

Article

Simultaneous Phycoremediation and Lipid Production by Microalgae Grown in Non-Sterilized and Sterilized Anaerobically Digested Brewery Effluent

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Abstract: Microalgae have the ability to utilize nutrients present in wastewater and generate biomass that is abundant in carbohydrates, lipids, and proteins. The ability of microalgae to integrate wastewater management and biofuel production makes them a promising solution for enhancing environmental sustainability. The objective of this study was to assess the potential of local microalgae, *Scenedesmus* sp., to simultaneously remediate wastewater and produce lipids. The microalgae were cultivated in anaerobically digested brewery effluent, both sterilized and non-sterilized, to evaluate their phycoremediation and lipid production capabilities. The phycoremediation study was investigated by measuring chemical oxygen demand (COD), total nitrogen (TN), ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), and total phosphorus (TP) removal from brewery effluent. Lipids were extracted from microalgal biomass without and with pretreatment methods, such as microwave, autoclave, osmotic stress, oven heating, and HCl digestion in a water bath, to enhance lipid extraction. Results indicate that *Scenedesmus* sp. achieves higher biomass production in non-sterilized brewery effluent compared to sterilized brewery effluent. Conversely, it attains higher lipid accumulation in sterilized brewery effluent compared to non-sterilized brewery effluent. *Scenedesmus* sp. also attained a higher removal of TP (69.32%) and COD (77.78%) in non-sterilized effluent, but TN (96.14%) in sterilized brewery effluent. The removal of $\text{NH}_4^+\text{-N}$ was nearly 100% in both effluents. The maximum lipid content obtained was 14.79%, which was enhanced by 39.06%, 23.89%, 15.81%, 11.61%, and 4.78% after microwave, HCl digestion, autoclave, osmotic, and oven heating pretreatments, respectively. The findings of this study demonstrate that local microalgae have a great potential for wastewater remediation with lipid production using appropriate pretreatment methods.

Keywords: brewery effluent; lipids; pretreatment; phycoremediation; *Scenedesmus* sp.



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1. Introduction

Wastewater management and remediation practices in various industries pose a challenge to many countries due to the presence of various organic and inorganic nutrients [1]. Nitrogen and phosphorus nutrient management is a primary requirement for wastewater management; if discharged into rivers and lakes, it can lead to eutrophication [2], which can then affect the sustainability of the environment. To mitigate this, different wastewater management strategies have been implemented. Microalgae have become a viable alternative treatment option for wastewater management due to their higher growth rate, biomass and lipid production, photosynthetic efficiency, and CO_2 fixation rate [3]. They offer the possibility of integrating biomass production with wastewater remediation. The microalgal biomass obtained from wastewater treatment can be converted into various biofuels, such

as biodiesel, bioethanol, or biogas [4,5]. However, lipid and carbohydrate extractions are required before biodiesel and bioethanol production from microalgal biomass.

Phycoremediation is the process of using microalgae to remove nutrients and other pollutants from wastewater [6]. *Chlorella* and *Scenedesmus* are fast-growing species that are commonly employed for phycoremediation and biofuel production [7]. For example, *Scenedesmus* sp. has been cultivated in various wastewater types, such as municipal wastewater, aerobically digested urban effluent, and domestic wastewater, for phycoremediation studies [7–9]. Moreover, it has the ability to accumulate a significant amount of lipids, which can be used for biofuel production [4]. The integration of wastewater remediation with lipid production using *Scenedesmus* sp. has been reported in several previous studies. For instance, Ansari et al. [10] reported a maximum lipid content of 30.85% with 42% COD (chemical oxygen demand), 77.7% NO_3^- -N, and 100% PO_4^{3-} -P removal using *Scenedesmus obliquus* in aquaculture wastewater. Gupta et al. [11] found the highest yield of 18.2% lipids with a removal efficiency of 76.5% NH_4^+ -N and 83.1% PO_4^{3-} -P using *Scenedesmus* sp. in domestic wastewater. Baldev et al. [7] cultivated *Scenedesmus* sp. in domestic wastewater and found a maximum 20.47% lipid content with 86% NO_3^- -N, 80% NH_3 -N, and 66% TP removal efficiencies. These studies show that microalgae have great potential for the integration of wastewater remediation with lipid production.

Among different wastewaters, anaerobically digested effluent (ADE) has currently gained attention for microalgae cultivation due to its richness in nitrogen and phosphorus nutrients [12]. Additionally, pathogens and algae predators found in wastewater have been inactivated during anaerobic digestion, thereby reducing the risk of contamination in microalgae culture [13]. The discharging of ADE without post-treatment may cause environmental pollution [14]. Therefore, the integration of microalgae cultivation with ADE treatment may be a viable approach to reduce pollution risk due to the effluent and produce valuable biomass [15]. Zhang et al. [16] also suggested that anaerobically digested effluent utilization for microalgae cultivation is a promising and cost-effective approach for biomass production. In particular, the use of food by-products and wastes such as brewery by-products and spent osmotic solutions as growth mediums can reduce operational costs by an average of 35% compared to standard cultivation methods [17]. Therefore, the use of anaerobically digested effluent as a production medium can be used to purify wastewater and produce biomass, which is rich in lipids, carbohydrates, proteins, and pigments that are used for different applications, including biofuel production [18,19].

In Ethiopia, most brewery industries use primary treatment with anaerobic digestion only or in combination with an aeration system for their wastewater treatment. The effluent from the anaerobic digester has a concentration ranging from 25–101 mg/L TN and 17.9–42.9 mg/L TP [20]. On the other hand, the effluent from an aeration system contains 92.4 mg/L TN, 22.5 mg/L NO_3^- -N, and 25.5 mg/L TP [21]. These concentrations indicate that the effluent either from anaerobic digester or aeration system requires further treatment before being discharged into rivers. As a result, the integration of microalgae cultivation with the existing wastewater treatment after anaerobic digestion in Ethiopia offers a cost-effective and sustainable way to remove nutrients and produce biomass for various uses. Therefore, the objective of this study is to evaluate the simultaneous phycoremediation and lipid production potential of local microalgae grown in non-sterile and sterilized anaerobically digested brewery effluents. Additionally, the study aims to enhance lipid extraction through the use of various pretreatment methods.

2. Methods and Materials

2.1. Microalgae and Brewery Wastewater

The isolation of the local microalga, *Scenedesmus* sp. was carried out from a water sample of a freshwater lake called Lake Ziway in Ethiopia. The isolation process and inoculum preparation are employed using autoclaved bold Basel medium (BBM) [22], which is a nutrient-rich medium that supports the growth of freshwater and green algae. The composition of BBM per liter includes: 175 mg KH_2PO_4 , 25 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 75 mg

MgSO₄·7H₂O, 250 mg NaNO₃, 75 mg K₂HPO₄, 25 mg NaCl, 11.42 mg H₃BO₃, 1 mL of microelement stock solution (which consists of: 8.82 g ZnSO₄·7H₂O, 1.44 g MnCl₂·4H₂O, 0.71 g MoO₃, 1.57 g CuSO₄·5H₂O, and 0.49 g Co (NO₃)₂·6H₂O per liter), 1 mL of solution 1 (which consists of: 50 g Na₂EDTA and 3.1 g KOH per liter), and 1 mL of solution 2 (which consist of: 4.98 g FeSO₄ and 1 mL concentrated H₂SO₄ per liter), and it has a final pH of 6.8. The isolation process involved a combination of pipetting, agar plating, and serial dilution methods described by Andersen and Kawachi [23]. Lake water samples were first enriched with BBM and incubated under 5500 lux light intensity and a 12:12 h light/dark cycle at room temperature. The enriched samples were then serially diluted in BBM and examined microscopically to isolate microalgae. After serial dilution, a sample from the most diluted culture was spread on an agar plate and incubated under the same conditions as the enriched samples. Following incubation, a single colony of algae was picked from the plate and transferred to a Petri dish containing BBM. The colony was then cultured under the same conditions as the enriched sample. Micro-pipetting was then employed to isolate a single microalga from a culture prepared from agar plating. Repeated serial dilution, agar plating, and micro-pipetting were performed until distinct unialgal colonies were obtained. All materials used in the isolation process were sterilized to prevent contamination with other microorganisms and to establish an axenic culture. Finally, morphological characteristics were employed to identify the local microalga as *Scenedesmus* sp. through microscopic observation based on Bellinger and Sigeo [24] and Shubert and Gärtner [25].

The brewery wastewater samples were collected from St. George Brewery Industry, Ethiopia, after being in an up-flow anaerobic sludge blanket (UASB) reactor. This wastewater is known as an anaerobically digested brewery effluent (hereafter, brewery effluent). The brewery effluent samples were taken using a sterilized sampling bottle and prepared as sterilized and non-sterilized after filtration using Whatman no. one filter paper at the laboratory of the Center for Environmental Science, Addis Ababa University. The sterilization of the brewery effluent samples was performed by autoclaving at 121 °C for 15 min. Sterilization is undertaken to inhibit the growth of competing microorganisms in brewery effluent. This allows for a comparison of microalgae growth in sterilized and non-sterilized effluent. Both effluents were characterized for COD, TN, NH₄⁺-N, TP, and PO₄³⁻-P based on standard methods.

2.2. Microalgae Cultivation in Brewery Effluent

Scenedesmus sp. was cultivated in batch mode using a 2 L sterilized conical flask as a photobioreactor [26]. It was separately cultured in flasks containing 1600 mL of sterilized and non-sterilized brewery effluents with 10% algal suspension inoculums [27]. The flasks were illuminated using six fluorescent lamps (18 W each, 108W total, PHILIPS, Holland) with a maximum of 5.5 klux light intensity [28] and a light/dark cycle of 12:12 at room temperature (18–24 °C). A time switcher (SUL 180 h, Zhejiang C&J Electrical Holding Co., Ltd., Zhejiang, China) was used for keeping the photoperiod of the culture. Aeration was supplied by an aerator (SB-648, Zhongshan Songbao Electric Co., Ltd., Zhongshan, China) with a 0.45 µm air filter to provide atmospheric CO₂ and mix the culture. The cultures were checked periodically in order to determine whether they were axenic or not during the experiment. The growth characteristics and the change in nutrient concentrations were investigated over the cultivation period. Finally, the biomass was collected by centrifugation and washed with distilled water. It was dried at 60 °C in an oven and stored at 4 °C until analysis.

2.3. Growth and Microalgal Biomass Production

The growth of microalgae was assessed by measuring the optical density (OD) at 680 nm using a JENWAY spectrophotometer (model 6705) [29]. The dry cell weight (DCW) of *Scenedesmus* sp. was determined on the basis of the standard methods for the total suspended solid [30]. The relationship between OD and DCW was established

through regression analysis, resulting in the following equations: $DCW \text{ (g/L)} = 0.95 * OD - 0.037$ ($R^2 = 0.992$) for non-sterilized effluent and $DCW \text{ (g/L)} = 0.96 * OD - 0.44$ ($R^2 = 0.987$) for sterilized brewery effluent. The specific growth rate, μ (day^{-1}), and biomass productivity, B_p (mg/L/d), during the cultivation periods were calculated by using Equations (1) and (2) [31], respectively. The doubling time (day) is calculated by dividing the natural log of 2.0 by the specific growth rate [32].

$$\mu \text{ (1/d)} = (\ln X_t - \ln X_o) / (t_t - t_o) \quad (1)$$

$$B_p \text{ (mg/L/d)} = (X_t - X_o) / (t_t - t_o) \quad (2)$$

where X_t is biomass concentration at a t_t and X_o is biomass concentration at a t_o (at the initial).

2.4. Nutrient Removal Efficiency and Removal Rate

COD and TN concentrations were measured using a HACH spectrophotometer (HACH, Loveland, CO, USA) according to HACH instructions [33]. The concentrations of TP and $\text{NH}_4^+\text{-N}$ were analyzed using the ascorbic acid method (persulfate digestion) and the phenate method, as stated in APHA [30], respectively. Their concentrations were measured using a JENWAY spectrophotometer. Removal percentage (R_f) and removal rate (R_r) were calculated by Equations (3) and (4), respectively, [34].

$$R_f \text{ (\%)} = \frac{C_o - C_f}{C_o} \times 100\% \quad (3)$$

where C_o (mg/L) is the raw effluent concentration, whereas C_f (mg/L) is the concentration at the end of microalgae cultivation.

$$R_r \text{ (mg/L/d)} = \frac{C_o - C_t}{t_t - t_o} \quad (4)$$

where C_o (mg/L) and C_t (mg/L) denote the concentration at the initial time, t_o , and at time t , respectively.

2.5. Removal Rate Constant, and Biomass Yield Coefficient

The removal of COD, nitrogen, and phosphorus nutrients from wastewater using microalgae can be described using a pseudo-first-order kinetic model [35]. The model for COD, nitrogen, and phosphorus nutrients can be expressed by an Equation (5).

$$\ln(C_t) = \ln(C_o) - kt \quad (5)$$

where k is the first-order rate constant and t is time. C_t and C_o are the concentrations of TN, $\text{NH}_4^+\text{-N}$, and TP (mg/L) at time t and at an initial time t , respectively. A plot of $\ln(C_t)$ versus t provides a straight line with slope k , which can be utilized to recognize situations where higher nutrient removal is achieved [36].

The yield coefficient based on the consumption of COD, nitrogen, and phosphorus nutrients (Y , $\text{g biomass/mg substrate}$) can be calculated using Equation (6) [37]

$$X_f - X_i = Y_{N \text{ or } P} (C_o - C_f) \quad (6)$$

where X_i (g/L) and C_o (mg/L) are the initial biomass and nutrient concentrations, while X_f (g/L) and C_t (mg/L) are the final biomass and nutrient concentrations (g/L), respectively. The slope obtained from the plot $[X_f - X_i]$ versus $[C_o - C_f]$ resulted in a biomass yield coefficient for COD (g X/mg COD), nitrogen nutrient (g X/mg N), or phosphorus nutrient (g X/mg P).

2.6. Lipid Extraction

Total lipids were determined according to modified Bligh and Dyer's method [38]. A mixture of chloroform and methanol (1:2 *v/v*) was used to extract lipid from microalgal biomass. The mixture was kept at room temperature overnight after shaking for 20 min at 150 rpm. The following day, the solvent ratio of chloroform, methanol, and water was adjusted to 2:2:1 by adding chloroform and water, and then the mixture was transferred into a separatory funnel. The chloroform layer containing lipids was washed with 1% NaCl to separate it from the methanol–aqueous layer. The lipids were separated from the organic layer by evaporating the solvent in an oven at 60 °C using a pre-weighed glass beaker. Finally, the total lipid contents were measured gravimetrically. The total lipid content and productivity were computed based on Equations (7) and (8), respectively [39].

$$\text{Lipid content (w/w, \%)} = W_L / B \times 100\% \quad (7)$$

where W_L is the weight of dried lipid in the dry weight and B is the dried microalgal biomass

$$\text{PL (mg/L/d)} = \text{LC} \times \text{Bp (mg/L/d)} \quad (8)$$

where PL is biomass productivity, LC is the lipid content, and Bp is the biomass productivity

2.7. Pretreatment for Lipid Extraction

The pretreatment of microalgal biomass was performed using various methods, including autoclave (Model, DIXONS, and ST3028, Dixons Surgical Instruments LTD, Wickford, UK), oven heating (Model, GX65B, Faithful instrument (Hebei) Co., Ltd., Huanghua, China), osmotic shock, acid digestion in a water bath (DK-98-II, Faithful instrument (Hebei) Co., Ltd., Huanghua, China), and microwave (ETHOS One, SK-10 and SK-12, Milestone Srl, Sorisole (BG), Italy). The procedure is as follows:

- Autoclave pretreatment: A dry microalgal biomass of 0.5 g was mixed with 15 mL of distilled water and subjected to autoclaving at 121 °C for 15 min;
- Microwave pretreatment: The same biomass was mixed with 15 mL of water and subjected to microwave radiation at 1000 W and 100 °C for 10 min;
- Osmotic stress pretreatment: Another portion of the biomass (0.5 g) was blended with 10 mL of a 10% NaCl solution and incubated for 48 h to induce osmotic stress;
- Oven heating: The biomass (0.5 g) was mixed with 15 mL of water and heated in an oven at 120 °C for 15 min;
- Acid digestion: The biomass (0.5 g) was mixed with 15 mL of 3 N HCl solution and digested at 80 °C for 1 h in a water bath.

The procedures for autoclave, microwave, and osmotic stress pretreatments were adapted from Lee et al. [40]. Following pretreatment, total lipids are extracted using a modified version of Bligh and Dyer's method [38]. This method involves a liquid–liquid extraction process using a mixture of chloroform and methanol to separate lipids from other cellular components.

2.8. Statistical Analysis

All analyses in this study were performed in triplicate. The results are illustrated in Figures and Tables. A paired sample *t*-test was employed using Microsoft Excel 2013 for nutrient removal efficiency and lipid content comparison between non-sterilized and sterilized brewery effluents. ANOVA with post hoc Tukey's honesty test was employed using R-software to compare the means of the growth characteristics and lipid contents obtained through different pretreatment methods. The results are significant at a level of 95% ($p < 0.05$).

3. Results and Discussion

3.1. Brewery Effluent Characterization

Table 1 displays the average characteristics of the brewery effluent used for this study. The concentrations of COD, TN, $\text{NH}_4^+\text{-N}$, TP, $\text{PO}_4^{3-}\text{-P}$, and pH of the brewery effluent were monitored before microalgae cultivation. The study found that the concentrations of COD, N, and P nutrients exceeded the national discharge limits recommended for brewery effluent. The recommended limits are 250 mg/L for COD, 40 mg/L for TN, 20 mg/L for $\text{NH}_4^+\text{-N}$, and 5 mg/L for TP [41].

Table 1. Non-sterilized and sterilized brewery effluent characteristics used in the experiments (mean $\pm$ sd).

Parameters	Concentration (mg/L, Except for pH)	
	Non-Sterilized Effluent	Sterilized Effluent
pH	7.47 $\pm$ 0.18	9.00 $\pm$ 0.11
COD	399 $\pm$ 29.14	408 $\pm$ 33.79
$\text{NH}_4^+\text{-N}$	41.52 $\pm$ 4.73	36.86 $\pm$ 4.18
TN	53.42 $\pm$ 6.19	47.50 $\pm$ 5.06
$\text{PO}_4^{3-}\text{-P}$	37.78 $\pm$ 2.64	39.28 $\pm$ 3.00
TP	50.01 $\pm$ 2.44	53.88 $\pm$ 2.44

Alayu and Leta [42] reported a maximum concentration of 614 mg/L COD, 101.4 mg/L TN, 32.5 mg/L TP, and 25 mg/L $\text{PO}_4^{3-}\text{-P}$ in anaerobically digested brewery effluent. Farooq et al. [43] and Darpito et al. [44] found an anaerobically treated brewery effluent with a concentration in the range of 50 to 75 mg/L TN, 10 to 55 mg/L TP, and 100 to 275 mg/L COD. These studies indicate that anaerobically digested brewery effluent has relatively higher carbon, nitrogen, and phosphorus nutrients that should be removed before discharging into the environment to prevent environmental deterioration.

The average concentrations of COD, nitrogen, and phosphorus nutrients in sterilized brewery effluent are also displayed in Table 1. The COD, $\text{PO}_4^{3-}\text{-P}$, and TP concentrations increase, whereas the TN and $\text{NH}_4^+\text{-N}$ concentrations decrease after brewery effluent sterilization. Sterilization using autoclaving is a very effective method to inhibit competitive microorganisms in microalgae culture, but it can cause changes to the chemical composition of wastewater. For instance, Gupta et al. [11] found autoclaving municipal wastewater increased TN and TP concentrations, while COD and $\text{NH}_4^+\text{-N}$ concentrations decreased. Li et al. [45] and Li et al. [46] observed an increase in COD, TN, and TP concentrations, compared to a decrease in $\text{NH}_4^+\text{-N}$ concentration after autoclaving highly concentrated municipal wastewater and cooking cocoon wastewater, respectively. These studies used autoclaving wastewater for microalgae growth, biomass production, and nutrient removal.

3.2. Growth and Biomass Production in Brewery Effluent

Microalgae can use nitrogen and phosphorus nutrients from wastewater to produce biomass that is abundant in lipids, carbohydrates, proteins, pigments, vitamins, and antioxidants. Additionally, this biomass can be utilized to produce a wide range of value-added products [47]. An increase in biomass concentration with a reduction in nutrients in wastewater shows nutrient utilization by microalgae. The *Scenedesmus* sp. cultures in BBM and sterilized brewery effluent were axenic or free of contamination throughout the cultivation period. The growth and biomass concentration of *Scenedesmus* sp. in BBM (as a control), non-sterilized, and sterilized brewery effluents during the cultivation period are shown in Figure 1. *Scenedesmus* sp. exhibited higher growth in BBM compared to that found in brewery effluent until the end of cultivation. However, it attained better growth in sterilized effluent for the first four days and in non-sterilized effluent from the 4th day to the 18th day. After the 18th day, the growth declined in all conditions; as a result, the 18th day marked the end of cultivation. The average biomass concentration,

specific growth rate, biomass productivity, and doubling time are summarized in Table 2. *Scenedesmus* sp. showed better growth characteristics in non-sterilized effluent; however, it exhibited a similar growth pattern in both effluents. A non-sterilized effluent resulted in a slightly higher biomass concentration, specific growth, and biomass productivity compared to sterilized effluent. This observation could be attributed to the presence of indigenous wastewater bacteria. The biomass concentration and productivity obtained in non-sterilized effluent were 5.85% and 1.67% higher than those obtained in sterilized effluent, respectively. The statistical test reveals that the biomass concentration obtained in BBM significantly differs from those obtained in non-sterilized effluent ($p = 0.007$) and sterilized effluent ($p = 0.001$). However, there are no significant differences in biomass concentration ($p = 0.48$), specific growth rate ($p = 0.11$), or biomass productivities ($p = 0.97$) between non-sterilized and sterilized brewery effluents. The closeness of growth characteristics in non-sterilized and sterilized effluents may be attributed to the inactivation of most bacteria or algae predictors during anaerobic processes in non-sterilized effluent [13]. Udaiyappana et al. [48] reported that non-sterilized palm oil mill wastewater grew *Scenedesmus* sp. better than sterilized effluent. As a result, it is convenient to cultivate microalgae using non-sterilized anaerobically treated brewery effluent for nutrient removal and biomass production.

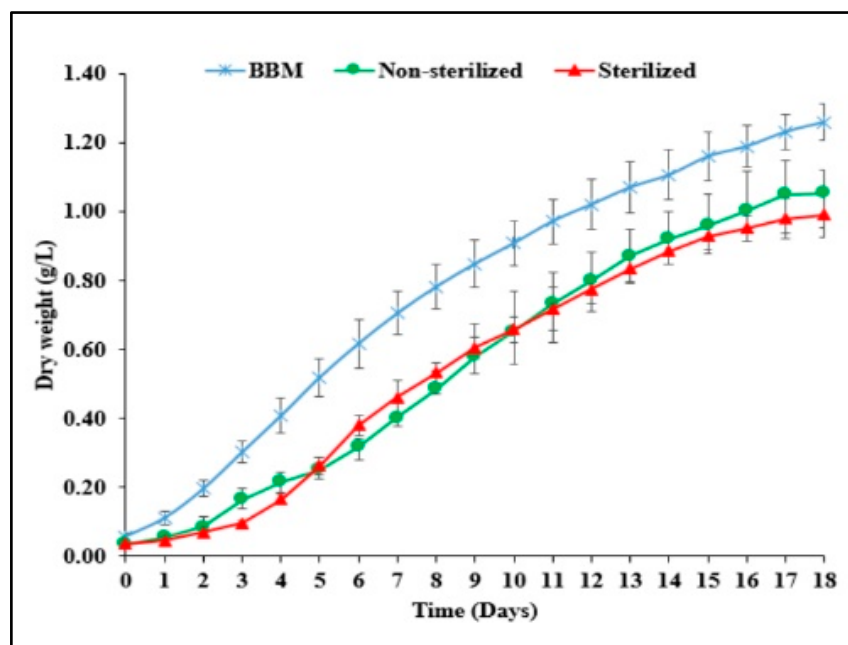


Figure 1. Variation of biomass production of *Scenedesmus* sp. in non-sterilized and sterilized brewery effluents over cultivation time.

Table 2. Summary of *Scenedesmus* sp. cultivation in BBM and brewery effluent. (Mean of $n = 4 \pm \text{SD}$).

Parameters	BBM	Brewery Effluent	
		Non-Sterilized	Sterilized
Biomass concentration (g/L)	1.26 ± 0.1^a	1.05 ± 0.10^b	0.992 ± 0.06^b
Specific growth rate (d^{-1})	0.34 ± 0.01^a	0.31 ± 0.02^{ac}	0.28 ± 0.02^c
Doubling time (d)	2.46 ± 0.97^a	2.43 ± 0.15^b	2.63 ± 0^c
Biomass productivity (mg/L/d)	93.30 ± 9.23^a	64.33 ± 6.26^b	63.27 ± 3.13^b

($p < 0.05$ for different letters horizontally).

The biomass concentration obtained in the present study was comparable with the results reported by Ferreira et al. [49], who found a maximum biomass productivity of 0.95 g/L when *Scenedesmus obliquus* was cultivated in brewery effluent. However, Darpito et al. [44] found a higher biomass concentration from *Chlorella protothecoides* grown

in brewery effluent. Furthermore, *Scenedesmus* sp. has been cultivated in several types of wastewater for growth characteristics and phycoremediation studies. For instance, Ansari et al. [27] achieved a maximum biomass concentration, specific growth rate, and biomass productivity of 0.45 g/L, 0.33 d⁻¹, and 58.7, respectively, for *Scenedesmus* sp. cultivated in institutional wastewater. Similarly, Tan et al. [31] reported a maximum biomass concentration, specific growth rate, and maximum biomass productivity of 0.59 g/L, 0.798 d⁻¹, and 99.33 mg/L/d for *Scenedesmus* sp. grown in 100% non-sterilized primary domestic wastewater. The variation in growth characteristics for *Scenedesmus* sp. among various studies might be due to the variations in wastewater types, cultivation period, and cultivation conditions [32].

3.3. Nutrient and COD Removal Efficiency

The phycoremediation study for non-sterilized and sterilized brewery effluents was investigated by measuring COD, TN, NH₄⁺-N, and TP removal using *Scenedesmus* sp. Microalgae assimilate carbon, nitrogen, and phosphorus nutrients from the wastewater for protein, nucleic acid, and phospholipid synthesis [50]. The brewery effluent used in this study contains relatively high concentrations of nitrogen and phosphorous, which should be removed before discharge into the environment. Figure 2a,b show the change in concentration of COD, TN, NH₄⁺-N, and TP over the cultivation period in non-sterilized and sterilized brewery effluents, respectively. It was observed that COD concentrations gradually decreased until day 6 in both effluents. After the sixth day, the COD concentration increased up to days 8 and 14 in non-sterilized and sterilized brewery effluents, respectively, and then dropped until the end of cultivation. Eventually, the average COD concentration in non-sterilized and sterilized effluents reached 89 ± 9.13 mg/L and 102.00 ± 11.38 mg/L from initial concentrations of 399.58 ± 29.14 and 408.17 ± 33.79 mg/L, respectively. The NH₄⁺-N concentration in both brewery effluents was nearly depleted and reached below 0.09 mg/L from 41.52 ± 4.73 mg/L in non-sterilized effluent and 36.86 ± 4.18 mg/L in sterilized effluent. The TN and TP concentrations in both brewery effluents gradually decreased until the end of cultivation. The final TN concentrations were dropped to 2.93 ± 0.44 and 1.97 ± 0.94 mg/L from an initial concentration of 53.42 ± 6.19 and 48.25 ± 5.07 mg/L in non-sterilized and sterilized effluents, respectively. The TP concentrations reached 15.29 ± 1.25 mg/L from 50.00 ± 2.64 mg/L in non-sterilized effluent and 17.32 ± 1.12 mg/L from 53.88 ± 2.44 mg/L in sterilized effluent. It was observed that TN and TP reduction trends were similar in both effluents. The final concentrations obtained for COD, TN, and NH₄⁺-N were below the national permissible standard for brewery effluent, whereas the TP concentration was not. The removal efficiency of COD, TN, NH₄⁺-N, and TP was 77.78%, >99%, 94.36%, and 69.32% in non-sterilized and 74.76%, >99%, 96.16%, and 67.77% in sterilized brewery effluent at the end of the experiment. There were no significant differences ($p > 0.05$) in TN, NH₄⁺-N, TP, and COD removal between non-sterilized and sterilized brewery effluents using *Scenedesmus* sp.

The removal efficiencies of COD, nitrogen, and phosphorus nutrients from brewery effluents and other wastewater types using microalgae have been reported in several previous studies. For example, Darpito et al. [44] found the average reductions in TN, TP, and COD using *Chlorella protothecoides* were 96%, 90%, and 68.46% from initial concentrations of 72.6 mg/L, 54.4 mg/L, and 225.4 mg/L, respectively. Ferreira et al. [49] also achieved a maximum removal of 89% N (as TKN), 22% P (as PO₄³⁻-P), and 62% COD using *Scenedesmus obliquus*. Moreover, regarding *Scenedesmus* sp., Kim et al. [51] cultivated three microalgae in anaerobically digested effluent for 18th day cultivation with initial concentrations of 118 mg/L TN and 35 mg/L TP, and they found the removal of 98% TN and 98% TP for *Scenedesmus* sp. Baldev et al. [7] reported 86% NO₃⁻-N, 80% NH₃-N, and 66% TP removal efficiencies from domestic wastewater with an initial concentration of 303.3 mg/L NO₃⁻-N, 3.5 µg/L NH₃-N, and 98.3 mg/L TP. Tan et al. [31] achieved a higher removal efficiency of TN, NH₄⁺-N, and TP by *Scenedesmus* sp. cultivated in non-sterilized primary domestic wastewater, reporting a removal of 94.81%, 98.88%, and 99.5%, respectively. These studies

showed that the removal of COD, TN, and TP varied with species for brewery wastewater and other wastewaters for *Scenedesmus* sp. The lower removal of phosphorus in this study compared to nitrogen was possibly due to phosphorus removal being affected by algal physiology, initial phosphate concentration, chemical form of available phosphate, light intensity, pH, temperature, and algae species [11]. Furthermore, it was reported that phosphorus removal strongly depends on the N/P ratio [52]. Xin et al. [53] reported that the optimal N/P ratio for growth of *Scenedesmus* sp. was in the range 5–12. The N/P ratio was found to be 1.07 for non-sterilized effluent and 0.89 for sterilized effluent in this study, and, thus, the brewery effluent was subject to nitrogen limitation. Since the removal of phosphorus is associated with N removal, the limitation of nitrogen in brewery effluent has contributed to the low uptake of phosphorus into biomass, irrespective of the P concentrations in the effluent [54]. The study conducted by Beuckels et al. [55] also showed that phosphorus removal was dependent on nitrogen concentration. As a result, the removal of phosphorus in the present study could be improved by adjusting the N/P ratio of brewery effluent. The decrease in COD concentration observed in this study suggests that *Scenedesmus* sp. is able to utilize the organic matter present in a brewery effluent as a source of energy and substrate for its growth, in addition to CO₂ [56]. However, organic matter releasing, mainly as extracellular polymeric substances (EPS), can contribute to an increase in COD concentration in microalgae culture [57]. The main components of EPS are polysaccharides, which account for about 45–95% and contain a variety of monomers, including glucose, galactose, mannose, arabinose, rhamnose, and fructose [58].

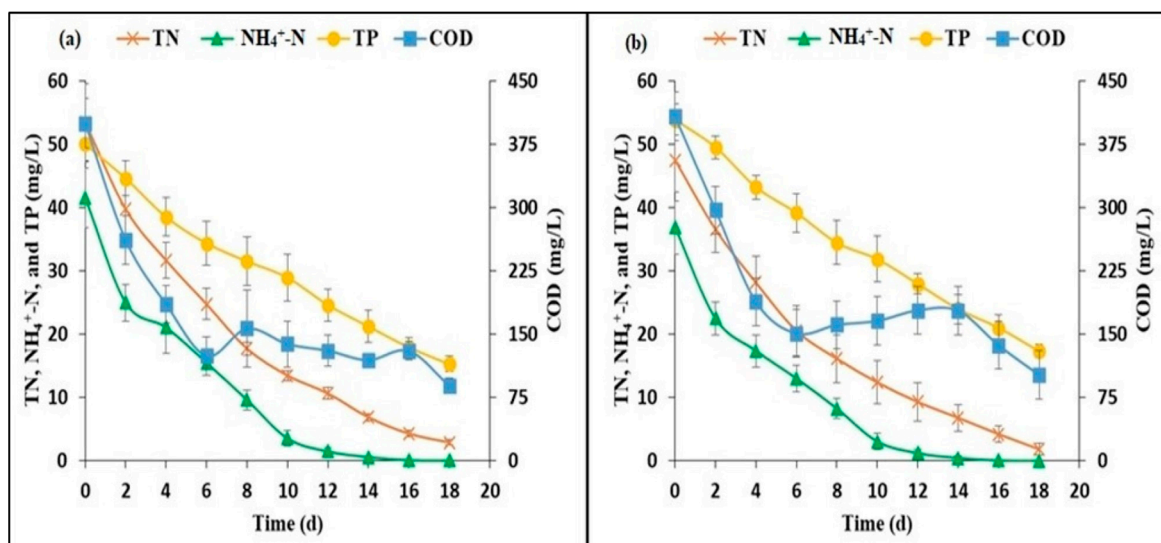


Figure 2. Change in TN, NH₄⁺-N, TP, and COD concentrations during the cultivation period in (a) non-sterilized and (b) sterilized effluents using local *Scenedesmus* sp.

The coefficient of determination (R^2) is a statistical measure used to assess the strength of the linear relationship between variables in regression analysis. [59]. In the case of this study, strong correlations ($R^2 > 0.93$) were observed between biomass production and the removal of TN, NH₄⁺-N, and TP in non-sterilized and sterilized brewery effluents. This suggests that the assimilation of TN, NH₄⁺-N, and TP by microalgae is responsible for biomass production in brewery effluent. However, a weak correlation ($R^2 < 0.62$) was found between biomass production and COD removal in non-sterilized and sterilized brewery effluents. This result indicates a weak association between COD removal and biomass production, suggesting that the concentration of COD does not significantly influence biomass production.

3.4. Nutrient and COD Removal Rate

The removal rates of COD, TN, $\text{NH}_4^+\text{-N}$, and TP in sterilized and non-sterilized brewery effluent by *Scenedesmus* sp. were computed to compare the extent of each nutrient removal per day. The consumption rates of COD, TN, $\text{NH}_4^+\text{-N}$, and TP were found to be 17.00 ± 2.38 , 2.53 ± 0.27 , 2.05 ± 0.23 , and 2.03 ± 0.16 mg/L/d in sterilized effluent and 17.50 ± 1.82 , 2.80 ± 0.36 , 2.30 ± 0.26 , and 1.93 ± 0.17 mg/L/d in non-sterilized effluent, respectively. These findings show that the consumption rate of TN is 1.25 times higher than that of TP in sterilized effluent and 1.45 times higher than that of TP in non-sterilized effluent. These indicate the removal of TN per day from both effluents is slightly higher than TP. The consumption rate of COD is 6.72 and 6.16 times TN and 8.38 and 8.94 times TP in sterilized and non-sterilized effluents, respectively. Nevertheless, the correlation between biomass production and COD removal (Figure 3) shows that assimilation is not considered the only mechanism for COD removal. Nayak et al. [60] reported the highest removal rate of 5.42 mg/L/d for $\text{NH}_4^+\text{-N}$ and 19.5 mg/L/d for COD by *Scenedesmus* sp. cultivated in domestic wastewater with a supply of 2.5% CO_2 . Yang et al. [32] reported that the removal rates of TN and TP ranged from 1.45–0.21 mg/L/d to 0.36–0.03 mg/L/d, respectively. Mennaa et al. [61] achieved the maximum removal rate of 4.65 mg/L/d TN and 11.98 mg/L/d TP using *Chlorella vulgaris* in urban wastewater, and Luo et al. [62] reported the maximum removal rates of 20 mg/L/d $\text{NH}_4^+\text{-N}$ and 0.93 mg/L/d TP by *Desmodesmus* sp. in piggery wastewater. The difference in nutrient removal rate regarding COD and nitrogen and phosphorus nutrients among various studies may be due to the types or compositions of wastewater, initial nutrient concentrations, cultivation conditions, and the species of microalgae [63].

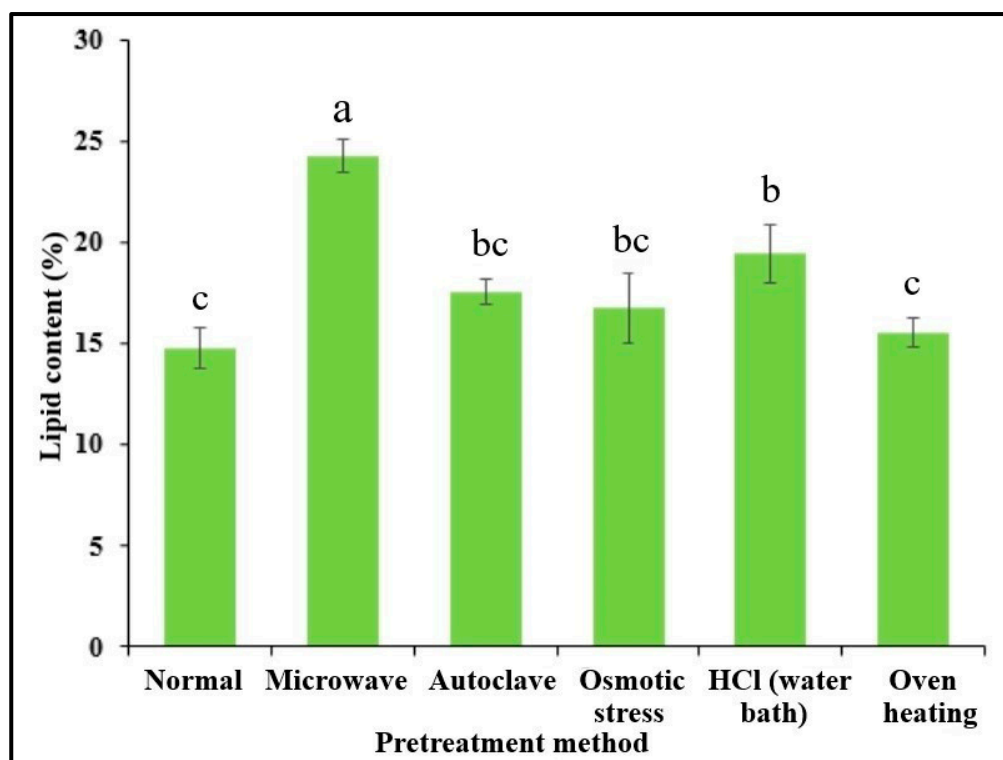


Figure 3. Lipid production from local *Scenedesmus* sp. using different pretreatment methods ($p < 0.05$ for different letters).

3.5. Nutrient Removal Rate Constant and Biomass Yield Coefficient

The decreasing trend of TN, $\text{NH}_4^+\text{-N}$, and TP concentrations with time is clearly observed in both brewery effluents (Figure 2). This process can be explained using the pseudo-first-order model. The removal rate constant and determination coefficient (R^2)

obtained from the pseudo-first-order equation are provided in Table 3. The removal rate constants for TN and TP removal were 0.16 d^{-1} and 0.06 d^{-1} in both effluents, respectively. The removal rate constant obtained for $\text{NH}_4^+\text{-N}$ was 0.37 d^{-1} in non-sterilized effluent and 0.38 d^{-1} in sterilized effluent. These results show that the nitrogen removal rate is faster than the phosphorus removal rate in both effluents. Moreover, the removal rate of $\text{NH}_4^+\text{-N}$ was more rapid than that of TN. The strong linear correlation ($R^2 > 0.94$) for TN, $\text{NH}_4^+\text{-N}$, and TP indicates that the removal of TN, $\text{NH}_4^+\text{-N}$, and TP fits the first-order kinetic model very well. However, the R^2 -value of COD is rated as moderate, showing that its removal due to assimilation is moderately explained by the first-order kinetic model. The removal rate constant found for TN using *Scenedesmus* sp. is similar to that of *Scenedesmus rubescens* cultivated in ammonium-containing wastewater but lower and higher than those cultivated in ammonium-containing wastewater with adjusting pH and nitrate-containing wastewater, respectively [64]. Luo et al. [62] found that *Desmodesmus* sp. cultivated in piggery wastewater had a lower removal rate constant in $\text{NH}_4^+\text{-N}$ but a higher removal rate constant in TP than those obtained in this study. Liu et al. [36] found a comparable removal rate constant for N under air injection with this study. However, they found a higher removal rate constant for P using *Chlorella vulgaris* in wastewater under the supply of air and CO_2 at different concentrations.

Table 3. Nutrient removal rate constant (k) and biomass yield coefficient (Y) of *Scenedesmus* sp. in non-sterilized and sterilized brewery effluent.

Parameter	Pseudo-First-Order Constant (k_{nutrient} , 1/d)				Biomass Yield Coefficient (Y, mg Biomass/mg Nutrient)			
	Non-Sterilized Effluent		Sterilized Effluent		Non-Sterilized Effluent		Sterilized Effluent	
	k	R^2	k	R^2	Y	R^2	Y	R^2
COD	0.06	0.76	0.05	0.69	0.006	0.64	0.005	0.54
TN	0.16	0.99	0.16	0.96	0.026	0.96	0.027	0.98
$\text{NH}_4^+\text{-N}$	0.37	0.94	0.38	0.94	0.035	0.93	0.039	0.96
TP	0.06	0.99	0.06	0.99	0.035	0.97	0.030	0.99

Biomass yield coefficients (Y) based on TN, $\text{NH}_4^+\text{-N}$, TP, and COD consumptions were determined to investigate the contribution of each nutrient to biomass production. The biomass yield coefficient obtained based on each nutrient concentration is also provided in Table 3. The biomass yield based on TN, $\text{NH}_4^+\text{-N}$, TP, and COD consumptions was found to be 0.027 g biomass/mg N, 0.039 g biomass/mg N, 0.030 g biomass/mg P, and 0.005 g biomass/mg COD in sterilized effluent, while 0.026 g biomass/mg N, 0.035 g biomass/mg N, 0.035 g biomass/mg P, and 0.006 g biomass/mg COD in non-sterilized effluent, respectively. These results show that the biomass yield based on the consumption of TN and $\text{NH}_4^+\text{-N}$ is slightly higher in sterilized effluent than in non-sterilized effluent. In addition, the biomass yield coefficient based on $\text{NH}_4^+\text{-N}$ is higher than that obtained based on TN, showing that $\text{NH}_4^+\text{-N}$ may contribute more to biomass formation. The yield coefficient based on TN and TP suggests that for the same amount of dry weight, *Scenedesmus* sp. consumed more nitrogen than phosphorus from brewery effluent. The R^2 -values obtained for TN, $\text{NH}_4^+\text{-N}$, and TP are over 0.93, indicating that the yield coefficients achieved from nitrogen and phosphorus nutrients are more suitable and appear more reliable. These indicate that nitrogen and phosphorus nutrient assimilation by microalgae greatly contributes to biomass production. However, the R^2 -value for COD is less than 0.65 in both effluents, suggesting that COD has little contribution to biomass formation. The biomass yield of *Scenedesmus* sp. is higher based on $\text{NH}_4^+\text{-N}$, but lower based on TP than those reported by Luo et al. [62] (0.01 g biomass/mg $\text{NH}_4^+\text{-N}$ and 0.21 g biomass/mg TP), who cultivated *Desmodesmus* sp. in piggery wastewater. The biomass yield coefficient obtained in this study based on TN was in the range of that reported by Yang et al. [32] (0.019–0.041 g biomass/mg of TN) using *Scenedesmus obliquus* in domestic wastewater. However, they achieved a higher biomass yield coefficient based on the consumption of

TP (0.174–0.279 g biomass/mg of TP). Liu et al. [36] reported the highest yield coefficient of 0.027 g biomass/mg N using *Chlorella vulgaris* in wastewater under the supply of 10% CO₂, which is comparable to those found in the present study. However, they reported a higher yield coefficient based on TP (0.042–0.217 g biomass/mg of TP) than this study under different percentages of carbon dioxide supply.

3.6. Lipid Production

The *Scenedesmus* sp. biomass obtained from non-sterilized and sterilized brewery effluents was analyzed for total lipid extraction. *Scenedesmus* sp. accumulated $13.67 \pm 0.31\%$ and $14.79 \pm 1.02\%$ total lipid in its biomass when grown in non-sterilized and sterilized brewery effluent, respectively. The lipid productivity could be 8.72 ± 0.19 mg/L/d in non-sterilized effluent and 9.58 ± 0.66 mg/L/d in sterilized effluent. The lipid content obtained in sterilized effluent was 8.19% higher than that obtained in non-sterilized effluent. However, the lipid contents found in non-sterilized and sterilized brewery effluent were not significantly different. Moreover, the lipid content of *Scenedesmus* sp. is classified under the range of moderate microalgal lipid contents, which are estimated at around 10% to 18%. Table 4 shows the lipid content and productivity of *Scenedesmus* sp. grown in various wastewaters. The lipid accumulation extent of *Scenedesmus* sp. has varied among different wastewaters and even within the same wastewater type. *Scenedesmus* sp. accumulated a similar content of lipids when it was cultivated in brewery effluent (this study), pig slaughterhouse wastewater [65], municipal wastewater [66], and institutional wastewater [27]. However, Silambarasan et al. [67] reported a higher lipid content of 33% for *Scenedesmus* sp. in municipal wastewater. Ansari et al. [10] and Ma et al. [68] found a lipid content of around 30% using *Scenedesmus* sp. grown in aquaculture and molasses wastewater, respectively. Mercado et al. [18] found the highest lipid content from *Scenedesmus* sp. grown in dairy wastewater compared to the other wastewater presented in Table 4, except that reported by Thangam et al. [69]. Baldev et al. [7] and Thangam et al. [69] found different lipid content values for *Scenedesmus* sp. grown in domestic wastewater. The variation in lipid content of *Scenedesmus* sp. with different wastewaters is perhaps due to several factors, such as light intensity and photoperiod, nutrient content, cultivation factor, CO₂ concentration, iron concentration, silica concentration, and culture aeration degree [70]. Among these factors, light intensity and nutrient availability are the most responsible factors for lipid accumulation in microalgae grown in wastewater. Furthermore, the lipid content variation in *Scenedesmus* sp. may be due to the extracting solvent used in the given study [65]. Generally, wastewater utilization for microalgae growth generally enhances the sustainability of lipid production from microalgal biomass and its subsequent conversion into biodiesel.

Table 4. Lipid content and productivity of *Scenedesmus* sp. grown in different wastewater streams.

Type of Wastewater	Culture Volume (Liter)	Culture Period (day)	Biomass Concentration (g/L)	Biomass Productivity (mg/L/d)	Lipid Content (%)	Lipid Productivity (mg/L/d)	Reference
Institution wastewater	0.25 *	12	0.445	58.70	13.00	7.63 +	[27]
Molasses wastewater	0.25 *	7	2.5	357.14 +	28.9	94.4	[68]
Municipal wastewater	3.00 *	4	0.217	54.20	12.50	6.77 +	[66]
Aquaculture wastewater	1.00 *	14	1.25	89.61	30.85	27.65	[10]
Dairy wastewater	0.15 **	11	-	1750	51.00	892.5 +	[18]
Domestic wastewater	1.00 *	22	0.836	-	20.47	8.56	[7]
Domestic wastewater	20.0 **	14	0.95	-	50.50	19.00	[69]
Pig slaughterhouse wastewater	40.0 **	11	0.2	18	16.25	0.274	[65]
Municipal wastewater	1.00 *	14	1.54	128.61	33.00	42.44 +	[67]
Non-sterilized brewery effluent	2.00 *	18	1.05	63.27	13.67	8.72	This study
Sterilized brewery effluent	2.00 *	18	0.992	64.33	14.79	9.58	This study

* Conical or Erlenmeyer flask; ** photobioreactor; + calculated.

3.7. Pretreatment for Lipid Extraction

This study found that the lipid content obtained in sterilized brewery effluent was relatively higher compared to non-sterilized under normal extraction methods. To further enhance lipid production, the microalgal biomass obtained from sterilized effluent underwent various pretreatment methods. These methods were employed to maximize lipid extraction from microalgal biomass and assess their potential for downstream processes. The contents of total lipids obtained after different pretreatment methods, namely autoclave, microwave, osmotic stress, oven heating, and acid digestion in a water bath, are depicted in Figure 3. All pretreatments performed in this study were able to disrupt the cell of the *Scenedesmus* sp., but their lipid yields differed. The maximum and minimum lipid contents were extracted from *Scenedesmus* sp. cells using microwave and oven heating, respectively. The statistical analysis shows that there are no significant differences ($p > 0.05$) in lipid contents among autoclave, osmotic, and HCl digestion pretreatments. However, the microwave pretreatment significantly differs ($p < 0.05$) from the other pretreatments. The highest lipid content obtained was $24.27 \pm 0.81\%$ using microwave pretreatment, which was followed by HCl digestion ($19.43 \pm 1.35\%$), autoclaving ($17.57 \pm 0.67\%$), osmotic shock ($16.73 \pm 1.76\%$), and oven heating ($15.53 \pm 0.69\%$). The total lipid achieved without pretreatment ($14.79 \pm 1.02\%$) was significantly different ($p < 0.05$) from microwave and HCl digestion pretreatments. However, it was not significantly different ($p > 0.05$) from osmotic stress, autoclave, and oven heating pretreatments. As a result, pretreatment is an option to maximize lipid content from microalgal biomass obtained after wastewater treatment. It is recommended to perform pretreatment before further processing. Additionally, it is suggested that the composition of fatty acids for each pretreatment method be examined in the future to determine the suitability of *Scenedesmus* sp. for biodiesel production.

Results from this study are in compliance with previous studies by Lee et al. [40], where the microwave pretreatment has been shown to extract higher lipid content from *Scenedesmus* sp. as compared to autoclave, bead-beating, microwave, ultrasonication, and osmotic shock. Likewise, Silva et al. [71] obtained a higher lipid content extraction from mixed cultures using microwave pretreatment compared to ultrasonication, autoclave, and electroflotation by alternating current pretreatments. However, Yu et al. [72] investigated different pretreatments, including autoclave, microwave, acid digestion, and sonication, for lipid extraction from *Chlorella sorokiniana*, and they reported that acid digestion provided a higher lipid content than the other pretreatments. Moreover, Prabakaran and Ravindran [73] found the highest lipid content using sonication from *Chlorella* sp. compared to microwave, autoclave, bead-beating, and osmotic shock. The different results in lipid content with different pretreatments are due to the variation in cell wall compositions and structures among microalgae taxonomic classes and families [74]. Moreover, the types of pretreatment methods used for cell disruption could also contribute to a variation in lipid extraction from microalgal biomass. The findings of most previous studies suggest that microwave pretreatment is an efficient method for lipid extraction from various microalgal biomasses [40,71]. This is because microwave radiation has an advantage over conventional heating in terms of quick heating, selective energy dissipation, and offering the same direction of heat and mass transfer [75].

4. Conclusions

This study investigated the potential of a local microalga, *Scenedesmus* sp., for phycoremediation and lipid production. The microalga was cultivated in both sterilized and non-sterilized brewery effluents for phycoremediation and lipid production. Maximum biomass production was obtained in the non-sterilized effluent, but there were no significant differences in biomass production, specific growth rate, or biomass productivity between the two types of effluents. This suggests that non-sterilized brewery effluent can be used for microalgae growth and biomass production. *Scenedesmus* sp. demonstrated efficient removal of nitrogen nutrients from both effluents, ensuring compliance with national discharge limits. The study also displayed a strong correlation between

biomass production and the removal of TN, $\text{NH}_4^+\text{-N}$, and TP. Notably, *Scenedesmus* sp. exhibited a better removal rate, removal rate constant, and biomass yield coefficient for nitrogen nutrients compared to phosphorus nutrients. Microwave pretreatment provided the highest lipid content, followed by HCl digestion in a water bath, autoclave, osmotic stress, and oven heating. The lipid content of *Scenedesmus* sp. was increased by 39.06% after microwave pretreatment from the value obtained without treatment. The results suggest that local microalga, *Scenedesmus* sp., has potential for phycoremediating wastewater and producing biomass for total lipid extraction under a suitable pretreatment method. Therefore, a pilot-scale study should be performed to investigate the practicability and efficiency of microalgal-based treatment of brewery effluent. Moreover, the potential of wastewater-grown local microalgae for biodiesel production should be explored to gain a comprehensive understanding of their applications.

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