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EFFECTS OF LIGHT INTENSITY ON BIOMASS YIELD IN MICROALGAE GROWN IN PEEL WASTE WATER

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ABSTRACT

In this study, effects of various types of peel waste water and microalgae strains in the growth medium and different light intensities on biomass production of microalgae were investigated. High Light with 595x10 Lux light intensity was determined as the optimal light for biomass production. The results indicated that microalgae growth with 180 ml water chestnut peel waste water was higher than that of other peels waste water and tap water. Maximum biomass concentration (1.076g/200ml) was obtained in 180 ml water chestnut peel waste water with 20 ml *Chlorella vulgaris* and followed by 180 ml water chestnut peel waste water with High Light with 595×10Lux light intensity and 20ml *Chlorella vulgaris* with High Light with 595×10Lux light intensity.

Key words : Biomass yield, Microalgae, Peel waste water and Light intensities.

Introduction

Microalgae are autotrophic organisms that harness light energy and inorganic nutrients to generate biomass enriched with valuable products. The biotechnological potential of microalgae stems from their biomass, which is rich in essential components such as lipids, starch, and alkanes. There are estimated to be between 200,000 and several million species of microalgae. Among the various factors influencing their growth, light plays a crucial role in the photosynthesis process, making it a key element in establishing optimal cultivation conditions. These conditions significantly impact the proliferation rate of algae and the production of biomass. This study aims to investigate the effects of light intensity and photoperiod on the growth and biochemical composition (carbohydrates, proteins) of two green algae species, *Tetranephris brasiliensis* and *Scenedesmus* sp., under laboratory conditions, as noted by Asfouri *et al.* (2019). Algae serve as a primary source of various nutrients, and their high protein content in different species positions them as a promising alternative source of proteins and oils. Additionally, algae are significant sources of vitamins, minerals, antioxidants and natural colorants, making the incorporation of whole biomass into food and feed beneficial for enhancing color, nutritional value and improving texture or resistance to oxidation. The integration of algae into conventional food products presents an opportunity to create healthier options (Kovaè, Simeunoviæ, Babiæ, Mišan and Milovanoviæ, 2013), as plant-based materials are abundant in various compounds beneficial for human health (Oniszczuk and Olech, 2016; Oniszczuk and Podgórski, 2015). In comparison to *Chlorella vulgaris*, *C. pyrenoidosa* exhibits a significantly faster growth rate. Additionally, *C. pyrenoidosa* boasts a superior protein content and an enhanced amino acid profile; however, it is also sensitive to toxic contaminants (Katsimichas *et al.*, 2023). The U.S. Food and Drug Administration classifies *C. pyrenoidosa* as Generally Recognized as Safe (GRAS) (Lisbôa *et al.*, 2014), indicating its suitability for use in food and pharmaceutical products. In addition, cultivation of microalgae in wastewater/waste as cheaper nutrient

sources adds more credits towards algae biofuels towards greener economy (Mata *et al*., 2010) Furthermore, in order to efficiently cultivate microalgae few parameters such as pH, temperature, dissolved oxygen concentration and light supply should be carefully considered to design efficient photo-bioreactor (Munoz and Guieysse, 2006). More importantly, light source is one of the major criteria for the microalgae growth because it can affect the growth and metabolism of microalgae (Show *et al*., 2017).

Materials and Methods

The study was performed in the Department of Food Science and Technology, SHUATS, Prayagraj, Uttar Pradesh, India

Raw material

Green pea peels and water chestnut peels were collected from local markets and thoroughly washed to remove any impurities. A tube light was also procured to provide a controlled light intensity for algae growth.

Selection of strains

Strains such as *Chlorella pyrenoidosa* and *Chlorella vulgaris*, were chosen from the collection of microalgal strains in the NCIM, CSIR-National Chemical Laboratory, Bhabha Road Pune and National Facility for Marine Marine Cyanobacterial Bhartidasan University, Tiruchirappalli. Strains were inoculated to the diluted samples separately.

Experimental design

The experiment was conducted in a factorial completely randomized design (FCRD) with three replications.

Wastewater Media

T_0 : 180 ml Tap Water

T_1: 180 ml green pea Peel wastewater

T_2: 180 ml Water chestnut peel wastewater

M1 : 20ml *Chlorella pyrenoidosa*

M2 : 20ml *Chlorella vulgaris*

Light Intensity

L1 : Sunlight

L2 : High Light Intensity (595x10Lux)

L3 : Medium Light Intensity (430x10Lux)

L4 : Low Light Intensity (128x10Lux)

Preparation of Wastewater Media

The green pea peels and water chestnut peels were collected and chopped into small pieces. One kilogram of the chopped peels was mixed with 10 liters of water in a container. The mixture was steeped for 24 hours, allowing the peels to release their nutrients into the water.

Algae cultivation

The wastewater media (180ml) were inoculated with 20 mL of *Chlorella pyrenoidosa* or *Chlorella vulgaris* microalgae strain. The glass bottles were placed under different light intensities:

1. Sunlight
2. High light intensity: 595 × 10 Lux
3. Medium light intensity: 430 × 10 Lux
3. Low light intensity: 128 × 10 Lux

Sterilisation

The equipment's and glassware mentioned in the list were properly cleaned and were rinsed and dried thoroughly to remove the detergent residues. All the glassware, pipette, rubber corks, thin rubber pipes, thin plastic pipes were sterilised in autoclave at 121^0C (15 psi) for 15 minutes.

Determination of physic chemical parameter of waste water media

pH

pH was estimated by electrometric method. Fifty ml of each sample was taken separately and the initial pH was determined by using digital pH meter (Digital pH meter 335).

Biological oxygen method (BOD)

The BOD was estimated by titrimetric method. BOD was analyzed immediately after dilution with tap water individually along with undiluted sample as such. Samples were taken in 100 ml BOD bottles and kept for incubation at 20 °C for 5 days in BOD incubator (NBO180/D). After incubation two ml of $MnSO_4$ (22.5 g in 1000 ml) and two ml of alkali azide iodide solution (0.5 g of sodium azide, 35 g of potassium hydroxide, 7.5 g of potassium iodide dissolved in water and the volume was made upto 100 ml) was added and mixed properly. Precipitate formed was dissolved by adding two ml concentrated H_2SO_4 and fifty ml of each sample was taken in a separate flask and titrated with 0.0125 N sodium thiosulphate solution using starch as indicator and BOD was calculated as follows.

Calculation:

$$\text{BOD (mg /l)} = \frac{(D5 - D0)}{\text{ml of sample}} \times 1000$$

Where, D0= Initial dissolved oxygen

D5 = dissolved oxygen after five days of incubation

Total dissolved solids

Total dissolved solids were estimated by gravimetric method. Sample of fifty ml was transferred to a pre

weighed evaporating dish (W1). It was evaporated to dryness on steam bath maintained at 80 °C. Evaporated sample was dried for at least one hour in an oven at 180 ± 2 °C. The samples were cooled in a desiccator and weighed (W2).

Calculation:

$$\text{Total dissolved solids (mg/l) (B)} = \frac{(W2 - W1)}{\text{ml of sample}} \times 1000$$

Dissolved oxygen (DO) (mg/L)

Calculate the amount of dissolved oxygen (DO) (mg/L) by using the following formula:

$$DO(mg/L) = \frac{8 - 1000 - N}{V} \times v$$

Where,

V = Volume of water sample used for titration

v = Volume of sodium thiosulfate (titrant)

N = Normality of titrant

8 = It is a constant since 1 ml of 0.025N sodium thiosulfate solution is equivalent to 0.2 mg oxygen

Biomass estimation (Richmond and Gobbelaar, 1986)

The biomass production of individual isolate was determined separately by homogenizing using glass beads of 2 mm and the flasks were kept under shaking condition for 20 minutes. The culture of 100 ml was filtered through a dried and pre-weighed Whatman No. 1 filter paper. This was dried in an oven at 60 °C until constant weight was obtained. The biomass yield was calculated as follows.

$$\text{Biomass yield (g/l)} = \frac{\text{Final weight of the filter paper (g)} - \text{Initial weight of the filter paper (g)}}{\text{Volume of the Sample taken (ml)}} \times 1000$$

Statistical analysis of the data

The data were subjected to Factorial CRD analysis (OPSTAT software) and interpretation of the data was carried out in accordance with Panse and Sukhatme (1985). The level of significance used in the 'F' and't' test was P=0.01. The critical difference values were calculated whenever the F test values were significant.

Results and Discussion

The physicochemical parameters of two different peels were determined in order to detect any type of adulteration and improper handling of plant material. The physico-chemical properties of two types of peels (Green pea peel and Water chestnut peel) were carried out. The results of different properties of Green pea peel and Water chestnut peel are presented in Table 1 and Fig. 1. The vitamin C of different peels varied from 81.31 in Green pea peel to 0.00 in the peels of Water chestnut. The Iron of different peels varied from 8.65 in Green pea peel to 0.00 in the peels of Water chestnut. The Energy (Kcal/100g) of different peels varied from 330.82 in Green pea peel to 316.07 in the peels of Water chestnut. The Carbohydrate of different peels varied from 69.27 in Green pea peel to 88.89 in the peels of Water chestnut. The Protein of different peels varied from 8.62 in Green pea peel to 0.86 in the peels of Water chestnut. The moisture of different peels varied from 9.69 in Green pea peel to 10.56 in the peels of Water chestnut. The Ash of different peels varied from 4.94 in Green pea peel to 4.56 in the peels of Water chestnut. The Crud fiber of different peels varied from 5.34 in Green pea peel to 2.41 in the peels of Water chestnut.

Initial physico-chemical properties of tap and green pea and water chestnut peel water

The data of pH of tap water and peels waste water is given in Table 2. The pH of tap water was (7.19). The pH of different peels varied from 7.05 in Green pea peel to 7.08 in the peels of Water chestnut. The DO (mg/L) of tap water was (4.47). The DO (mg/L) of different peels varied from 5.17 in Green pea peel to 5.12 in the peels of Water chestnut. The BOD (mg/L) of tap water

Table 1 : Initial physico-chemical properties of peels.

Physico-chemical properties	Peels	
	Green pea peel	Water chestnut peel
Vitamin C	81.31	0.00
Iron	8.65	0.00
Energy	330.82	316.07
Carbohydrate	69.27	88.89
Protein	8.62	0.86
Fat	2.14	0.23
Moisture	9.69	10.56
Ash	4.94	4.56
Crud fiber	5.34	2.41

Table 2 : Initial physico-chemical properties of tap and green pea and water chestnut peel water.

Physico-chemical properties	Tap water and peel water		
	Tap water	Green pea peel water	Water chestnut peel water
pH	7.19	7.05	7.08
DO (mg/L)	4.47	5.17	5.12
BOD (mg/L)	0.83	10.2	10.25
TDS (mg/L)	666.67	681.12	685.15

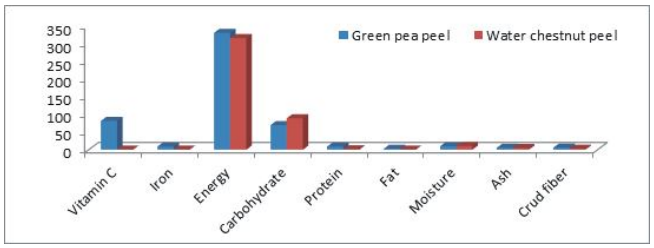

Fig. 1 : Initial physico-chemical properties of peels.

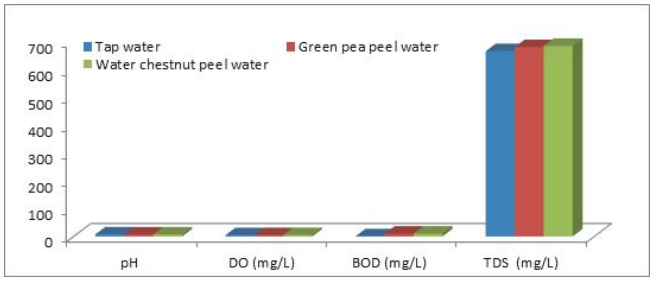

Fig. 2 : Initial physico-chemical properties of tap and green pea and water chestnut peel water.

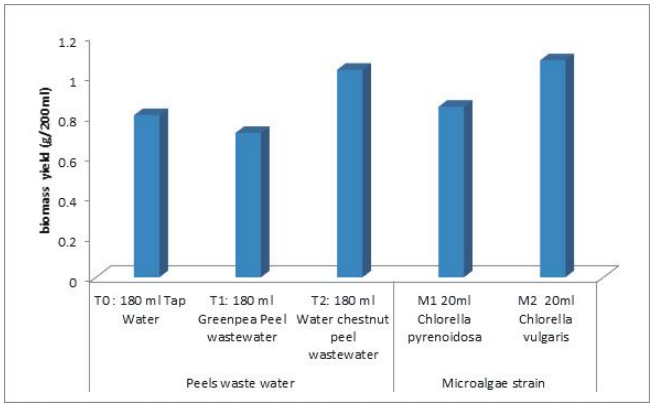

Fig. 3 : Interactive effect due to use of peels waste water and microalgae strain on biomass yield (g/200ml).

was (0.83). The BOD (mg/L) of different peels varied from 10.2 in Green pea peel to 10.25 in the peels of Water chestnut. The TDS (mg/L) of tap water was (666.67). The TDS (mg/L) of different peels varied from 681.12 in Green pea peel to 685.15 in the peels of Water chestnut.

Interactive effect due to use of peels water, microalgae strain and light intensity on biomass yield

In the interactions between the type of peels waste water and microalgae strains the highest amount of biomass (1.07 g/200ml) was obtained in treatment T2M2: 180 ml Water chestnut peel wastewater x 20ml *Chlorella vulgaris*. Whereas, the minimum biomass yield (0.680) was found in T1:M2 (180 ml Greenpea Peel wastewater x 20ml Chlorella vulgaris). Since the calculated F value is less than tabulated F value due to interaction of peels waste water x microalgae strain at 2, 48 degree of freedom on 5% probability level so our null hypothesis will be rejected therefore it can be concluded from the

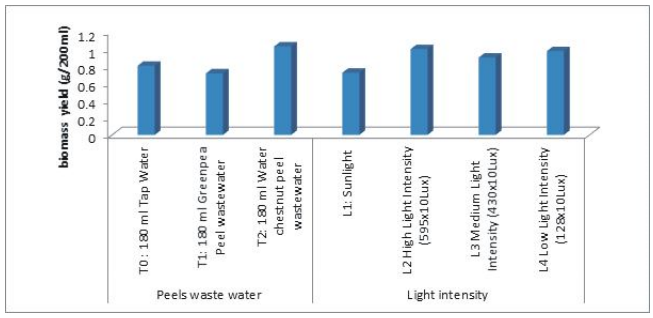

Fig. 4 : Interactive effect due to use of peels waste water and light intensity on biomass yield biomass yield (g/200ml).

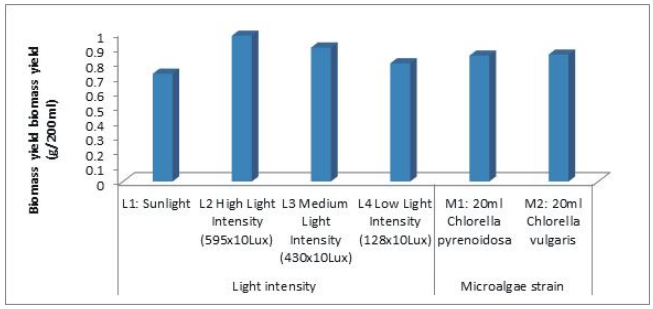

Fig. 5 : Interactive effect due to use of microalgae strain and intensity on biomass yield biomass yield (g/200ml).

given data that there is significant difference between the interaction of different variables. In the interactions between the type of peels waste water and light intensities the highest amount of biomass (1.088 g/200ml) was obtained in treatment T2L2: 180 ml Water chestnut peel wastewater x High Light Intensity (595x10Lux). Whereas the minimum biomass yield (0.518) was found in T1:L1 (180 ml Greenpea Peel wastewater x Sunlight. Since the calculated F value is less than tabulated F value due to interaction of peels waste water x light intensity at 6, 48 degree of freedom on 5% probability level so our null hypothesis will be rejected therefore it can be concluded from the given data that there is significant difference between the interaction of different variables. Photosynthesis and the corresponding biomass production indeed depends on photonic flux (Carvalho *et al.*, 2011). When light intensity is insufficient, microalgae consumed carbohydrates during photorespiration; although, they are unlikely to cause fatal damage. Excessive light intensity makes photosystems overload, pigments bleach and finally break in photosystem II (Jeong *et al.*, 2012). Adequate illumination is an essential factor for microalgae growth (Carvalho *et al.*, 2006). The obtained results are in agreement with those of Zhao *et al.* (2013), who have reported that the microalgae *Chlorella* sp. growth was low under insufficient or excessive light intensities.

In the interactions between the type of microalgae strains and light intensities the highest amount of biomass

Table 3 : Interactive effect due to use of peels waste water and microalgae strain on biomass yield biomass yield (g/200ml).

	M1 20ml ***Chlorella pyrenoidosa***	**M2 20ml** ***Chlorella vulgaris***	**Mean**
T_0 : 180 ml Tap Water	0.804	0.803	**0.804**
T_1: 180 ml Greenpea Peel wastewater	0.752	0.680	**0.716**
T_2: 180 ml Water chestnut peel wastewater	0.982	1.076	**1.029**
Mean	**0.846**	**0.853**	
	C.D.	**SE(d)**	**SE(m)**
	0.033	**0.017**	**0.012**

Table 4 : Interactive effect due to use of peels waste water and light intensity on biomass yield biomass yield (g/200ml).

	L1	**L2**	**L3**	**L4**	**Mean A**
T_0 : 180 ml Tap Water	0.630	0.957	0.895	0.733	**0.804**
T_1: 180 ml Greenpea Peel wastewater	0.518	0.895	0.778	0.672	**0.716**
T_2: 180 ml Water chestnut peel wastewater	1.025	1.088	1.025	0.977	**1.029**
Mean C	**0.724**	**0.980**	**0.900**	**0.794**	
	C.D.	**SE(d)**	**SE(m)**		
	0.047	0.024	0.017		

Table 5 : Interactive effect due to use of microalgae strain and intensity on biomass yield biomass yield (g/200ml).

	L1	**L2**	**L3**	**L4**	**Mean B**
M1: 20ml *Chlorella pyrenoidosa*	0.714	0.981	0.897	0.791	**0.846**
M2: 20ml Chlorella vulgaris	0.734	0.979	0.902	0.797	**0.853**
Mean C	**0.724**	**0.980**	**0.900**	**0.794**	
	C.D.	**SE(d)**	**SE(m)**		
	N/A	**0.019**	**0.014**		

(0.979 g/200ml) was obtained in treatment M2L2: 20ml *Chlorella vulgaris* x High Light Intensity (595x10Lux). Despite a better photosynthetic performance at medium light treatment than low and high light treatments, the high light treatment achieved higher biomass yield and nutrient removal. This could be attributed to several factors, including 1) the self-sustaining and adaptive capacity of algal biofilm to various forms of stress (Gao *et al*., 2024; Wang *et al*., 2021; Brock and Brock, 1969). The fluorescence activities were based on the cells on the biofilm surface directly affected by light treatments, while the cells beneath were not assessed. Therefore, the photosynthetic performance does not reflect the entire biofilm growth and activities as suggested by Kula *et al*. (2017). The high cell density at high light treatment could have contributed to the stress, as noted by Wakjera *et al*. (2019) and Mkpuma *et al*. (2023a). Whereas, the minimum biomass yield (0.714) was found in M1:L1 (180 ml Greenpea Peel wastewater x Sunlight. Since, the calculated F value is less than tabulated F value due to interaction of peels waste water x microalgae strain x light intensity at 3, 6, 48 degree of freedom on 5% probability level so our null hypothesis will be rejected therefore it can be concluded from the given data that there is significant difference between the interaction of different variables. The major pigment groups present in microalgae are chlorophylls, phycobilins, and carotenoids (carotenes and xanthophylls) (Carvalho *et al*., 2011). The color of incident light ideally should match with the pigment absorption band which corresponds to the lowest excited state. In the case of chlorophyll, absorption bands are present in blue as well as red spectral regions Matthijs *et al*. (1996). The excess energy present in the blue photons is wasted as heat. Blue light at first glance does not seem to be very well fit for photosynthesis and, for that reason, may be considered redundant. Thus, the chlorophyll in *C. vulgaris* could efficiently absorb the red rather than blue light wavelength. But, low intensities of blue light may play an essential role in regulation of cell growth and metabolism (Matthijs *et al*., 1996). However, both

Table 6 : ANOVA Interactive effect due to use of peels water, microalgae strain and light intensity on biomass yield biomass yield (g/200ml).

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Due to peel waste water	2	1.252	0.626	376.404	< 0.001
Due to microalgae strains	1	0.001	0.001	0.553	0.461
Int peel waste water X microalgae strains	2	0.083	0.041	24.879	< 0.001
Due to light intensity	3	0.689	0.230	138.057	< 0.001
Int Due to peel waste water X light intensity	6	0.212	0.035	21.237	< 0.001
Int microalgae strains X light intensity	3	0.001	0.000	0.232	0.874
Int T × M × L	6	0.063	0.010	6.276	< 0.001
Error	48	0.080	0.002		
Total	71	2.379			

natural white light and warm white light are composed of visible light wavelengths (consist of red and blue wavelengths), they have different relative photon flux curves. Luminous intensity of warm white light is lower compared to natural white light in blue wavelength. Therefore, warm white light showed better results than natural white light. Also, Kim *et al*. (2012) have found the production rate of *Scenedesmus* sp. microalgae was highest under white light, followed in order by red, blue and green lights.

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