Transforming Tofu Waste into a Growth Medium: Boosting Biomass and Proximate Content of Microalgae

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***Abstract*— This study explores the potential of tofu waste as a cost-effective alternative growth medium for cultivating *Spirulina* sp. and *Nannochloropsis oculata*, addressing the high costs of traditional nutrient media that limit large-scale applications. Despite the nutrient richness of tofu waste, its use as a sustainable growth substrate remains underexplored. This research aims to fill this gap by evaluating the growth performance and nutritional suitability of these microalgae in tofu-based media compared to standard controls. The cultivation process was conducted in a closed photobioreactor system, with harvesting methods including flocculation, centrifugation, and filtration. Results showed that tofu waste media supported biomass production comparable to standard cultivation media, with the highest biomass concentrations recorded at the 20% tofu waste treatment, yielding 0.23 ± 0.05 g L^{-1} for *Spirulina* sp. and 0.53 ± 0.2 g L^{-1} for *Nannochloropsis oculata*. At this concentration, the final COD levels were 840.84 mg $L^{-1}$and 825.90 mg L^{-1}, respectively. The lipid and protein contents were 2.44% and 1.71% for *Spirulina* sp., and 1.21% and 1.50% for *Nannochloropsis oculata*, respectively. These findings demonstrate that tofu waste can serve as an effective and low-cost growth substrate for *Spirulina* sp. and *Nannochloropsis oculata*, promoting circular economy principles within many sectors such as energy, food, and agriculture. This study underscores the potential of waste utilization to enhance the sustainability and economic viability of microalgae cultivation.**

Keywords—*Lipid, Microalgae, Nannochloropsis oculata, Spirulina* Sp., *Tofu Wastewater*

I. INTRODUCTION

The Indonesian tofu industry has been established for a long time, aided by easily accessible raw materials and straightforward production methods that enable the sector to be operated on a household scale. Despite its very nutritious products, the production process has an adverse effect on the environment because it produces solid and liquid waste. Protein, carbohydrate, and other organic elements are still present in both wastes [1]. However, tofu waste can pollute the environment and emit disagreeable aromas if it is disposed of directly [2]. Wastewater from tofu can be used as a microalgae growth medium since it contains essential nutrients like N, P, K, and Mg [3]. Widayat et al. [4] claim that tofu wastewater contains organic stuff, specifically 40–60% protein and lipid, along with other compounds that are 25–50% carbohydrate and 10% lipid. Tofu wastewater is therefore an ideal medium for microalgae growth, while also serving as a solution to environmental contamination.

Microalgae have gained significant public attention due to their dual ability to remediate wastewater and produce high-value compounds. Microalgae are eukaryotic, single-celled microorganisms which are capable of thriving in both freshwater and saltwater environments. As photosynthetic organisms, they utilize carbon dioxide and sunlight to produce biomass and oxygen. Microalgae are also rich in a variety of bioactive substances and nutrients, including proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments, phycobiliproteins, enzymes, and other essential compounds [5]. Microalgae are therefore necessary due to their numerous advantages in a number of fields. They can be used as a food supply, medicinal, cosmetic, and renewable energy source [6]. For this reason, microalgae cultivation is a crucial key factor to maximizing biomass productivity.

The process of growing or multiplying the number of microalgae cells to achieve the required biomass is known as microalgae cultivation [7]. A number of factors must be taken into account during the cultivation process in order for it to function effectively and efficiently. The most suitable medium should be used, depending on the type of

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microalgae, when determining the ideal growing system and environment, including temperature, pH, light intensity, and nutrition [8]. Considering these variables can have an impact on biomass's high lipid content and cell density. In this study, tofu wastewater is utilized as the growth medium because it contains abundant nutrients required by microalgae, exhibiting similar characteristics to synthetic media such as Walne, BG-12, and Kw21. Synthetic media are not only expensive but also a major contributor to the high costs of microalgae cultivation. Using tofu wastewater offers a cost-effective and sustainable alternative, addressing these economic challenges while promoting waste utilization.

The two types of microalgae most commonly cultivated for their high-value compounds are *Spirulina* sp. and *Nannochloropsis oculata*. *Spirulina* sp. is a type of blue-green algae that is classified into cyanobacteria, single-celled and spiral-shaped [9]. Meanwhile, a single-celled marine algae belonging to the *Eustigmatophyceae* family is called *Nannochloropsis oculata* [10]. *Spirulina* sp. and *Nannochloropsis oculata* are two possible microalgae to use in the synthesis of lipids as raw materials for biodiesel extraction [11],[12]. The commercial availability of *Spirulina* sp. in large quantities and the high fatty acid content of *Nannochloropsis oculata* make it the preferred strain [13].

The innovative aspect of transforming tofu waste into a growth medium for microalgae cultivation lies in utilizing an abundant by-product from tofu production—often considered waste—as a nutrient-rich substrate. Tofu production generates significant volumes of wastewater containing proteins, carbohydrates, and minerals that can be repurposed. This nutrient profile makes tofu waste highly suitable for microalgae cultivation, potentially offering a low-cost alternative to conventional growth media [14]. Previous studies have shown that agricultural by-products and industrial waste can successfully support microalgae biomass production [15]. According to Putri et al. [16], high biomass, lipid, and protein were produced by *Spirulina* sp. and *Nannochloropsis oculata* growing in 15% v/v and 20% v/v POME wastewater produced 4.67 ± 0.95 g/L ; 4.43 ± 0.36 g/L biomass, 0.87%; 1.11% lipid and 1.03%; 0.86% protein. Six marine microalgae were successful in remediating effluent from seafood processing amounting the biomass of 3.95 ± 0.24 g/L (*Spirulina* sp.), 3.58 ± 0.1 g/L (*Chlorella vulgaris*), 4.77 ± 0.53 g/L (*Porphyridium cruentum*), 2.61 ± 0.01 g/L (*Tetraselmis chuii*), 5.15 ± 1.27 g/L (*Nannochloropsis oculata*), 3.98 ± 0.06 g/L (*Chaetoceros calcitrans*), while *Nannochloropsis oculata* generating lipid and protein (20.05 ± 1.36% and 32.41 ± 2.21%) according to Dewi et al. [17] *Chlorella vulgaris* and *Nannochloropsis oculata* were successfully grown in tofu wastewater, according to Taufikurahman et al. [18], yielding high lipid at 4% v/v tofu wastewater and high biomassa at 6% v/v tofu wastewater. Several studies have explored the impact of environmental parameters on microalgae growth rates. Tian et al. [19] showed that the maximum specific growth rate of *Desmodesmus* sp. using turtle aquaculture wastewater with a light intensity of 24 h/d was obtained at a temperature of 30ºC which produced 58.20% protein and 10.24% lipid.

Additionally, while many studies focus on algae cultivation for food products and supplements, few investigate its use for biofuels along with bioremediation. Although the effects of tofu wastewater, cultivation time, and photoperiod have been explored for microalgae species such as *C. pyrenoidosa* [20], *Euglena* sp. [21], *Spirulina plantensis* [22], *Chlorella vulgaris* [23], *Chlorella* sp. [24], and *Arthrospira platensis* [25] , limited research has been done on the impact of tofu wastewater at concentrations of 5-10% on *Spirulina* sp. and *Nannochloropsis oculata* with a light photoperiod (L/D) of 24:0 hours for 14 days to assess their growth performance. As previously noted, utilizing tofu waste in this manner can improve the economic and environmental sustainability of both tofu production and microalgae cultivation, offering a dual solution for waste management and bioresource optimization [26]. Thus, the purpose of this study was to investigate how tofu wastewater concentration and cultivation period affected the growth of microalgae. *Spirulina* sp. and *Nannochloropsis oculata* were cultivated in tofu wastewater using a closed photobioreactor system at five concentrations (0%, 5%, 10%, 15%, and 20%) for 14 days to assess their growth performance. Tofu wastewater has been found to be an efficient medium for growing *Spirulina* sp. and *Nannochloropsis oculata*, hence the work marks a significant scientific advancement. The nutrients in Tofu Wastewater can support the fast growth of microalgae at an ideal concentration, allowing for the generation of significant amounts of biomass, lipids and COD reduction.

II. METHOD

A. *Cultivation Spirulina* sp. *and Nannochloropsis Oculata in Tofu Wastewater*

Spirulina sp. and *Nannochloropsis oculata* microalgae were obtained from the Jembrana Marine and Fisheries Polytechnic, Bali, Indonesia. The wastewater from the tofu business in Balikpapan, East Kalimantan, Indonesia, was acquired.

TABLE **1**. shows the characteristics of tofu wastewater.

TABLE 1.
CHARACTERIZATION OF TOFU WASTEWATER

Parameter	Unit	Result
Physical Test		
pH	-	4.43
Temperature	°C	24.6
TDS	mg/L	969
TSS	mg/L	199

Parameter	Unit	Result
Turbidity	NTU	153
Salinity	%	0.4
	Chemical Test	
Ammonia as N	mg/L	0.66
BOD5	mg/L	971.9
COD	mg/L	2,776.85
Nitrate as N	mg/L	1.2
Oil & Grease	mg/L	0.21
Total Phosphate	mg/L	1.68
Total N	mg/L	96

B. Experimental

A batch system using a polyethylene flask and continuous aeration was used to cultivate microalgae with a total volume of 1000 ml (Figure 1). A total of 20% (v/v) microalgae were incorporated in the flask with the addition of saline water and tofu wastewater at 0%, 5%, 10%, 15%, 20% (v/v) concentrations. The cultivation was maintained at 5000 lux of light intensity, pH 8–9, 20–30°C, and 30 ppt of medium. For 14 days, daily analyses of pH, temperature, salinity, COD, and absorbance were carried out. The highest biomass concentration was then analyzed to obtain its proximate content.

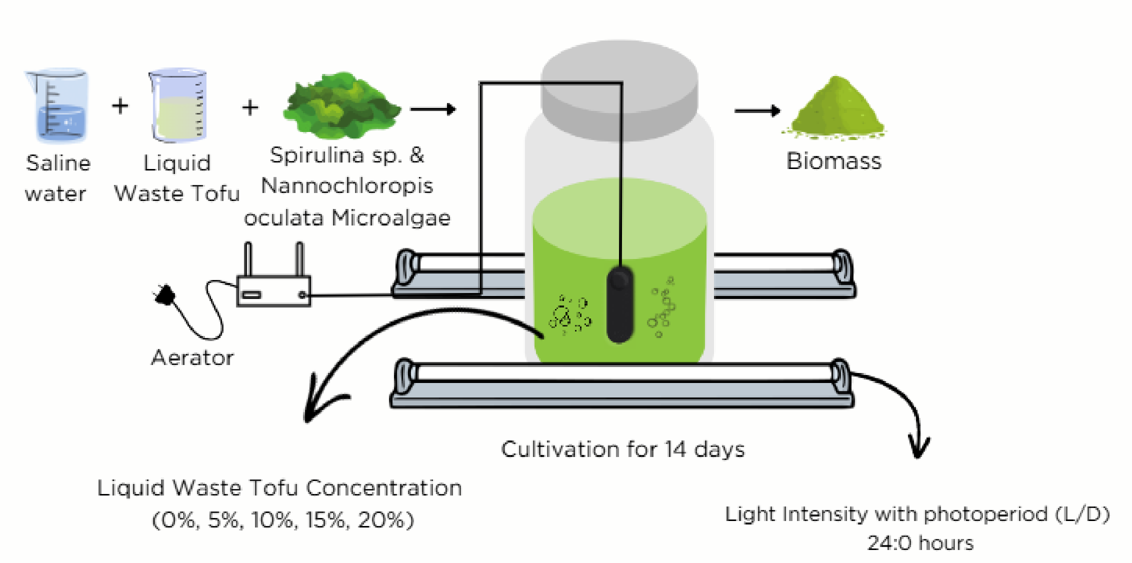


Figure 1. Microalgae Cultivation Setup

C. Optical Density (OD)

Optical Density (OD) analysis was performed using thermo scientific uv-vis spectrophotometer AQUAMATE8100 Model in Serial Number 9A8Y234004 at 680 nm. A volume pipette was used to take 5 ml of each sample, which was then put into the cuvette until ¾ of it was full. The analysis was conducted to ascertain the biomass of microalgae. Cell development is shown by an increase in the OD value, but cell death or suppression of microalgae cell growth may be indicated by a reduction in the OD value. The standard curve was then used to calibrate the OD into biomass concentration as shown in the supplementary materials.

D. Proximate Analysis

Proximate analysis is a chemical analysis to determine the basic composition of microalgae biomass and to give a general picture of the biomass quality that is generated during the cultivation process. Proximate analysis of the microalgae biomass includes the determination of water content using a gravimetric procedure in accordance with SNI 2354.2:2015. Lipid content was evaluated through solid-liquid extraction using a Soxhlet apparatus, based on SNI 2354.3-2017. Using the method described in SNI 01-2354.4-2006, the total nitrogen was measured to determine the protein concentration. Ash content was evaluated by drying and ash the sample, following SNI 2354.1:2010.

E. Chemical Oxygen Demand Analysis

The COD analysis was conducted using the Dewi et al technique [27]. To determine the COD content in the sample, 10 ml of 0.01 N $C_2H_2O_4$, 5 ml of 4 N H_2SO_4, and $KMnO_4$ solution were required. To standardize the KMnO4 solution, add 10 ml $C_2H_2O_4$ 0.01 N and 5 ml H_2SO_4 4 N to an Erlenmeyer flask and heat the mixture to 70-80°C. The solution was then titrated with $KMnO_4$ until the color turned red (b mL). The normality of $KMnO_4$ was estimated solution was estimated using the following equation (1):

$$N_{KMnO_4} = \frac{N_{C_2H_2O_4} \times V_{C_2H_2O_4}}{V_{KMnO_4}} \quad (1)$$

Where N and V are the normality and volume (ml), respectively

The COD content was determined by combining 10 ml of sample, 5 ml of 4 N H_2SO_4, and 3.2 ml of $KMnO_4$ solution in an Erlenmeyer. The mixture was then heated to temperatures ranging from 70 to 80 degrees Celsius. Then, 10 mL of 0.01 N $C_2H_2O_4$ was added. The solution was titrated with standardized $KMnO_4$ and the needed volume was measured (a ml). COD was computed using the equation (2) below:

$$COD = \left[(a \times b)N_{KMnO_4} - (V \times N)_{C_2H_2O_4}\right] \times 8000 \qquad (2)$$

Where COD, N, and V are the chemical oxygen demand (ppm), normality, and volume (ml), respectively

F. Data Analysis

IBM SPSS Statistics 26 was used to statistically analysis the data using t-test analysis. The dependent t-test was done in each microalga, while the independent t-test was conducted within four groups (moisture, ash, lipid, and protein content).

III. Results and Discussion

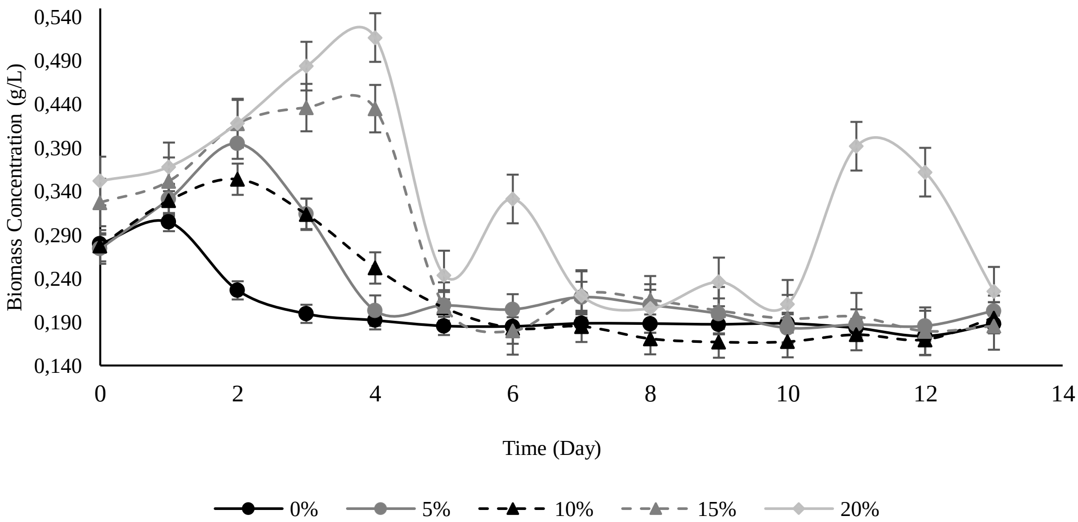
A. Effect of Tofu Wastewater on Biomass Concentration

Figure 2 and Figure 3 depicts the biomass concentration of *Spirulina* sp. and *Nannochloropsis oculata* when cultivated in tofu wastewater at various concentrations: 0%, 5%, 10%, 15%, and 20%. The biomass concentration is measured over a 14-day period. These microalgae species' growth dynamics and total biomass production will be examined in relation to the addition of tofu wastewater.

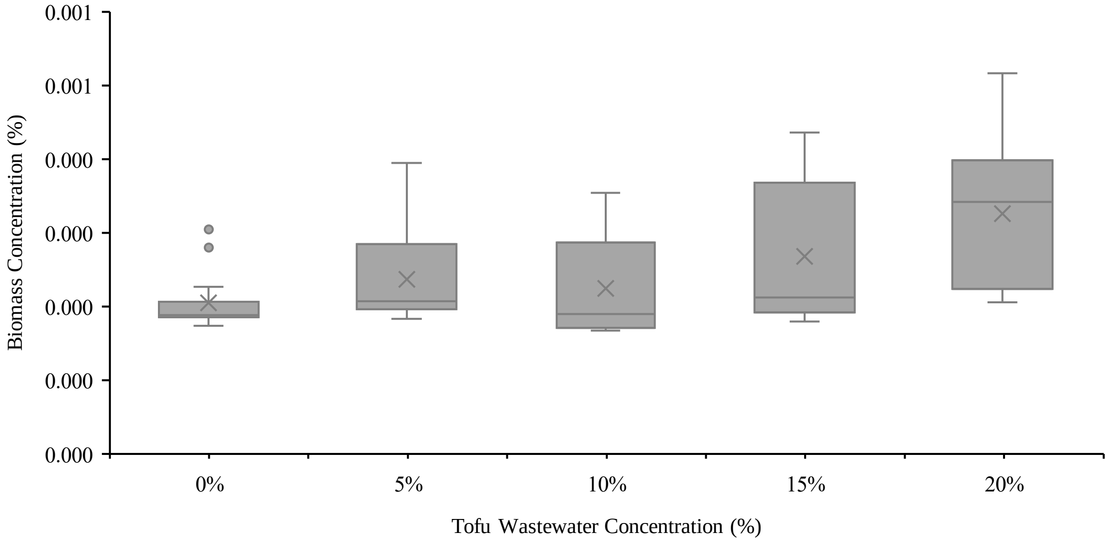
According to the findings, the biomass concentration of *Spirulina* sp. shows a varying response to different tofu wastewater concentrations over time. Initially, at day 0, the biomass concentration was low across all tofu wastewater treatments, reflecting the start of the cultivation process amounting of 0.27 ± 0.01 – 0.35 ± 0.05 g/L. This phase is called the lag phase or the initial period of slow growth in a microalgae culture. During this phase, the organisms are adapting to their new environment, synthesizing necessary enzymes and proteins, and preparing for rapid cell division [28]. Additionally, when *Spirulina* sp. grows on wastewater with a concentration of 15-20%, the lag phase was clearly visible; however, when the waste concentration is lower, the lag phase only lasted for 24 hours.

During the first 4 days, there is a noticeable increase in biomass concentration for all treatments, with the 20% tofu wastewater concentration reaching the highest peak (0.51 ± 0.16 g/L) and control is the lowest one (0.19± 0.01 g/L). This suggests that high concentrations of tofu wastewater (20%) provide optimal conditions for the early growth phase demonstrating that the microalgae in this percentage were able to make the most of the waste [28], [29]. Day 4 is also the exponential period. Cells reproduce and expand exponentially during this phase, causing the population to double at a steady rate and producing a large increase in biomass [30], [17]. After day 4, the biomass concentration of *Spirulina* sp. begins to decline entering the stationery and death phases.

The 0% tofu wastewater concentration (control) shows a more stable but lower overall biomass production throughout the experiment, suggesting that without any tofu wastewater, growth is slower but steady (0.19 ± 0.02 g/L on day 13). The 5% tofu wastewater treatment also shows a relatively stable growth pattern but at a slightly lower biomass concentration than the 10% treatment accounting for 0.20 ± 0.01 and 0.19 ± 0.02 g/L.



(a)

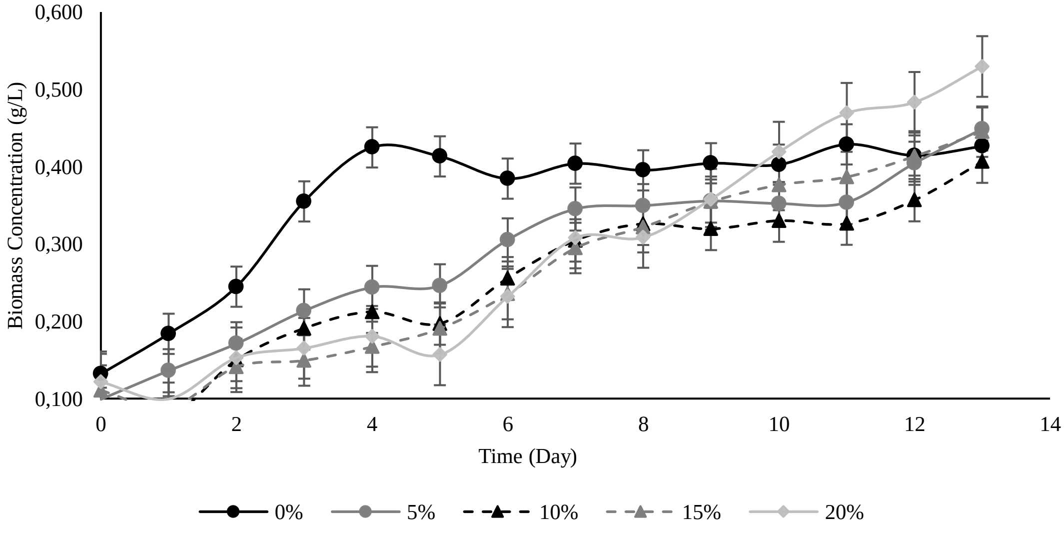


(b)

Figure 2. Biomass Concentration of *Spirulina* sp. at various Tofu Wastewater Concentration for 14 Days; (a) Biomass Concentration of *Spirulina* sp. (b) Box Plot of Biomass Concentration of *Spirulina* sp. Average values are shown (n = 3).

All treatments' biomass concentrations seem to decrease considerably by the conclusion of the experiment, particularly those with greater tofu wastewater concentrations (15 and 20%). This decline might be attributed to nutrient depletion or the accumulation of inhibitory compounds in the medium. Prayitno and Dewi et al. [31], [32] mentioned that after more than a week, the nutrients in the medium were significantly decreasing making the microalgae cells' rate of growth slow down. Thus, there will be an equal number of living cells and dead cells. Additionally, the proliferation of the cells is inhibited by the accumulation of secondary metabolites in the media [21], [33]. When a cell enters the death phase because of a lack of nutrition, there are more dead cells than living cells.

In this investigation, it was demonstrated that microalgae in this fraction were able to fully utilize the waste since high concentrations of tofu wastewater did not impair the microalgae's growth. Nevertheless, in Ajijah's 2019 [25] study, *Spirulina platensis* had the highest biomass yield at a 3% concentration of tofu wastewater. This may be due to species variations as well as increased pollutant loads (Table 1) in comparison to Ajijah et al.'s study [25], which found that the levels of COD, phosphate, and nitrate were merely 374 mg/L, 0.2 mg/L, and 13.7 mg/L, respectively. Similarly, *Spirulina* sp. grew at its best when a very high quantity of tofu wastewater was introduced, with a biomass absorbance of 0.86 to 0.91 [18]. Musa et al. [34] also found that *Chlorella pyrenoidosa* resulted the highest growth rate when 20% of tofu wastewater was added which was 0.826 1/day. Tofu wastewater is abundant in a variety of organic and inorganic substances, including metal ions, phosphate, sulfate, NH4+, vitamins, organic acids, monosaccharides, oligosaccharides, and other nutrients [20], [35]. Monosaccharide and oligosaccharide, which microalgae may efficiently use as an additional carbon source, are among the nutrients that tofu wastewater can provide in sufficient amounts for algal growth [36].



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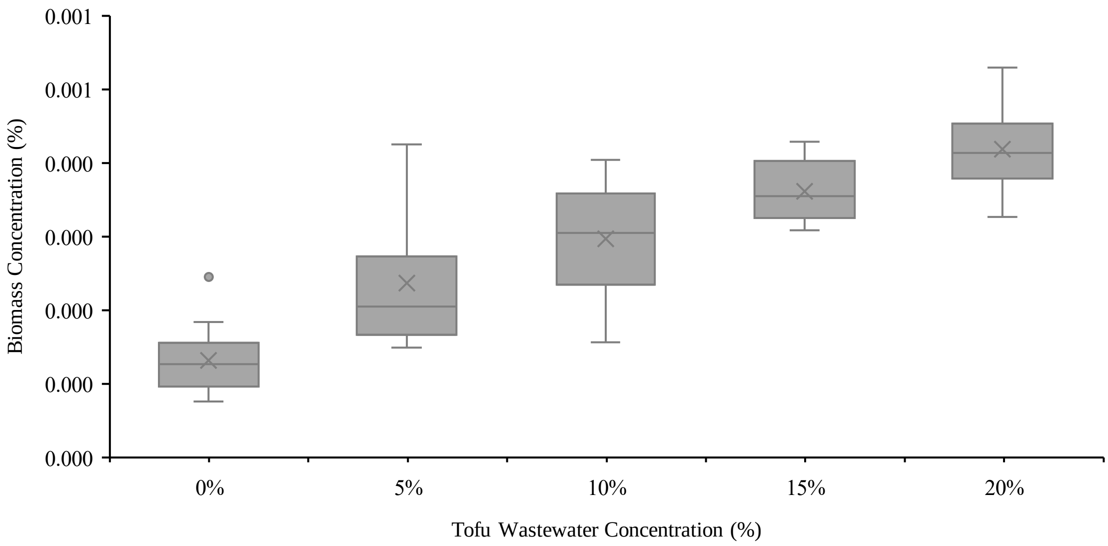


(b)
Figure 3. Biomass Concentration of *Nannochloropsis oculata* at various Tofu Wastewater Concentration for 14 Days; (a) Biomass Concentration of *Nannochloropsis oculata* (b) Box Plot of Biomass Concentration of *Nannochloropsis oculata.* Average values are shown (n = 3).

The boxplot (Figure 2b) presents the relationship between tofu wastewater concentration and the resulting biomass concentration of *Spirulina* sp. At 0% tofu wastewater (control), the biomass concentration is the lowest, averaging around 0.20%, with a small interquartile range, indicating a more consistent growth pattern but limited biomass production. As the concentration of tofu wastewater increases to 5%, 10%, 15%, and 20%, there is a noticeable rise in biomass concentration, with the highest median values observed at 15% and 20%, suggesting that higher concentrations of tofu wastewater promote more robust *Spirulina* growth. The distribution in these higher concentrations is wider, indicating more variability in growth outcomes. This is because the tofu wastewater contains nutrients such as phosphate and nitrogen, which *Spirulina* sp. utilized to fuel its growth. Thus, as the wastewater content dropped, *Spirulina* sp. biomass also dropped, suggesting that there was less available nitrogen and phosphorus [6], [34].

The results suggest that tofu wastewater, likely due to its nutrient content, supports *Spirulina* sp. cultivation effectively at concentrations higher than 5%. This may imply that the organic material and nutrients in tofu wastewater provide a favorable environment for *Spirulina* growth, though the variability at higher concentrations (e.g., 20%) could indicate differences in how *Spirulina* sp. responds to excessive nutrients or possible inhibitory effects [37]. Overall, the research points to tofu wastewater as a viable medium for enhancing biomass production, especially at concentrations of 15-20%.

In contrast, Figure 3a illustrates the biomass concentration of *Nannochloropsis oculata* across different tofu wastewater concentrations. The trend is different compared to *Spirulina* sp. From day 0 to day 5, there was a consistent increase in biomass for all treatments. The 0% tofu wastewater (control) treatment reached the highest biomass concentration (0.42 ± 0.01 g/L) on day 5 and maintained a relatively high level throughout the experiment. Interestingly, while the control treatment performs the best overall, the other tofu wastewater concentrations (5%, 10%, 15%, and 20%) showed a more gradual and sustained increase in biomass over time, without the steep decline observed in *Spirulina* sp. The 20% tofu wastewater treatment consistently outperformed the higher tofu wastewater concentrations and shows steady growth, peaking 0.53 ± 0.20 g/L by day 13. The 5%, 10%, and 15% show similar trends but at lower biomass levels, indicating that higher TW concentrations could be utilized by *Nannochloropsis oculata*. When compared to *Spirulina* sp., *Nannochloropsis oculata* produced the most biomass, which is 2-folds. Hafidzah et al. [38] found that *Nannochloropsis oculata* has great potential for bioremediation. According to earlier research by Şirin & Sillanpää [39], they can also endure high salinity levels.

When grown in wastewater from seafood processing, *Nannochlorpsis oculata* produced more biomass than *Spirulina* sp., *Chlorella vulgaris, Porphyridium cruentum, Tetraselmis chuii, and Chaetoceros calcitrans*, according to a previous study [40]. Additionally, *Nannochloropsis oculata* can thrive when grown in municipal wastewater, according to Sofiyah &Suryawan [41]. Therefore, *Nannochloropsis oculata* is thought to be useful for wastewater remediation, especially because of its capacity to eliminate pollutants and absorb nutrients from a variety of wastewater streams. Nitrogen (N), phosphorous (P), and other substances commonly found in wastewater are removed by this microalga, which is renowned for its high nutrient uptake efficiency [20]. These characteristics make it a feasible choice for bioremediation and sustainable wastewater treatment.

The data show that at 0% tofu wastewater (control), the biomass concentration is the lowest, with a median of around 0.15%, and a narrow interquartile range, indicating consistent but low growth. As the tofu wastewater concentration increases, the median biomass concentration steadily rises, with the highest median recorded at 20%, approximately 0.35%. This suggests that higher concentrations of tofu wastewater provide an environment that better supports the growth of *Nannochloropsis oculata*.

The increasing trend from 0% to 20% tofu wastewater concentrations highlights the positive influence of tofu wastewater on biomass production, likely due to the presence of nutrients necessary for microalgae growth [42], [43]. However, the widening interquartile ranges at higher concentrations, especially at 20%, indicate greater variability in the biomass yield. This could suggest that while higher tofu wastewater concentrations generally promote better growth, the response of *Nannochloropsis oculata* may vary depending on other factors, such as nutrient balance or potential inhibitory effects from excess organic content. Wang et al. [44] discovered that tofu wastewater may quadruple *Chlorella pyrenoidosa* biomass productivity when compared to the BG-11 medium under mixotrophic conditions. Similarly, Nur et al. [45] found that mixotrophic growing of microalgae TW increased *Spirulina platensis* biomass production. Therefore, tofu wastewater proves to be a promising medium for enhancing the biomass production of *Nannochloropsis oculata*.

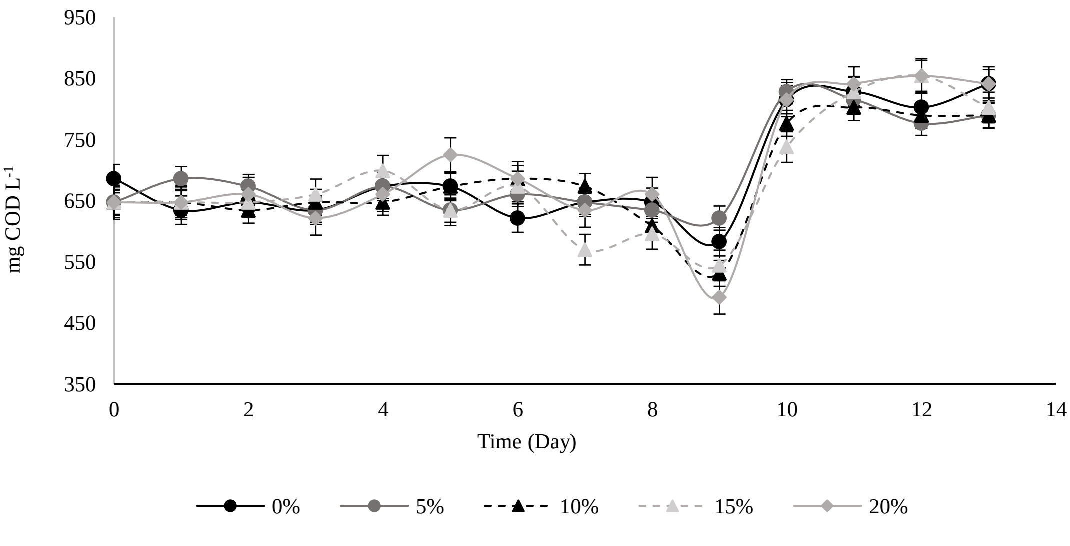
B. *Effect of Microalgae on COD Reduction*

A chemical technique for calculating the amount of oxygen needed to oxidize materials in a water sample is called chemical oxygen demand, or COD [46]. A high COD level suggests that the water contains a lot of reducing chemicals, primarily organic materials. It also indicates the amount of reducing compounds present in the water [47]. According to reports, *Spirulina* sp. has an effective removal efficiency against COD in the starting content range of 600 mg COD L^{-1}, although the efficiency varies greatly from 2% to 90% [48],[16]. *Spirulina* sp. has been used in wastewater treatment as a mixotrophy cyanobacteria because of its ability to use both organic and inorganic carbon for biomass growth [49], [50].

Riano et al. (2012) [51] also discovered that, after 37 days of cultivation, *Spirulina* sp. eliminated 30% of COD at an initial COD concentration of 181 mg L^{-1}. However, all five concentrations' end COD contents did not significantly decrease from the initial COD content at the experiment's initial content range of 647 mg L^{-1} (Fig. 4a). After cultivation, the COD contents of the five concentrations gradually decreased with the cultivation time of 9 days with an average decrease around 100 mg L^{-1} and a significant increase after the 9th day of cultivation around 200 to 300 mg L^{-1}. During the 14-day cultivation, the maximum COD removal efficiency of *Spirulina* sp. was on day 9 with 20% concentration of 24%. This shows a linear relationship between COD and tofu waste concentration in *Spirulina* sp. microalgae during the cultivation process between day 1 to day 9. The reduction in COD content suggests that *Spirulina* sp. can absorb organic matter, such as exopolysaccharides released, to support its growth [52],[53]. Furthermore, as cultivation time grew, COD levels fell, implying that microalgae development is linked to the consumption of available organic matter in wastewater. As a result, the wastewater's organic content was found to have decreased [54].

However, on the 10th day, all concentrations gave a very significant increase in COD, namely an increase of 200-300 mg L^{-1}. The increase in COD content may be due to the death of some microalgae cells after inoculation of microalgae transfer from media to wastewater [47] and also allows the available organic matter in the tofu waste to be depleted. Thus, there was a significant increase as seen in the 9th day peak (Figure 4a).

Similar to *Spirulina* sp., *Nannochloropsis oculata* likewise underwent a drop on the first day of cultivation, followed by a progressive increase on the second day until the fourteenth day, as illustrated in Figure 4b. A significant increase occurred at a concentration of 15%, while at a concentration of 20% there was only an increase in COD around 25%. The increase in COD in the culture of *Spirulina* sp. and *Nannochloropsis oculata* with tofu wastewater is caused by bacteria digesting dead microalgae.



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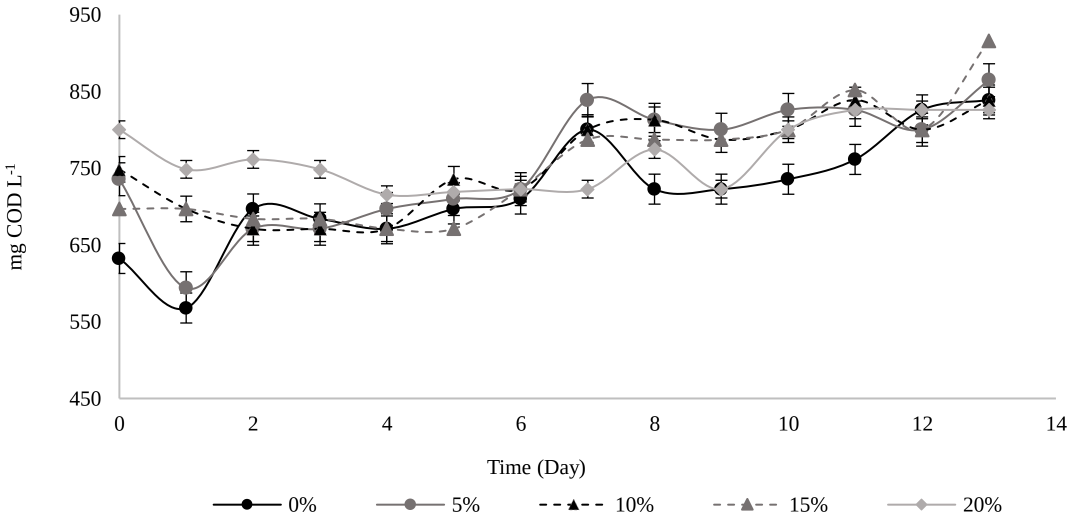

(b)
Figure 4. COD result at various Tofu Wastewater concentrations of 14 Days;
(a) *Spirulina* sp. (b) *Nannochloropsis oculate*

According to Ramraj et al. (2015) [55], there are two possible causes for the increase in COD: the first is the bacterial decomposing of old microalgae, and the second is the release of organic matter following the fixation of carbon dioxide by microalgae. The growth of algae and the increase in COD in the wastewater are attributed to a "carbon imbalance" in the system. As a result, higher COD levels may arise from an imbalance between bacteria and microalgae or from extended cultivation periods [55][56]. In conventional wastewater treatment using microalgae, COD removal is inefficient, as microalgae release organic compounds, which ultimately lead to higher COD levels [56][57].

Microalgae may generally lower dissolved COD in wastewater during their early development stages. However, during the stationary growth phase, they create organic molecules, which may cause COD levels to rise again. These released compounds can sometimes lead to cell death or act as growth inhibitors, hindering further growth [57]. Cultivating *Spirulina* sp. and *Nannochloropsis oculata using* wastewater media poses a significant challenge due to the variability in the nutritional composition of the wastewater, which can greatly influence the microalgae's biomass, growth, and subsequent nutrient removal. Such that the COD decrease throughout the cultivation phase might also be impacted.

C. Proximate Analysis of Microalgae Biomass

The chart illustrates the proximate composition of *Spirulina* sp. and *Nannochloropsis oculata* cultivated in tofu wastewater, comparing their moisture, ash, lipid, and protein contents (Figure 5). The ash content, indicating mineral presence, differs significantly between the two species. *Nannochloropsis oculata* has a higher ash content of 6.11%, compared to 4.61% in *Spirulina* sp. This disparity can be linked to the nutrient absorption and mineral accumulation properties of *Nannochloropsis oculata*. Previous study mentioned that certain microalgae have the ability of accumulating more minerals when cultivated in wastewater environments [26], [58]. In addition, *Spirulina* sp. shows a notably higher moisture content of 90.41%, compared to 86.55% for *Nannochloropsis oculata*, which could be attributed to the distinct structural differences between these microalgae species. High moisture content in microalgae often reflects water retention capabilities related to the cell wall and cytoplasmic components, a characteristic commonly observed in *Spirulina* sp. due to its larger cellular size and more gelatinous structure [59],[60].

The lipid content also varies, with *Spirulina* sp. showing a slightly higher lipid concentration (2.44%) than *Nannochloropsis oculata* (1.21%), which may relate to the different metabolic adaptations of these species in response to environmental stressors present in tofu wastewater, such as high organic matter and nitrogen levels. Additionally, turbidity in microalgae could decrease light penetration, which affects the algae's lipid and protein content as well as its productivity (photosynthesis) [61]. *C. sorokiniana* grown in aquaculture effluent had lipid levels of 24.57% and 30.15%, respectively. Compared to the biomass generated in synthetic medium, which included 35.75% fat and 28.64% protein, these values were lower [62]. In terms of protein content, it is a crucial parameter for assessing the potential of microalgae as a nutritional supplement. *Spirulina* sp. displays a protein content of 1.71%, which is marginally higher than the 1.50% in *Nannochloropsis oculata*. This difference may be a result of *Spirulina* sp.'s nitrogen assimilation capacity, which facilitates protein synthesis more effectively in nutrient-rich wastewater environments. Prior research also reported that *Spirulina* sp. has a higher protein content than other microalgae, and is considered a promising source of protein [63], [64]. According to Hadiyanto et al. [65], the protein content in *Spirulina* sp ranges from 60-70%, and lipid 20-50% (dry weight). Spirulina sp. microalgae is renowned for its high protein content, which can constitute up to 80% of its biomass [66]. While in *Nannochloropsis oculata* (dry weight) the lipid content ranged from 12.51 ± 1.01% and protein 34.11 ± 0.70% (dry weight) [65][66].

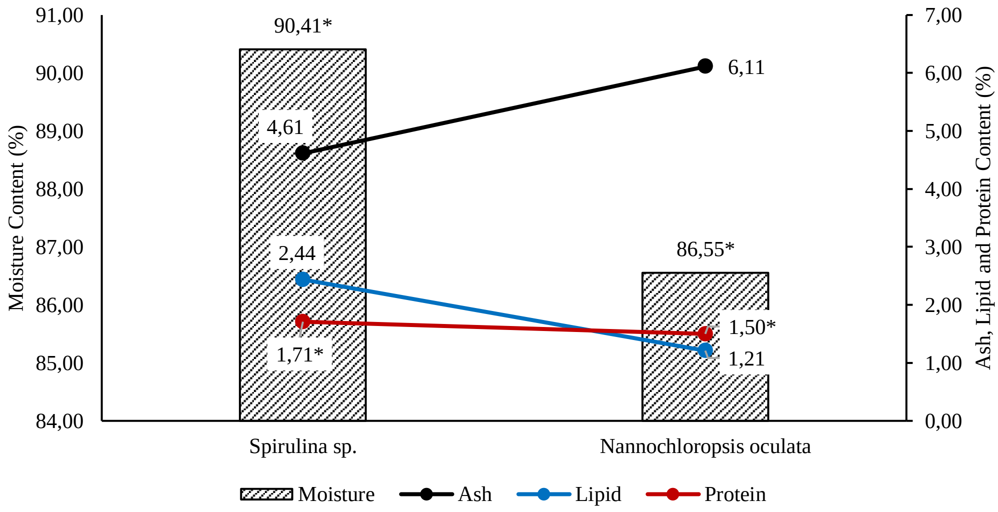

Figure 5. Proximate Content of *Spirulina* sp. and *Nannochloropsis oculata*.
Asterisk (*) is significantly different ($p < 0.05$).

Overall, these results underscore the differential nutrient composition and possible functional applications of Spirulina sp. and *Nannochloropsis oculata* cultivated in tofu wastewater, with implications for their potential in bioremediation and as nutritional resources. To further enhance their proximate content, optimizing the cultivation conditions in tofu wastewater could be effective, such as adjusting pH, light intensity, and nutrient supplementation. Adding trace minerals or nitrogen sources may boost protein synthesis, while regulating carbon sources can enhance lipid accumulation [67],[68]. Additionally, controlling the duration of cultivation and implementing stress conditions, like nitrogen limitation for lipid enrichment, could improve the overall nutritional profile and make these microalgae more viable for commercial applications in food and feed industries [69],[70],[71],[72],[73],[74],[75],[76].

IV. Conclusion

In order to compare the nutritional appropriateness and growth rates of *Spirulina sp.* and *Nannochloropsis oculata* in tofu-based medium to standard controls, this study will substitute tofu waste for traditional culture media. The research demonstrates the viability of using tofu waste as a nutrient-rich growth medium for *Spirulina sp.* and *Nannochloropsis oculata* over 14 days in a closed photobioreactor system, offering an effective alternative to often costly conventional nutrient media. This study supports the idea of a circular economy by using tofu waste, which is normally thrown away, to create microalgae. The growth performance of *Spirulina* sp. and *Nannochloropsis oculata* in tofu waste media, assessed through biomass yield and proximate content, closely matches that of standard media, confirming its potential as a viable substitute. This approach not only enhances biomass production but also provides an environmentally friendly solution by reducing COD levels. Furthermore, adding tofu waste to the growth of *Spirulina* sp. and *Nannochloropsis oculata* increases the viability of producing microalgae for use in the food, feed, and biofuel sectors. The findings of this study highlight the importance of interdisciplinary approaches in tackling global sustainability challenges through innovative resource management and waste utilization strategies.

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