28
Yellow	Y1	Y2	Y3	36 – 37	26 – 28
White	W1	W2	W3	34 – 35	26 – 28

2.3 Analytical methods

The filtered supernatant samples were analyzed for total dissolved organic carbon (DOC), dissolved phosphorus (DP), and total dissolved nitrogen (DN). Total dissolved organic carbon (DOC) and nitrogen (DN) were measured using a TOC-L analyzer (Make: Shimadzu). The DP was measured using method 4500 P-C described in the Standard Methods for the Examination of Water and Wastewater [35]. The 10200 H method in Standard Methods for the Examination of Water and Wastewater was used to determine the chlorophyll concentration in the obtained biomass sample [35].

The removal efficiency was computed by deducting the final concentration from the initial concentration and dividing the result by the initial concentration. The obtained decimal number was multiplied by 100 to convert it to a percentage. The correlation matrix for five parameters – intensity, chlorophyll (Chl), DOC removal (%), DN removal (%), and DP removal (%) for different wavelengths was plotted using Excel Statistical toolpak.

3 Results and discussion

3.1 Chlorophyll concentration

Chlorophyll is an important pigment that converts light energy into chemical energy during the photosynthetic process by producing organic molecules. Chlorophylls effectively absorb light in the red and blue spectral regions. Each chlorophyll also has its unique absorption spectrum. Algal cells have chlorophyll-a as the primary pigment and other chlorophylls (b, c, d, and f) as minor pigments. The seed culture used in this study was a mixed algal culture with a, b, and c pigments. The total chlorophyll concentration (Chl_a + Chl_b + Chl_c) for various wavelengths at different light intensities is shown in Figure 2. It was observed that the chlorophyll concentration reached its peak value within 12 hours of the experiment and then started decreasing.

At light intensity I1, the maximum chlorophyll concentration, 3.5 mg/L was observed in red light spectra. The next highest chlorophyll concentration was observed in B1 and there was not much difference in W1 and Y1. The order of chlorophyll content at I1 light intensity is red > blue > white > yellow. At light intensity I2, it was observed that the chlorophyll concentration was higher in white and yellow wavelengths. A similar pattern was observed at I3 where the highest chlorophyll concentration was observed in yellow and white wavelengths and lower concentration was observed at B3 and R3. The sequence of chlorophyll at I2 and I3 is White = Yellow > Blue > Red.

The effect of light intensity for blue, red, white and yellow wavelengths is shown in Figure 3. The chlorophyll concentrations have been shown to be affected differently by varying the light intensity for different wavelengths. In blue and red-light illumination reactors, the low-light intensity (100 µmol/m2-s) test

reactors showed higher chlorophyll concentrations compared to the high-light conditions (B1> B2> B3; R1> R2> R3). Whereas, high- light intensity test reactors had higher chlorophyll concentrations than low light intensity test reactors in both yellow and white light wavelength reactors. The maximum chlorophyll concentration was observed at red light wavelength with low intensity. The next maximum concentration was observed in yellow wavelength. Red and yellow light, each has longer wavelengths, can be used to avoid photoinhibition. Shorter wavelengths of blue light can be more energetic and efficient for photosynthesis, while causing photoinhibition at higher intensities.

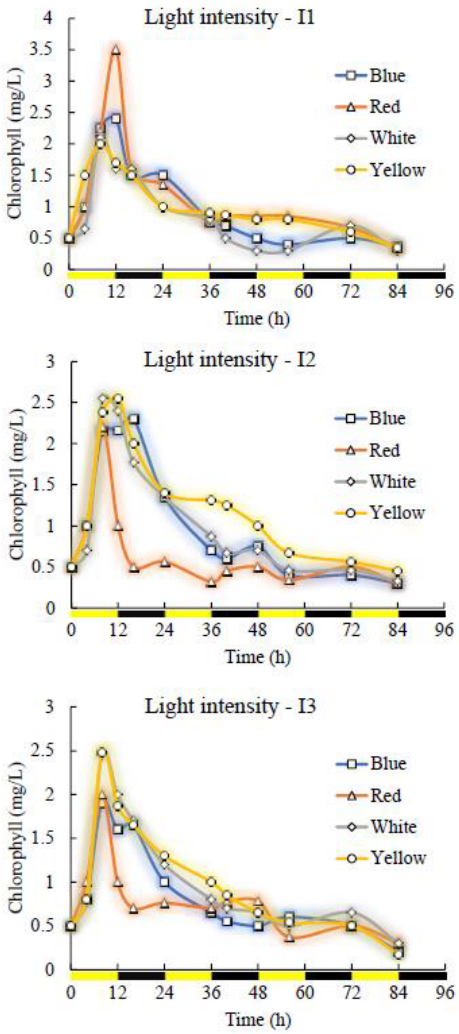

Figure 2. Total chlorophyll concentration for various wavelengths at different light intensities

The biomass production in R2 and R3 is lower than in other wavelengths due to the extensively absorbed red light, which reduces light utilization efficiency in algae [36]. The average chlorophyll concentrations were detected in white light because the emission spectrum band of white light completely covers that of red light, blue light and other growth-inefficient light wavelengths.

3.2 Carbon and nutrient removal

The average dissolved carbon removal at different wavelengths and intensities is shown in Figure 4. At I1 light intensity, the DOC removal at different light colors decreased in the order red > yellow > white > blue. The DOC removal decreased in order of blue> red > white > yellow at I2 and I3 light intensity. The highest DOC removal of 84% was observed in blue illumination test reactors (B2 and B3). The DOC removal increased with the increase in the intensity of blue light. The removal was more or less constant in red and white wavelengths whereas the removal efficiency decreased with an increase in intensity for yellow wavelength. The lowest

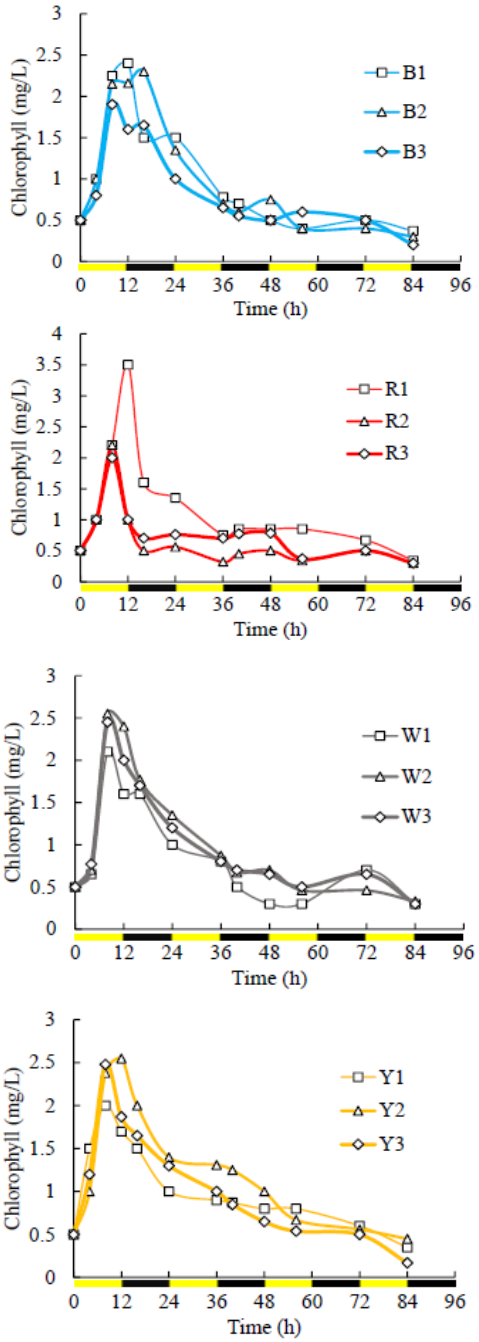

Figure 3. Effect of intensity on chlorophyll concentration

DOC removal of 44% was observed in B1. Overall, the order of DOC removal is B3 > B2 > R1 > Y1 ≥ R2 ≥ R3 > W1 > W2 > W3 > Y2 >Y3 > B1 at different intensities. The chlorophyll concentration and the organic carbon removal had not shown any relation indicating that a major portion of carbon was removed by the bacterial culture.

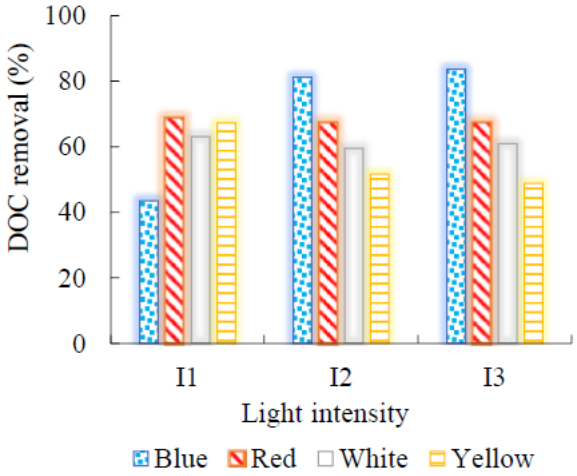

Figure 4. Dissolved carbon removal at different wavelengths and intensities

The average dissolved nitrogen removal at different wavelengths and intensities is shown in Figure 5. At I1 light intensity, the DN removal at different light colors decreased in the order red > blue > white > yellow. The DN removal decreased in order of yellow > red > white > blue at I2 light intensity. At I3 light intensity, the DN removal is in the order yellow > white > red > blue. The highest DN removal of 52% was observed in the red light test reactor (R1). The lowest was observed in B2 with 32%. The overall DN removal at different intensities is R1 > Y3> W3 > R3 > Y2 > B3 > R2 > B1 > W1 > W2 > Y1 > B2.

The average dissolved phosphorous removal at different wavelengths and intensities is shown in Figure 6. At I1 light intensity, the DP removal at different light colors decreased in the order red > yellow > white > blue. The DP removal decreased in order of yellow > red > white > blue at I2 light intensity. At I3 light intensity, the DP removal is in the order yellow > red > white > blue. The highest DP removal of 81% was observed in yellow wavelength test reactors (Y1, Y2, Y3) and white wavelength W2. The lowest was observed in B1 with 64% removal. The overall DP removal is Y3> Y2> Y1> W3> R1> R2> R3> W2> B3> B2> W1> B1. The low C/N/P ratio might be one of the reasons for low nutrient removal in test reactors. The higher chlorophyll concentration in red light and higher nutrient removal in R1 indicates that the algae had played role in removing nutrients from the wastewater.

During their cell and life cycles, algae undergo morphologic changes that are influenced by light quality and nutrition availability. In general, microalgae consume more nitrates when exposed to blue light than when exposed to red light. At this wavelength, nitrate and phosphate reductases are activated, leading to increased absorption of these nutrients [37,38]. However, in this study red and yellow wavelength has shown higher nutrient removal.

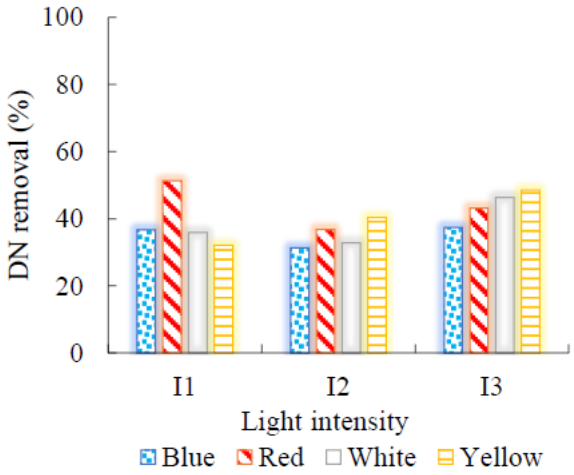

Figure 5. Dissolved phosphorus removal at different wavelengths and intensities

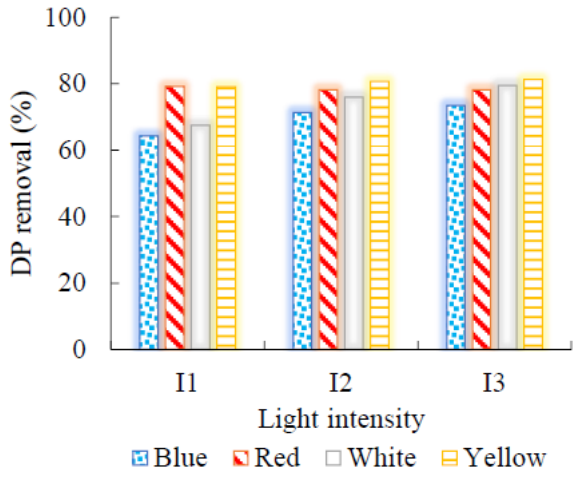

Figure 6. Dissolved phosphorus removal at different wavelengths and intensities

The correlation matrix for five parameters – intensity, chlorophyll (Chl), DOC removal (%), DN removal (%), and DP removal (%) for different wavelengths are given in Table 2. It was observed that each wavelength responded differently with intensity. In blue wavelength, it was observed that the intensity and chlorophyll concentration are negatively related, whereas it is strongly correlated to the carbon and nutrient removal. The chlorophyll concentration has a negative correlation with carbon and nutrient removal. In red wavelength reactors, it was observed that the intensity has a negative correlation with all the other parameters - algal growth, carbon and nutrient removal. The chlorophyll concentration has a strong correlation with carbon and nutrient removal. In white wavelength, it was

observed that the intensity has a strong correlation with chlorophyll concentration and nutrient removal whereas negative relation with carbon removal. In yellow wavelength, the intensity has shown a positive correlation with chlorophyll and nutrient removal.

Table 2. Correlation coefficient matrix for different light wavelengths

BLUE	*Intensity*	*Chl*	*DOC (%)*	*DN (%)*	*DP (%)*
Intensity	1				
Chl	-1.00	1			
DOC (%)	0.89	-0.88	1		
DN (%)	0.10	-0.12	-0.36	1	
DP (%)	0.96	-0.95	0.99	-0.20	1
RED	*Intensity*	*Chl*	*DOC (%)*	*DN (%)*	*DP (%)*
Intensity	1				
Chl	-0.92	1			
DOC (%)	-0.87	0.99	1		
DN (%)	-0.56	0.84	0.89	1	
DP (%)	-0.87	0.99	1.00	0.90	1
WHITE	*Intensity*	*Chl*	*DOC (%)*	*DN (%)*	*DP (%)*
Intensity	1				
Chl	0.84	1			
DOC (%)	-0.60	-0.94	1		
DN (%)	0.74	0.25	0.11	1	
DP (%)	0.97	0.94	-0.76	0.56	1
YELLOW	*Intensity*	*Chl*	*DOC (%)*	*DN (%)*	*DP (%)*
Intensity	1				
Chl	0.77	1			
DOC (%)	-0.93	-0.95	1		
DN (%)	1.00	0.77	-0.93	1	
DP (%)	0.97	0.91	-0.99	0.97	1

In blue, white and yellow light wavelengths the chlorophyll concentration has shown a negative correlation to carbon removal. The high carbon removal in B2 and B3, a negative correlation of light intensity to chlorophyll concentration, and negative relation of chlorophyll to carbon removal indicated that bacteria have played a major role in eliminating the carbon from wastewater. Although there was a positive correlation between light intensity and chlorophyll in white and yellow wavelengths, the chlorophyll has shown negative relation to carbon removal indicating no role of algae in carbon removal. There might be a release of extracellular compounds by the biomass which resulted in relatively low carbon removal in yellow and white wavelengths. The bacterial consortium mineralizes organic carbon mostly by heterotrophic metabolism, which is a common mechanism in wastewater treatment plants. Photoautotrophic and mixotrophic processes are present in microalgae [39]. In their photoautotrophic metabolism, microalgae use light and CO_2 (or inorganic carbon) as energy and carbon sources. Microalgae have a mixotrophic metabolism in the presence of both light and organic carbon. Organic carbon is utilized in the algal-bacterial system via mixotrophic or heterotrophic metabolism [40]. Several algae species, including *Chlorella* and *Scenedesmus*, can use organic carbon molecules as a carbon source when exposed to sunlight [41]. Extracellular organic carbon compounds like polysaccharides, nucleic acids, proteins, lipids and other dissolved organic substances are released by autotrophic microalgae and lead to an increase in organic carbon concentration [42]. As per literature, algae will release 7-50 percent of the absorbed carbon back into the environment [43]. Under normal conditions, certain algal species can synthesize these organic compounds; others can produce them in response to low nutrient stress or other stressful conditions (e.g., unfavorable light, pH, or temperature), and others can produce them after the decomposition of algal cells [44].

Chlorophyll concentration has a negative correlation with nutrient removal in the blue wavelength, but a positive correlation in the red, white, and yellow wavelengths. As the intensity of red light increased, nutrient removal and chlorophyll concentration both dropped. Chlorophyll concertation and nutrient removal increased as light intensities in the white and yellow wavelengths increased. This suggests that microalgae have a significant role in wastewater nutrient removal. The nutrient removal by microalgae may be attributed to the assimilation process of the microalgae, precipitation of the insoluble nutrient, release in the form of gas due to aeration or stir, and bio-sorption by algal cells [45,46]. In general, microalgae can assimilate nitrogen in various forms including ammonium, nitrate, nitrite, and urea although ammonium is the preferred form for nitrogen assimilation [47].

In red and white wavelengths, carbon and nitrogen removal are positively related, but in blue and yellow wavelengths, they are negatively related. The carbon and phosphorus removal are positively related in blue and red. In microalgae, the utilization of nutrients and carbon and nutrient are interrelated. Microalgae use the tricarboxylic acid cycle to generate energy from the carbon that has been stored. [48]. The resultant energy is utilized to absorb inorganic nitrogen (ammonium) to produce the glutamine and glutamate amino acids, which need energy in the form of ATP and NADP and carbon skeletons in the form of 2-oxoglutarate and oxaloacetate [49,50].

The nitrogen removal has shown a positive correlation to phosphorus removal in red, white and yellow wavelengths. Microalgae metabolize nitrogen predominantly into proteins that are necessary for the synthesis of ribosomes and ribosomal RNA. The primary function of phosphorus absorption is the storage of the synthesized ribosomal RNA. Therefore, adequate quantity of nitrogen is required for the uptake of phosphate in order to prevent inhibition of cell's capacity to synthesize proteins [51]. Phosphorus uptake by microalgae is generally influenced by several additional elements, including light intensity, temperature, pH, algae physiology, phosphate concentration, and chemical form of accessible phosphate [52]. Compared to other species of algae, green algae require significantly more N and P. Algal growth stoichiometry states that in order to eliminate 1 mg of phosphorus, 5.4–7.2 mg of nitrogen

are required [53,54]. This implies that P removal from municipal wastewater is significantly influenced by the nitrogen to phosphorous ratio, which also influences biomass growth and nutrient removal [52].

In addition to the wastewater characteristics, other factors that influence the algal wastewater treatment include the photobioreactor design, light intensity, and light period. This study demonstrates that compared to white and yellow light, blue and red light wavelengths are better suited for mixed algal-bacterial systems. Since only monochromatic light was used in this experiment, it was necessary to examine the effect of different light modes and dichromatic light illumination on mixed algal-bacterial culture. It is also crucial to study how the light affects the destruction of micropollutants along with carbon and nutrients.

Due to the strict pollution controls recommended by circular economy principles before wastewater is discharged and reused in the environment, existing wastewater treatment plants are currently having difficulties. Many technologies, including pyrolysis, anaerobic digestion, and hydrothermal carbonization, are now being researched and used to improve energy recovery from residual biosolids and eliminate excessive solids discharge [55,56]. A sustainable wastewater treatment and disposal waste management solution is being constrained by excessive solid waste landfilling, ineffective recycling, high energy consumption, and greenhouse gas emissions by existing treatment technologies. These drawbacks can be addressed by using algae-bacterial systems. Carbon fixation, nutrient uptake, and the consequent generation of value-added biomass, which may be used as a substrate for the production of biodiesel and bioethanol, are the potential environmental and economic advantages of integrating microalgae in wastewater treatment systems. However, since using artificial lighting might raise treatment costs, it is also necessary to do a cost-analysis for the photobioreactor when examining how light affects algal-bacterial systems.

4 Conclusion

Algal growth was regulated by the wavelength and intensity of light, which in turn influenced the overall effectiveness of the algal-bacterial system in wastewater treatment. The concentration of chlorophyll has a significant impact on nutrient removal. The R1 wavelength of low-intensity red light resulted in a better nutrient removal efficiency. Higher carbon removal was achieved using the blue wavelength with I3 intensity. Overall, the wavelength of blue light at I3 can be considered the best wavelength for removing carbon and nutrients. At I1, the red wavelength can be considered the second-best wavelength. In comparison to white and yellow light wavelengths, blue and red light wavelengths are more suited for algal-bacterial systems, according to this study.

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* Corresponding author: keerthi.katam@mahindrauniversity.edu.in