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# Biochemical composition of *Tetradesmus obliquus* grown in municipal wastewater towards biofuel production

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**Abstract:**
Global energy is undergoing a transformation driven by the imperative to mitigate climate impact, enhance energy security, and promote sustainable development. Biofuel production has emerged as a promising alternative to fossil fuels, offering renewable and environmentally sustainable sources of energy. Biofuels are derived from organic materials such as crops, agricultural residues, forestry waste, and microorganisms, which undergo conversion processes to produce liquid or gaseous fuels suitable for transportation, heating, and electricity generation. This study aims to maximize the production of biomass, protein, lipid and carbohydrate in a newly isolated microalga, *Tetradesmus obliquus* RESP2 cultivated using a combination of municipal waste water and Bold's Basal Medium (BBM). The experimental design involved at varying the composition of growth medium to determine the most optimal conditions for algal growth and biochemical accumulation. After thorough experimentation and analysis, the results revealed that the optimal combination of waste water and BBM medium (75%:25%) yielded a maximum concentrationof biomass 16.2 g/L, lipid 5.53 g/L, carbohydrate 1.48 g/L and protein 4.48 g/L. These findings demonstrate the efficacy of utilizing municipal wastewater as a nutrient source in combination with BBM medium for the cultivation of *T. obliquus*. The optimized conditions identified in this study hold promise for enhancing the efficiency and sustainability of microalgae based biofuel production processes.
**Keywords:** *Tetradesmus*, Wastewater, Optimization, Biomass, Lipid

## 1. Introduction

Biofuel's have gained traction in the past decade as a sustainable alternative to fossil fuels, driven by concerns over climate change and energy security (Demirbas, 2009). Technological innovation in biofuel sector creates advance research in the field of biotechnology, agronomy and process engineering in order to improve the yields, reducing production costs and expanding the range of feedstocks (Davis *et al.*, 2011). Biofuel production has emerged as a critical component to address climate change, enhance energy security, and stimulate economic growth (Nigam and Singh, 2011).

Microalgae offers a promising feedstock for sustainable energy production due to several key factors such as highlipid content, rapid growth rates, carbon sequestration, nutrient recycling, genetic engineeringand versatile cultivation methods (Chisti, 2007; Borowitzka, 2013). Additionally, microalgae cultivation can yield valuable co-products and contributing to the overall economic viability (Harun *et al.,* 2010). Despite the challenge on high production costs, several studies have been conducted to optimize the microalgae-based biofuel production for widespread adoption (Ho *et al.,* 2014).

The significance of using microalgae in conjunction with municipal wastewater have its dual capability of waste remediation and biomass production. This approach exploits on the efficient utilization of nutrients present in wastewater leading to high biomass productivity without the need of costly synthetic fertilizers (Li *et al.,* 2008). Presence of nitrogen and phosphorus in municipal wastewater serves as a major nutrient for microalgae growth. This approach not only treats wastewater but also produces biomass that can be harvested and processed into valuable products such as biofuels, animal feed and other bioactive compounds (Rawat *et al.,* 2011). The scalability and adaptability of this approach make it suitable for implementation in various fields, offering a sustainable solution for both urban and rural communities (Rawat *et al.,* 2011).

Their adaptable cultivation methods can offer for algae growth in diverse environments, including saline water and wastewater (Borowitzka, 2013). The microalgae lipid contents reaching up to 50% of their dry biomass of *Tetradesmus* sp**.** strains offer a significant potential for biodiesel production (Hu *et al.,* 2008). Moreover, *Tetradesmus* sp. can efficiently utilize nutrients present in different growth media, such as wastewater and agricultural runoff, reducing the need for synthetic fertilizers and associated costs (Borowitzka, 2013). Overall, these highlights show the potential of *Tetradesmus* sp. as a valuable candidate for sustainable biofuel production, supporting the transition towards renewable energy sources.

Previous studies have extensively investigated the optimization parameters and biochemical analysis during microalgae cultivation (Wang *et al.,* 2019). Optimization parameters typically include factors such as pH, salinity, temperature and light intensity, which directly influence microalgae growth and lipid accumulation (Cheirsilp and Torpee, 2012). Researchers have conducted experiments to determine the optimal conditions for each parameter to maximize the biomass productivity and accumulation of various biomolecules. Lipid content is of particular interest due to its significance in biodiesel production. Previous studies have employed various analytical techniques, including spectrophotometry, chromatography, and mass spectrometry, to quantify these biochemical components accurately (Harun *et al.,* 2010; Mata *et al.,* 2010).

For instance, *Chlorella vulgaris* can yield up to 1.20 g/L/day of biomass under optimal conditions and has a lipid content of around 28-32% of dry biomass. It contains protein ranged between 42-58% of dry weight (Converti *et al.,* 2009; Griffiths and Harrison, 2009; Becker, 2007). *Nannochloropsis* sp. is another microalga known for its high lipid content, which are high as 50-60% of dry weight, with biomass productivity reaching up to 0.64 g/L/day (Rodolfi *et al.,* 2009). *Spirulina platensis*, renowned for its high protein content of 60-70% of dry weight, also demonstrates considerable biomass productivity around 1.0-1.5g/L/day, though it has a lower lipid content of 6-8%. *Scenedesmus obliquus*, studied for wastewater treatment and biofuel production, can produce about 0.026 g/L/day of biomass with a lipid content of 20-30% and a protein content of 40-45% (Martinez *et al.,* 2000).

The study aims to determine the best suitable conditions for cultivating the selected microalga, *T. obliquus* in municipal wastewater to maximize growth and lipid accumulation, alongside analyzing its biochemical composition. It seeks to assess the feasibility of using these microalgae as a biofuel feedstock and evaluate the environmental implications of its cultivation in wastewater.

## 2. Materials and Methods

### 2.1 Wastewater collection and analysis

The municipal wastewater samples were collected from the Sewage Treatment Plant (STP) in Coimbatore, Tamil Nadu, India. The wastewater samples were filtered to separate the visible particles and solids prior to use for algae cultivation. Then, the samples were used to analyse the various physicochemical parameters such as Temperature, pH, Dissolved Oxygen (DO), and Total Dissolved Solids (TDS) by using Multiparameter Analyzer (Hanna Instruments, USA). Additionally, the concentration of nitrate, sodium, potassium and calcium were determined according to the standard method and procedure (APHA, 2005) using Flame photometer (Labtronics: Model LT – 66).

### 2.2 Isolation and identification

Algae samples were collected from the saltpan of Adirampattinam, Tamil Nadu and transported to laboratory for maintain the integrity. The samples were observed for microalgae under the microscope. The sample were transferred to Bold Basal Medium (BBM) and incubated at 24 ± 1°C in a controlled room, illuminated with fluorescent lamps atan irradiance of 30µEm $^{-2}$ s $^{-1}$, under a 12/12 h light-dark cycle for 12 days. Further, the samples were subjected to isolation process using serially diluted up to $10^{-7}$. From this, 100µL of the sample was spread on the BBM agar medium. The colonies of *Tetradesmus* sp. developed on the plates were picked and again transferred to BBM medium. The isolated microalgae underwent detailed observation under a light microscope, focusing on morphological traits like cell size, shape and other features. The morphological features of isolate was confirmed by using identification manual on ‘‘Phytoplankton of Indian Seas’’ (Santhanam *et al.,* 1987). Additionally, molecular techniques were used to validate morphological identification of the isolated species.

### 2.3 Molecular identification of the isolated microalgae

The selected isolate was subjected to molecular identification in order to ascertain its systematic position. For the isolation of genomic DNA, the experiment was performed using Plant DNA Kit (HiMedia Laboratories Pvt Ltd, India) according to the instructions given by the manufacturer. The primers used were LROR + (5' ACC CGC TGA ACT TAA GC 3') and LR7 (5' TAC TAC CAC CAA GAT CT 3') to perform a Polymerase Chain Reaction (PCR). The PCR conditions were set as mentioned below: initial denaturation for 2 min at 95°C; 25 cycles for 30 sec at 95°C; 30 s at 51°C; 1 min at 72°C; and a final extension for 10 min at 72°C. The amplified product was sequenced at Yaazh Xenomics, TICEL bio park Phase -III, Coimbatore, Tamil Nadu, India. The obtained sequences were then analyzed using the Basic Local Alignment Search Tool (BLAST) on the server of the National Centre for Biotechnology Information (NCBI, Bethesda, MA, USA) to identify the closely related sequences. Further, the nucleotide sequence was submitted to NCBI and got an accession number.

### 2.4 Cultivation of *Tetradesmus* sp. in wastewater as a supplement

The cultivation of *Tetradesmus* sp. utilized municipal wastewater, which was combined with reverse osmosis (RO) water and Bold's Basal Medium (BBM) to create various combinations. Different ratios were employed: first, a 25%:75% ratio of wastewater collected after the grit removal chamber (WWC) to BBM medium and RO water (i.e., WWC+ BBM and WWC + RO); second, a 50%:50% ratio of WWC to BBM and RO (i.e., WWC + BBM and WWC + RO); and third, a 75%:25% ratio of WWC to BBM and RO (i.e., WWC + BBM and WWC + RO). Additionally, BBM and WWC were maintained separately as controls. These concentrations were prepared in 250 mL Erlenmeyer flasks, each containing 200 mL of media in triplicates. Subsequently, 10% v/v cells of 14 days old *Tetradesmus* sp. culture were inoculated into the culture flasks and incubated under controlled conditions. Samples were collected at every two day intervals to analyze the Optical Density (OD) of the culture, biomass, lipid, carbohydrate and protein.

### 2.5 Optimization of cultivation parameters

#### 2.5.1 pH

To optimize pH for algal growth, the process entails adjusting the pH of the growth medium within a range spanning from 4 to 11. Individual batches of medium were prepared and modified using either acidic or basic solutions. Algal cultures were subsequently inoculated into each batch under controlled conditions. Growth progression is closely monitored, and the pH yielding maximum growth and biomass production was identified based on observed growth rates.

#### 2.5.2 Temperature

Optimizing temperature for algal growth entails experimenting with different temperatures such as 18°C, 28°C and 40°C to ascertain the optimal range for maximum growth and biomolecules. Multiple batches of algal cultures were subjected to varying temperatures during incubation.

#### 2.5.3 Light intensity

To optimize light intensity for algal growth, the process involves adjusting the intensity of light to maximize productivity. Various light sources are tested at different intensities, typically ranging like 3000 lux, 5000 lux and 10000 lux to determine their effectiveness in promoting algal growth. The optimal medium is then determined based on parameters such as growth rate and biomass accumulation. This optimization process plays a crucial role in enhancing algal biomass production for diverse applications, including biofuel production and wastewater treatment.

### 2.6 Analytical Techniques

#### 2.6.1 Optical density

The measurement of algal density, starts by procuring the representative sample after they are thoroughly mixed. Then a portion of this culture is transferred into a cuvette and the optical density (OD) was measured using spectrophotometer using wavelength of 680 nm (Lee *et al.,* 2013). In a meanwhile, a calibration curve was drawn using the comparative correlation of OD values and dry weight of the algal samples.

#### 2.6.2 Determination of biomass

To determine the biomass of the culture, the sample underwent harvesting via centrifugation at 10,000 rpm for 10 minutes, followed by two washes. Subsequently, the weight of the pellet biomass was determined by measuring the volume difference between thetube containing the biomass and an empty tube. The findings were then expressed as Cell Weight in grams per liter (Lee *et al.,* 2013).

Biomass productivity (mgL-1day-1) = Bx - B0 / Tx - T0

Bx = biomass produced on day x

B0 = biomass produced on day 0

Tx = time x used for the production of biomass (days)

T0 = starting time

#### 2.6.3 Extraction and estimation of lipid

Lipid extraction from the biomass occurred at regular intervals of every two days, following the protocol outlined by Folch *et al.* (1957). The biomass underwent treatment with a mixture of chloroform and methanol in a 2:1 volume-to-volume ratio. Following theaddition of physiological saline (900 mg in 100 ml of water), the mixture was vortexed for 10

seconds, resulting in the formation of a two-layer structure, with chloroform settling in the lower phase. The chloroform phase was separated and allowed to air dry overnight, facilitating the evaporation of chloroform and yielding dried lipid for estimation. Addition of 0.5 mL sulfuric acid was added to the dried lipid, followed by heating the mixture at 70°C for10 minutes and subsequent natural cooling to ambient temperature. After incubating the mixture for 30 minutes at room temperature, the reacted sample was measured at 520 nm. For further analysis, 0.2 mL of lipid containing sulfuric acid was combined with 5 mL of vanillin reagent (comprising 200 mg of vanillin mixed with 80 mL orthophosphoric acid and 20 mL water). Cholesterol (HiMedia Laboratories, Mumbai, India) served as the reference standard for this experiment.

Total Lipid content (%) = Amount of lipid produced / Biomass taken x 100

Lipid productivity (mg/L/day) = Total lipid content x Biomass productivity

**2.6.4 Estimation of carbohydrate**

The carbohydrate content was determined following the standard protocol outlined by Dubois *et al.* (1956). For each sample, 1 mL of sample solution was mixed with 1 mL of 5% (w/v) Phenol and 5 mL of concentrated sulfuric acid (96%), with caution taken to maintain cooling during the addition of sulfuric acid. The mixture was then incubated at 30°C for 20 minutes. Subsequently, the absorbance of the sample was measured at 490 nm using a UV spectrophotometer immediately after incubation. Further, standard solutions with concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were prepared and their absorbance were recorded. The total carbohydrate content of the samples was calculated using theequation ($y = 0.1279x + 0.0923$) derived from the standard calibration curve.

**2.6.5 Extraction and estimation of protein**

The extraction and estimation of protein from the sample were conducted following the method described by Waterborg, (2009)**.** About 0.1 mL of either the sample or standard was added to 0.1 mL of 2 N NaOH, and the mixture was hydrolysed at 100°C for 10 minutes using a boiling water bath. The resulting hydrolysate was allowed to cool at room temperature before adding 1 mL of freshly prepared complex-forming reagent, followed by a 10 minute incubation at room temperature. Further, 0.1 mL of Folin reagent was added and mixed using a vortex mixer, and let the combined solution stand at room temperature for 30 - 60 minutes, ensuring the time does not exceed to 60 minutes. The absorbance was measured at 750 nm for protein concentrations below 500 µg/mL. Finally, a standard curve was constructed using the related absorbance to initial protein concentration for determination of unknown protein concentrations.

## 3. Results and Discussion

### 3.1 Isolation and identification of *T. obliquus*

The microalgae RESP2 was obtained from a saltpan located in Adirampattinam, Tamil Nadu, India, utilizing plating techniques for isolation. Morphological identification of the microalgae was conducted using standard manuals and classification keys (Santhanam *et al.*, 1987). Observations revealed that the isolate exhibited a typically cylindrical shape, with cells ranging from 5 to 20 µm in diameter, each enclosed by a rigid, cellulose-based cell wall (Cho and Lee, 2024). Based on these microscopic observations, the isolated microalgae were tentatively classified as belonging to the genus *Tetradesmus* sp., within the family *Scenedesmaceae*, part of the class Chlorophyceae. Subsequently, the isolated species were inoculated into the BBM medium and this medium provided optimal support for its growth.

Molecular identification of the green algae was carried out through 18S rRNA sequencing. After the PCR amplification, a partial gene sequence was achieved and submitted to GenBank. These sequences were deposited to the National Centre for Biotechnology Information (NCBI) and received the accession number: OR674051. The obtained sequence was compared to the sequences available in the Nucleotide collection (nt) BLAST. It revealed

closest similarity (98.82% identity) with the partial sequences of 18S rRNA from *Tetradesmus* sp. (Fig.1). Hence, the isolate was identified as *Tetradesmus obliquus* RESP2. Further, this isolate was taken for cultivation studies in different media as alternatives to the microalgal growth medium.

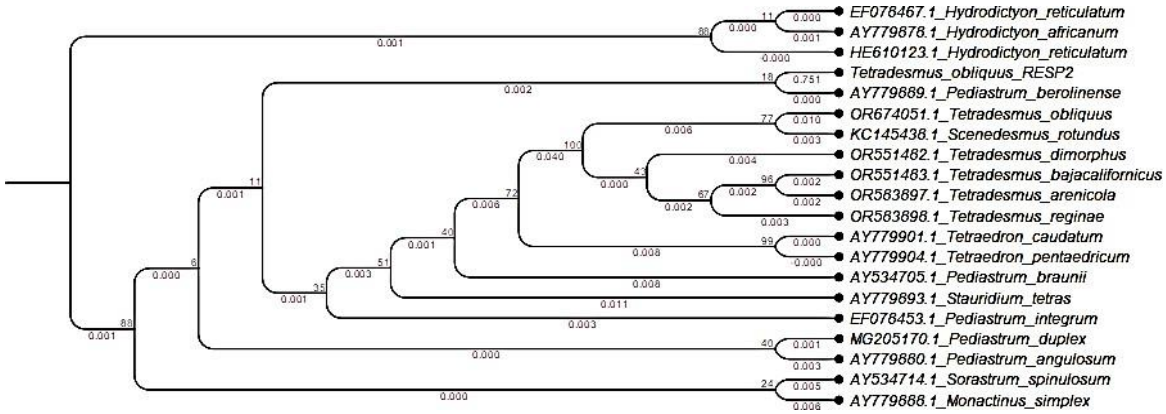


**Fig.1: Distance tree of BLAST results obtained by querying the sequence OR674051: *T. obliquus* RESP2 against the nucleotide collection.**

Wastewaters from the treatment plant, specifically WWC (Wastewater collected after the grit removal chamber), were gathered and subjected for physicochemical analysis to evaluate their potential as alternatives to traditional microalgal growth media. The parameters such as pH, temperature, concentrations of DO, total dissolved solids, salinity, nitrate, sodium, calcium, and potassium in WWC samples were recorded in table 1. Throughout this study, *T. obliquus* demonstrated the ability to thrive in wastewater, exhibiting a standard growth curve encompassing lag and exponential phases. WWC, rich in nutrients notably that have supported the algal growth and it was used as an alternative nutrient source for isolated species. The genus *Tetradesmus*, along with other microalgal genera, has been extensively studied due to its rapid growth rates and adaptability to various wastewater conditions (Gupta*et al.*, 2016; Singh *et al.*, 2016; Zhang *et al.*, 2008; Martinez *et al.*, 2000). However, the uptake of nutrients from wastewater depends significantly on species-specific factors, initial concentrations of the medium, and cultivation conditions.

| **Parameters** | **Municipal wastewater** |
|---|---|
| pH | 6.61 |
| TDS (ppm) | 682 |
| Salinity (ppm) | 769 |
| Temp ($^0$C) | 27 |
| Potassium (ppm) | 30.2 |
| Calcium (ppm) | 49.7 |
| Sodium (ppm) | 204 |
| DO (ppm) | 3.6 |
| Nitrate (ppm) | 270 |

**Table 1: Showing the physicochemical analysis of wastewater samples (WWC) collected from the Grit Removal Chamber of Sewage Treatment Plant in Coimbatore, Tamil Nadu**

### 3.2 Influence of light intensity

Optimization experiments revealed that a light intensity of 3000 lux has resulted in high content of wet biomass (9.2 g/L), lipid (3.15 g/L) and protein (2.57 g/L) were observed within 12 days. Simultaneously, the higher carbohydrate were recorded as 1.28 g/L in 5000 lux light intensity (Fig. 2).

This underscores the crucial role of light intensity in driving metabolic processes such as biomass synthesis and lipid accumulation in microalgae growth (Grobbelaar, 2010). The findings highlight the importance of optimizing light conditions for maximizing biomass and lipid yields in biotechnological applications, offering valuable insights for sustainable biofuel production and environmental remediation. Furthermore, the observed peak in biomass and lipid production at a light intensity of 3000 lux suggests an optimal balance between photosynthetic activity and cellular growth. These findings contribute valuable insights into the optimization of light conditions for enhancing productivity and efficiency in biotechnological processes, with implications for sustainable energy production.

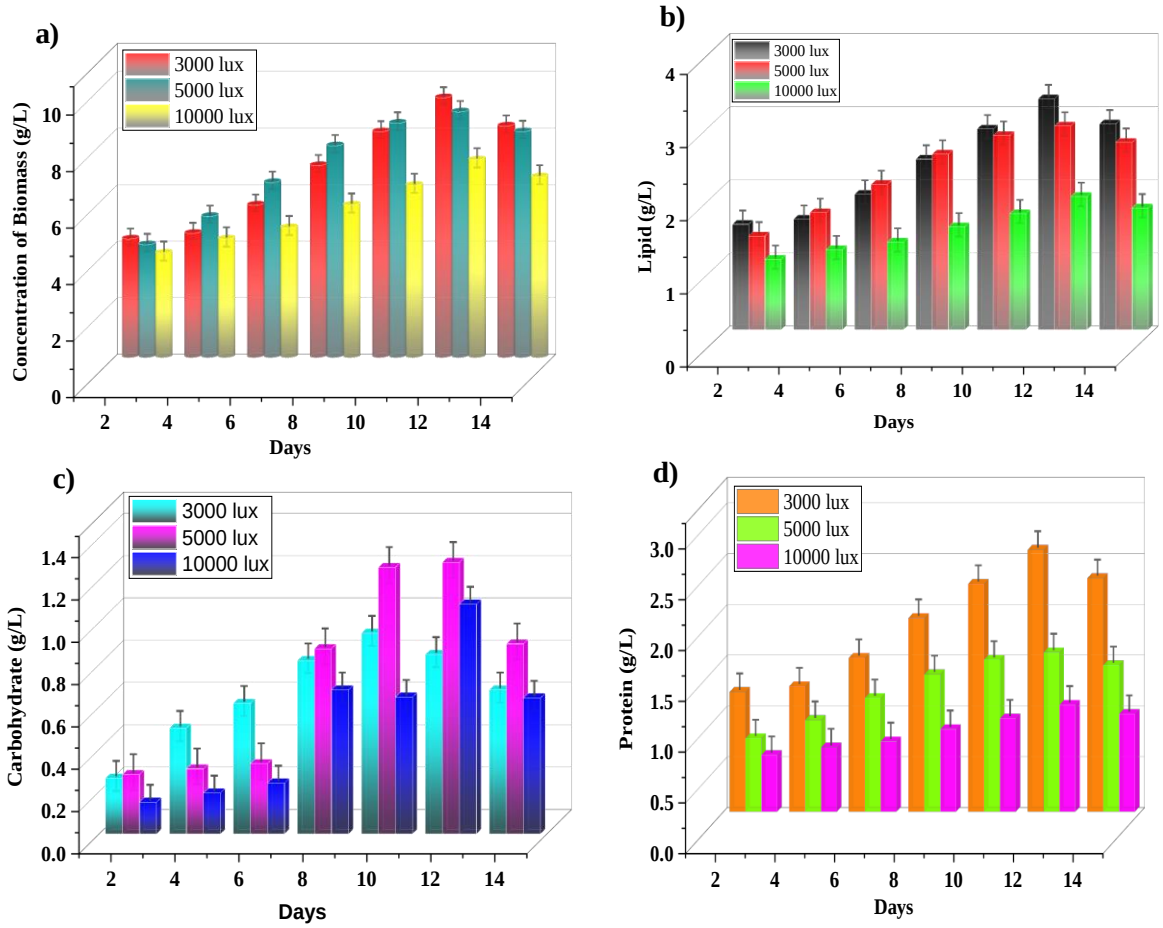


**Fig. 2. Influence of light intensity on (a) Biomass, (b) Lipid (c) Carbohydrate and (d) Protein content of *T. obliquus***

### 3.3 Influence of Temperature

Temperature profoundly influences metabolic activities and growth rates in microorganisms, with optimal temperatures ensuring efficient enzymatic reactions and nutrient uptake kinetics. Optimization experiments observed the maximum wet biomass of 11.2 g/L at 28 °C on day 12 (Fig. 3a), at the same day, concentration of lipid (3.87 g/L) (Fig. 3b), carbohydrate (1.2 g/L) (Fig. 3c) and protein (2.91 g/L) (Fig. 3d) were recorded within 12 days. Lower temperatures, like 18°C, may hinder metabolic rates, while higher temperatures, such as 40°C, could induce stress responses, impacting cell viability and lipid synthesis pathways. Therefore, temperature optimization plays a critical role in maximizing biomass and lipid yields for various biotechnological applications (Buono *et al.*, 2016).Moreover, temperature optimization is crucial for maintaining optimal enzymatic reactions and nutrient uptake kinetics in microorganisms.

The peak in biomass and lipid production was observed at 28°C indicates an ideal balance between metabolic activity and cellular growth. Conversely, lower temperatures like 18°C may slow metabolic rates, while higher temperatures such as 40°C could trigger stress responses, affecting cell viability and lipid synthesis pathways. Thus, precise temperature control is essential for maximizing biomass and lipid yields, offering significant potential for diverse biotechnological applications in sustainable biofuel production and environmental remediation.

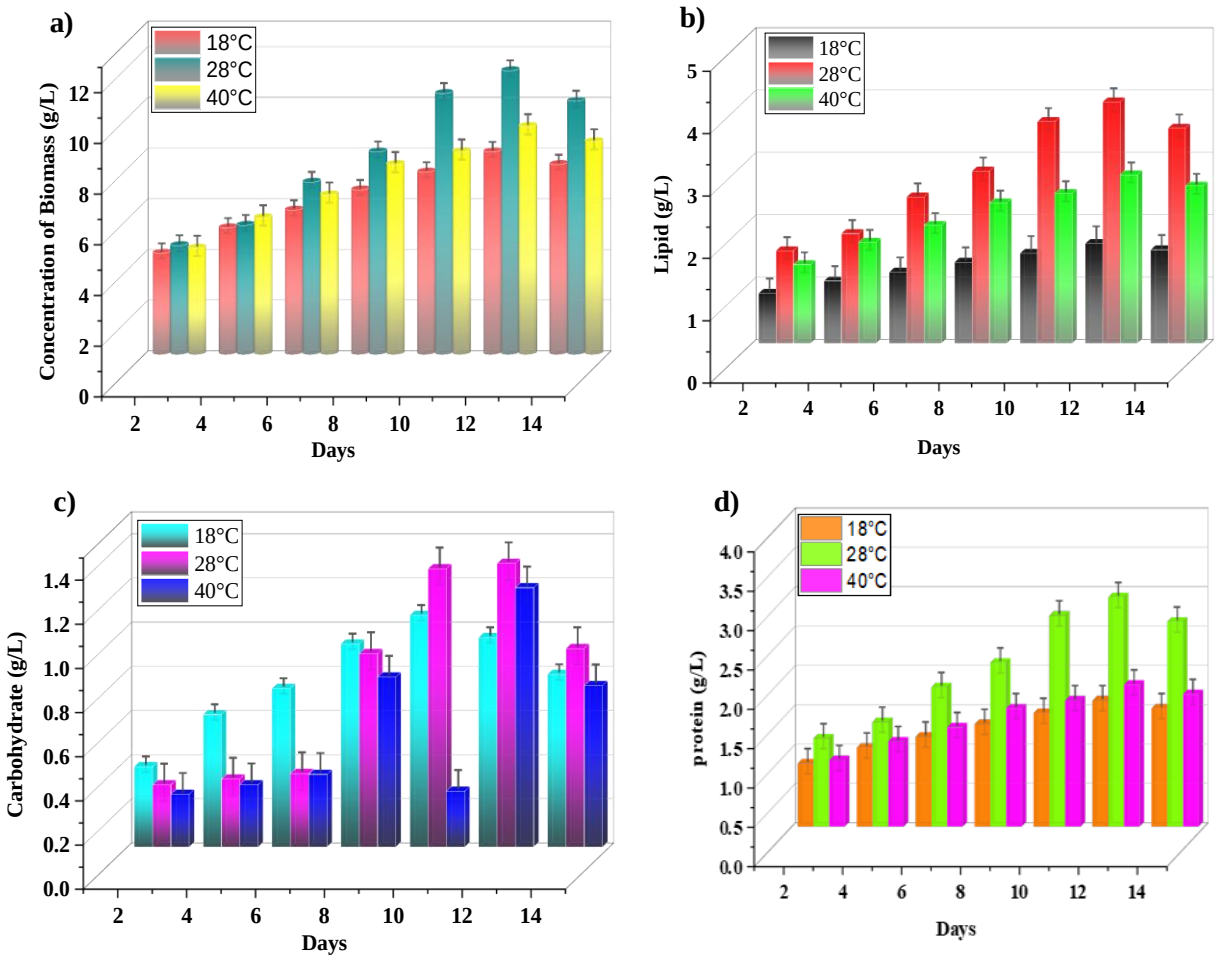


**Fig. 3. Influence of Temperature on (a) Biomass (b) Lipid (c) Carbohydrate and (d) Protein content of *T. obliquus***

### 3.4 Influence of pH

Optimizing pH levels is crucial for enhancing biomass and lipid production in algal cultures. In this study, pH 8 was identified as the optimal condition, yielding a maximum biomass concentration of 11.8 g/L (Fig. 4a), along with a lipid (4.14 g/L) (Fig. 4b), carbohydrate (1.13 g/L) (Fig. 4c) and protein (3.3 g/L) (Fig. 4d) were observed on day 12. These results highlight the significance of maintaining a slightly alkaline pH for promoting metabolic processes conducive to biomass synthesis and lipid accumulation. These findings offer valuable insights for sustainable development in biofuel production and environmental remediation (Bibi *et al.*, 2022). From this experiment, lipid percentage was calculated as 35%. Further, emphasizes the effectiveness of pH optimization in enhancing lipid production, crucial for various biotechnological applications. Intricate relationship between pH and metabolic processes provides better understanding for optimizing microbial cultures and maximizing their productivity for industrial-scale applications (Singh *et al.*, 2019).

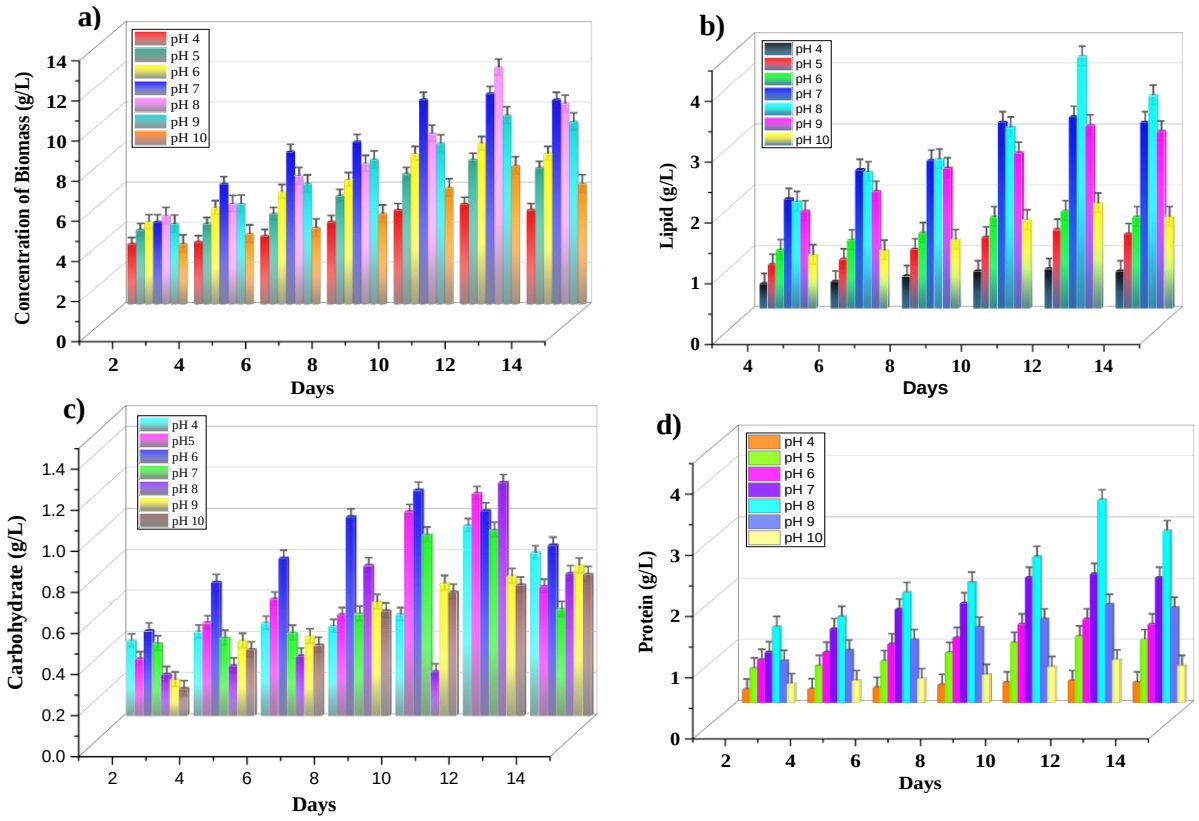


**Fig. 4. Influence of pH on (a) Biomass (b) Lipid (c) Carbohydrate and (d) Protein content of *T. obliquus***

To determine the optimal concentration of municipal wastewater, *T. obliquus* was cultured separately in WWC at varying concentrations using BBM media and RO water. Parameters such as biomass, carbohydrate, protein and lipid were assessed throughout the study period. The biomass content at a combination of 75:25 % ratio of WWC + BBM. The maximum biomass (wet) concentration was reached around 16.22 g/L on day 12 (Fig. 5a), with a biomass productivity of 1.42 mg/L/day. Harini *et al.* **(**2023) reported that the diatom was cultivated with the composition of municipal wastewater (80%) and De Walne's medium (20%). It was noticed that maximum biomass concentration of 17.225 mg/L and the productivity of 1.46 mg/L/day were recorded after 14$^{th}$ day. In a study by Cheng *et al.* (2020), microalgae species such as *Tribonema* sp. and *Synechocystis* sp. achieved maximum biomass production of 1.54 and 1.78 g/L, respectively. Martinez *et al.* (2000) reported that *Scenedesmus obliquus* cultivated in municipal wastewater was produced a substantial amount of biomass as 0.026 g/L. Additionally, *Chlorella zofingiensis* and *Chlorella minutissima* thrived in municipal wastewater, yielding biomass levels of approximately 2.5 g/L and 0.995 g/L, respectively (Zhou *et al.*, 2018; Fatima *et al.*, 2020).

The lipid concentration of *T. obliquus* cultivated in different ratios as presented in Fig. 5b. The maximum lipid content of 5.53 g/L and productivity of 0.353 g/L/day were observed at the 75%:25% combination of WWC + BBM on the 12$^{th}$ day. The previous study revealed that the *Amphora* sp. RRSE1 was able to produce maximum concentration of lipid 10.9 mg/L with the productivity of 0.92 mg/L/day after 14$^{th}$ days of culturing in wastewater and De Walne's medium (Harini *et al.*, 2023). This finding aligns with previous reports indicating the maximum lipid content in *Scenedesmus obliquus* (0.008 g/L), *Chlorella zofingiensis* (0.625 g/L) and *Chlorella minutissima* (0.139 g/L) when utilizing municipal wastewater as a nutrient source (Zhou *et al.*, 2018; Fatima *et al.*, 2020). Our results demonstrate that the lipid content was reached up to 35% under the aforementioned combinations.

This value is comparable to those of lipid-producing microalgae such as *Scenedesmus rubescens*, which has been reported to exhibit a 22% of lipid content (Abou- Shanab *et al.*, 2013). Notably, the newly isolated strain, *T. obliquus* collected from the saltpan of Tamil Nadu, exhibited the ability to produce a significant amount of biomass and lipid in municipal wastewater supplemented with BBM medium.

The carbohydrate concentration of *T. obliquus* cultivated in different ratios was presented in Fig. 5c. The maximum carbohydrate content of 1.48 g/L were observed at the 75%:25% combination of WWC + BBM on the 12$^{th}$ day. This observation aligns with existing literature on microalgae carbohydrate concentrations. Previous studies have reported comparable values ranging from 6.0 mg/mL to 8.5 mg/mL under similar cultivation conditions and nutrient compositions. This consistency in carbohydrate content underscores the reliability and reproducibility of the experimental findings. Furthermore, the observed carbohydrate concentration highlights the potential of utilizing wastewater and BBM combination as an effective medium for cultivating *T. obliquus* for carbohydrate-rich biomass production, which could be further explored for various biotechnological applications, including biofuel production. Further, the maximum protein were recorded as 4.86 g/L in the combination of 75%: 25 % (WWC + BBM) on the 12$^{th}$ day shown in Fig. 5d. The previous study showed the *Tetradesmus* sp. SVMIICT4 have produced the maximum carbohydrate (21.48 mg/g) and protein (19.52 mg/g), when utilizing dairy wastewater as a supplemented nutrient source.

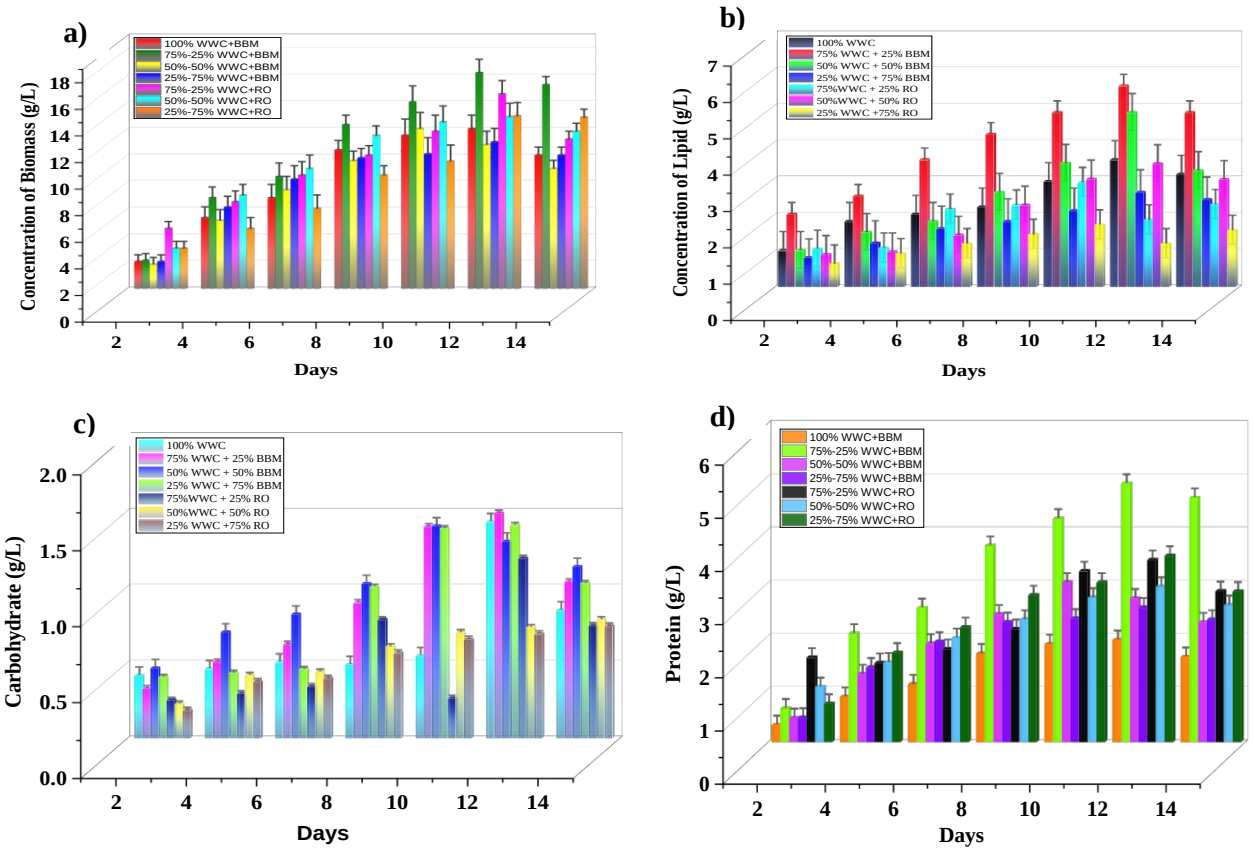


**Fig. 5. Influence of different wastewater ratio on (a) Biomass (b) Lipid (c) Carbohydrate and (d) Protein content of *T. obliquus***

## 4. Conclusion

In addition to the environmental remediation, the utilization of wastewater for algal cultivation offers a sustainable solution for biofuel production. In this respect, the cultivation of *T. obliquus* was studied under various culture conditions which can enhance the biomolecules accumulation in particular lipid. The experiments revealed that the combination of 75% wastewater and 25% Bold's Basal Medium supports the maximum biomass concentration of 16.22 g/L on day 12, with a productivity of 1.42 mg/L/day. Moreover, this combination also enhances the concentration of lipid (5.53 g/L), carbohydrate (1.48 g/L), and significant amount of proteins. Regarding the culture conditions, pH 8, light intensity 3000 lux, and a temperature 28 °C were supported the maximum production of all biomolecules from this study. Continuing research in algal cultivation using wastewater in biorefinery aspects helps to reduce the production cost.

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