Sigma J Eng Nat Sci, Vol. 44, No. 2, pp. 781–793, April, 2026

Sigma Journal of Engineering and Natural Sciences
Web page info: https://sigma.yildiz.edu.tr
DOI: 10.14744/sigma.2026.2010

Research Article

The effect of different light intensities on the growth of *Chlorella Vulgaris* in advanced biologically treated poultry slaughterhouse wastewater

Gamze KATIRCIOĞLU SINMAZ[1,*], İsmail Ayhan ŞENGİL[1]

[1]*Department of Environmental Engineering, Sakarya University, Sakarya, 54050, Türkiye*

ARTICLE INFO

Article history
Received: 17 October 2024
Revised: 02 January 2025
Accepted: 13 February 2025

Keywords:
Chlorella Vulgaris; FAME; Light Intensity; Lipid; Wastewater

ABSTRACT

Determining the optimal light intensity is essential for the growth of *Chlorella vulgaris* and the production of suitable biomass for biodiesel. This study aims to investigate the cultivation of *Chlorella vulgaris* under optimal light intensity to enhance biodiesel production while advancing the biological treatment of poultry slaughterhouse wastewater. To fill the relevant gap in the literature, the suitability of the biologically treated wastewater for microalgae growth was investigated at 20, 60, 100, and 140 µmolphoton/m²s light intensities with alkaline pH adjustment and pH shocking methods. Optimal phosphate and ammonium removal (up to 100%) was achieved using the alkaline pH method at 100 and 140 µmolphoton/m²s. In terms of biodiesel production, the most suitable result was obtained with the pH shocking method and the lipid ratio was calculated as 20% in the dry biomass of the C5.2 samples at 100 µ molphoton/m²s.The highest chlorophyll-a concentration (3.32 mg/L) was measured in the same sample at 140 µmol photons/m²s. Fatty acid methyl ester species obtained predominantly C16 and C18 fatty acids at all light intensities. According to the results, *Chlorella vulgaris* grown at 100 µmolphoton/m²s is optimal for biodiesel production due to its high lipid content and fatty acid methyl ester types. Kinetic analyses showed that phosphate and ammonium removal predictability with *Chlorella vulgaris* followed first-order kinetics with R^2 values of 96% and 85%, respectively. Under optimal light intensity, integrating *Chlorella vulgaris* cultivation into a biological slaughterhouse wastewater treatment system enables advanced treatment with real-world applicability.

Cite this article as: Katırcıoğlu Sınmaz G, Şengil İA. The effect of different light intensities on the growth of *Chlorella Vulgaris* in advanced biologically treated poultry slaughterhouse wastewater. Sigma J Eng Nat Sci 2026;44(2):781–793.

INTRODUCTION

The continued rise in CO_2 levels in the atmosphere is disquieting. The most crucial advantage of biodiesel obtained from oilseed plants and microalgae is that the carbon dioxide in the atmosphere is used via photosynthesis. According to the rapid growth rate, high lipid content and long-chain fatty acid methyl ester profile of microalgae, they are considered as a suitable source for biodiesel

***Corresponding author.**
*E-mail address: gkatircioglu@sakarya.edu.tr

This paper was recommended for publication in revised form by Editor-in-Chief Ahmet Selim Dalkilic

production [1]. The fatty acid methyl ester composition of microalgae determines the quality of the produced biodiesel [2]. For this reason, studies on biodiesel production from microalgae have gained speed in recent years.

Microalgal growth and lipid production are directly affected by abiotic conditions such as essential nutrients, light, temperature, CO_2, pH, and growth phase [3]. Light intensity is remarkably significant for developing microalgae culture in the presence of sufficient nutrients. Since microalgae photosynthesize and grow by carbon fixation in the air with the presence of light, more biomass is expected to be formed as the light intensity increases [4]. Nonetheless, excessive light intensity inhibits microalgae growth [5]. While the microalgae biomass increased with the increase in light intensity, lipid production mainly increased depending on the microalgae species and other environmental conditions [6,7] and decreased in some studies [8,9] and unaffected by light intensity [10]. While developing microalgae culture, using wastewater as a nutrient provides both-sided benefits by providing advanced treatment of wastewater compared to traditional treatment methods and obtaining biodiesel from the developed microalgae biomass [11]. Microalgae take the nutrients necessary for their growth from wastewater in NH_4^+ and PO_4^{3-} forms [12]. As the light intensity increases, the removal of nitrogenous and phosphorous compounds in wastewater increases [13]. While ammonium can be removed from wastewater with high efficiency at low light intensity (14 μmolphoton / m^2s), it is also possible to remove phosphate with lower efficiency [14]. Light is one of the most important parameters that *Chlorella vulgaris* needs during photosynthesis to develop. However, light's wavelength and light intensity are essential for the lipid content and fatty acid methyl ester (FAME) composition of the microalgae biomass [3].

While some microorganisms and microalgae can live in a consortium, there is competition with some organisms [12]. When biologically treated wastewater is used as a nutrient medium, eliminating wastewater from harmful microorganisms is very important for developing microalgae. For *Chlorella ellipsoidea* cultures grown using secondary treatment domestic wastewater effluent, wastewater was filtered and sterilized before the experiment [15]. In another study where *Chlorella sp.* was grown using domestic wastewater treatment plant effluent, filtration and UV radiation methods were used to solve the rotifer and bacteria problem [16]. In a study in which *Nannochlropsis salina* was grown in an artificial nutrient medium supplemented with rotifers, the hydrodynamic cavitation method was used to remove rotifers, and a high rate of rotifer removal efficiency was obtained [17]. In addition to these methods, lower cost and large scale applicable methods should be developed. In a previous study, pH shocking method was used to remove harmful organisms from biologically treated slaughterhouse wastewater. The pH shocking time and period, which give the most suitable result for microalgae development, have been determined [18]. In a recent study, the pH of the culture medium was adjusted to alkaline in order to destroy the microorganisms in biologically treated poultry slaughterhouse wastewater that inhibit *Chlorella vulgaris* growth. *Chlorella vulgaris* culture was developed successfully at pH 10.5, which is unsuitable for the survival of rotifers [19].

Studies on utilizing poultry slaughterhouse wastewater for microalgae cultivation have gained attention. These wastewaters have undergone various pretreatment processes and have been used to grow different microalgae species. In studies on microalgae cultivation, poultry slaughterhouse wastewaters have been used in various forms: untreated [20], autoclaved [21], filtered and autoclaved [22], or pretreated with acid precipitation [23]. Research gaps that need to be addressed include developing sustainable and cost-effective methods to eliminate harmful microorganisms in biologically treated slaughterhouse wastewater and investigating the suitable light intensity for growing *Chlorella vulgaris*. Therewithal, there is a gap in the literature regarding the effect of light intensity on the cultivation of *Chlorella vulgaris* with biologically treated poultry slaughterhouse wastewater

This study aims to determine the optimal light intensity for developing *Chlorella vulgaris* culture in biologically treated poultry slaughterhouse wastewater. At the same time, it was aimed to grow *Chlorella vulgaris* culture suitable for biodiesel production in biologically treated poultry slaughterhouse wastewater. In all light intensity studies, it was planned to investigate the sufficient nutrient supply of slaughterhouse wastewater for microalgal development with *Chlorella vulgaris* cultures grown in BG-11 nutrient medium as a control group. In order to monitor microalgae growth, total time dependent biomass and chlorophyll-a determinations were made. At the same time, the amounts of NH_4^+ and PO_4^{3-} were measured over time in order to follow the advanced treatment of slaughterhouse wastewater. Kinetic model calculations were made for microalgae growth and advanced treatment of slaughterhouse wastewater.

MATERIALS AND METHODS

Culture Condition

Within the scope of the study, it was aimed to examine the effect of *Chlorella vulgaris* on the advanced treatment of biologically treated poultry slaughterhouse wastewater and at the same time to compare the pH shocking and alkali culture (fixed pH at 10.5) methods used in the removal of harmful microorganisms in the slaughterhouse wastewater. *Chlorella vulgaris* is grown in the laboratories of the Environmental Engineering Department of Sakarya University and fed regularly with sterilized BG-11 nutrient medium. BG-11 is extensively used as a nutrient medium for the growth of blue-green algae species. In 1 liter of BG-11 nutrient medium contains 1.5 g $NaNO_3$, 0.075 g $MgSO_4.7H_2O$, 0.04 g K_2HPO_4, 0.036 g $CaCl_2.2H_2O$, 0.02

Table 1. Biologically treated poultry slaughterhouse wastewater

Parameter	Value
COD (mg/L)	98
Oil & Gres (mg/L)	20
NH_4^+-N (mg/L)	96.4
NO_3^--N (mg/L)	20.5
PO_4^{3-}-P (mg/L)	1.82
pH	6.98

g Na_2CO_3, 0.006 g citric acid, 0.006 g ferric ammonium citrate, 0.001 g EDTA (disodium magnesium salt), and 1 ml trace element mix [24]. *Chlorella vulgaris* culture at a rate of 10% by volume has been inoculated into the samples. The slaughterhouse wastewater was collected as 24 hours composite after the biologically treatment of poultry slaughterhouse wastewater at Sakarya – Turkiye (Table 1). A 24-hour composite biologically treated poultry slaughterhouse wastewater sample was obtained from the biological treatment plant outlet of a private chicken meat producer. Microalgae growth experiments were started within 2 hours after the biologically treated poultry wastewater sample was taken. Experiments were carried out with at least three replicates and average values are given for all results.

With the intention of developing *Chlorella vulgaris* in biologically treated slaughterhouse wastewater, the cultures were growth at room temperature with continuous white light illumination of 4 different light intensities, 20 - 60 - 100 - 140 μmol photons $m^{-2}s^{-1}$ in 1 L erlenmeyer flasks for 12 days. Cultivation was carried out continuously on a shaker at 150 rpm. For each light intensity, four different samples were prepared. These samples were named as BG11, BG11pH, C5.2 and pH10.5. Two of the samples contain BG11 as a nutrient medium, while two contain biologically treated slaughterhouse wastewater. BG11 nutrient medium provides all the nutrients required for the growth of *Chlorella vulgaris*. In order to compare the effect of biologically treated poultry slaughterhouse wastewater on the growth of *Chlorella vulgaris*, samples containing BG11 nutrient medium were also studied. Some rotifers and bacteria harm microalgal growth in the biologically treated poultry slaughterhouse wastewater that used in the study. Rotifers, in particular, consume microalgae, and therefore, algae death occurs in a short time. Two different methods were applied to inhibit predators without damaging the microalgae cells; pH stabilization at alkaline pH (pH10.5) and pH shocking. pH methods were preferred in order to cause minimum damage to microalgae life (not to add extra chemicals) and to present a different method as a contribution to the literature.

- BG11 is a control sample and its pH has not been interfered with.
- The pH of the C5.2 sample was adjusted to pH 2 for 5 minutes every 2 days to eliminate harmful microorganisms in the biologically treated slaughterhouse wastewater, adjusted to pH 8 at the end of the 5th minute. This process is called pH shocking.
- BG11pH sample was prepared to examine the effect of pH shocking on microalgae grown in nutrient medium containing BG-11. The pH shock process was carried out under the same conditions as the C5.2 sample.
- pH10.5 sample was prepared to determine the growth of microalgae in the Biologically treated slaughterhouse wastewater in alkaline condition (pH10.5) and to compare with pH shocking conditions.

Predatory microorganisms at low and high pH levels cannot survive in biologically treated water. However, to not adversely affect the microalgae's life, the pH change is carried out within 5 minutes with this method called pH shocking [18]. Also *Chlorella* vulgaris microalgae can live in alkaline conditions up to pH 11 [19].

Analytical Methods

Microalgae culture has been developed in the biologically treated water of poultry slaughterhouse and BG-11 nutrient medium. In order to determine the nutrient removal in samples, ammonium ($N\text{-}NH_4^+$), phosphate ($P\text{-}PO_4^{3-}$) analyses have been conducted at beginning and every four days [25]. Before the nutrient analysis, the samples were centrifuged at 14000 rpm for 15 minutes at 4°C in order to separate the grown biomass from the samples. Microalgae growth has been monitored by total suspended solids (mg/L) and, chlorophyll-a at beginning and every four days [25,26]. NH_4 and PO_4^{3-} test kits were purchased from MERCK. Ammonium ($N\text{–}NH_4$) analogous to the method: APHA 4500-NH_3 F), phosphate ($P\text{–}PO_4$) analogous to method APHA 4500-P C analyses have been conducted with spectrophotometric methods (APHA 2017). The MERCK brand Pharo 3600 spectrophotometer has been used for the analyses.

Lipid Extraction and Transesterification

Developed biomass has been harvested by sedimentation. The harvested biomass has been dried entirely at 40°C in the vacuum oven. In order to get efficient results from lipid extraction, the cell wall has been broken down in an autoclave with 50 ml of distilled water containing 0.5% nitric acid at 121°C for 30 min following the method by [27]. The total lipid of dried microalgae biomass has been extracted with a mixture of chloroform and methanol (2:1 v/v) for 3h. The solvent was centrifuged at 3000 rpm for 5 min and dried in a vacuum oven at 40°C. The weight of the extracted total lipid was verified gravimetrically [28,29].

The transesterification method that used was modified from [29]. Acidic esterification method was chosen for transesterification and was carried out in a water bath with sulfuric acid and methanol. The fatty acid methyl esters formed as a result of esterification were transferred to the

n-heptane phase for injection to the gas chromatograph. The fatty acid profile was determined on a gas chromatograph (Shimadzu - QP 2010 GC/MS) equipped with a GC/MS detector and a highly polar Rt2560 capillary column (100 m x 0.25 mm x 0.20 µm) [30].

Kinetic Model

Kinetic model calculations were made according to reaction time and reaction rate constant. The regression coefficient values were calculated according to the kinetic model calculations. For the growth of microalgae, first-order and second-order kinetic models were examined in the kinetic calculations for biomass and chlorophyll-a increases. The k and R^2 values were calculated for the investigated kinetic models. The kinetic equations (Eq.1) and (Eq. 2) are as given below [31]:

$$1^{st} \text{ order kinetics: } \ln\frac{C_0}{C} = k.t \quad (1)$$

$$2^{nd} \text{ order kinetics: } \frac{1}{C} - \frac{1}{C_0} = k.t \quad (2)$$

Co: the initial concentration of biomass

C: concentration of biomass in t time

k: reaction rate constants.

First-order and second-order kinetic models were also examined in the kinetic calculations for removing PO_4^{3-} and NH_4^+ from the biologically treated poultry slaughterhouse wastewater by microalgae.

RESULTS AND DISCUSSION

Nutrient Removal from Wastewater Depending on Light Intensity

Chlorella vulgaris cultures have been developed in BG11, BG11pH, C5.2, and pH10.5 samples for 12 days at 20, 60, 100, and 140 µmolphoton/m^2s light intensities. Since the initial ammonium and phosphate amounts of the samples containing BG11 and the biologically treated poultry slaughterhouse wastewater samples were different, the results were examined in terms of the removal rates calculated with the values measured at the end of the 12th day.

The lowest NH_4^+ removal was obtained at 20 µmolphoton/m^2s compared to other light intensities. However, 77.95% NH_4^+ removal was achieved in the biologically treated poultry slaughterhouse wastewater sample, C5.2 at 20 µmolphoton/m^2s (Figure 1). For all light intensities, the NH_4^+ removal at the end of the 12th day was almost 100% for pH 10.5 sample. In the samples containing BG11, NH_4^+ removal increased as the light intensity increased. In the pH-shocked BG11pH sample, more NH_4^+ removal was obtained than the control sample BG11.

PO_4^{3-} removal increased with the increase of light intensity up to 100 µmol photon/m^2s for C5.2 and pH 10.5 samples. Since the removal amounts were relatively high, it tended to be fixed at 100 and 140 µmol photon/m^2s. Another reason for this fixation trend is that microalgae growth is similar and at high concentrations at 100 and 140 µmol photon/m^2s. 100% PO_4^{3-} removal was achieved for C5.2 and pH 10.5 samples at 100 and 140 µmol photon/m^2s (Figure 2).

As the light intensity increased, nutrient removals increased, and more than 70 percent nutrient removal was obtained even at the lowest light intensity in samples containing wastewater. For this reason, in addition to the purpose of cultivating *Chlorella vulgaris* culture in the biologically treated poultry slaughterhouse wastewater for biodiesel production, high efficiency was obtained at low light intensities in order to increase nutrient removal. In a study performed at 14 µmol/m^2s at low light intensity, 93.5% of ammonium and 11.1% of phosphate were removed with anaerobic wastewater treatment secondary effluent after 8 days of development of *Chlorella vulgaris* [14]. In another study, 79.63% nitrate and 100% phosphate were removed

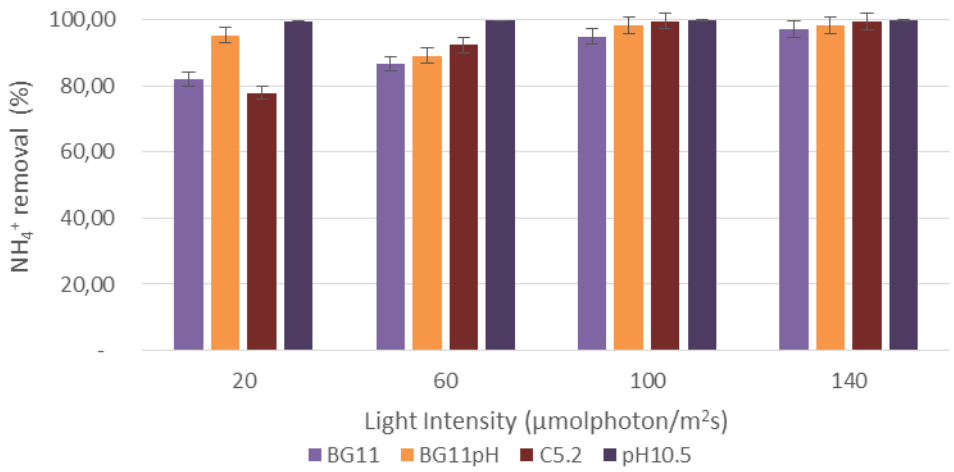


Figure 1. NH_4^+ removal at 12th day depending on light intensity.

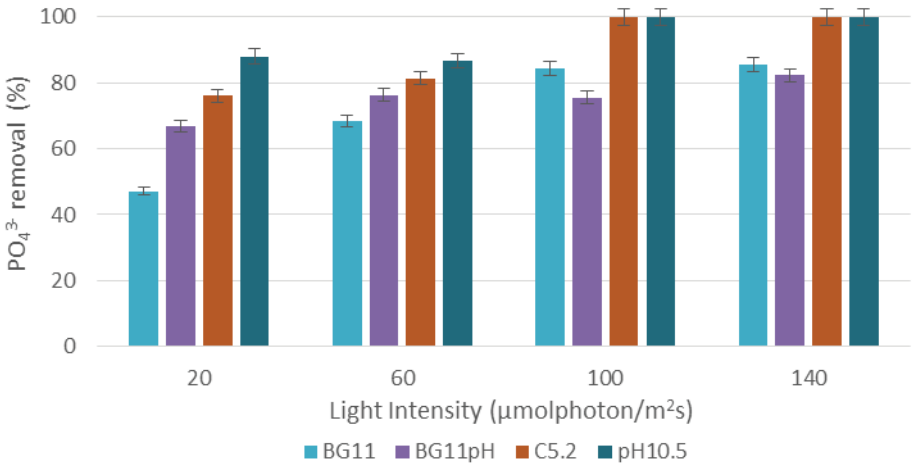


Figure 2. PO_4^{3-} removal at 12th day depending on light intensity.

from the domestic wastewater treated with *Chlorella vulgaris* after a 14-day culture period at a light intensity of 3500 lux (≈ 50 µmolphoton/m²s) [32]. In a study investigating the effect of light intensity in wastewater with different types of microalgae, it was found that as light intensity increased, total nitrogen (TN) removal for *Chlorella vulgaris* decreased, while total phosphorus (TP) removal increased. At the end of 8 days, it was calculated that total nitrogen and total phosphorus were removed by 75% with all microalgae species [7]. In a study conducted by Solmaz and Işık, nutrient removal results were obtained as 50.08% for NH_4-N and 4.54% for PO_4-P from synthetic wastewater with a culture of a mixture of different microalgae species [33].

In a study, total nitrogen and total phosphorus removal in pretreated slaughterhouse wastewater with mixed microalgae culture using a photobioreactor were obtained as 70.2% and 96.2%, respectively, after 7 days [22]. In another study, nitrate and phosphate removal efficiencies were obtained as 18% and 48%, respectively, with *Chlorella pyrenoidosa* culture in autoclaved and diluted poultry slaughterhouse wastewater [21]. When the studies in the literature are examined; Although nutrient removal rates are related to microalgae growth, it is understood that they vary with initial concentration, culture time, and microalgae species [7,14,32]. It has also been proven that as the light intensity increases, microalgae growth increases up to a point, and that very high light intensity will be inhibitory for microalgae growth and nutrient removal [5].

Chlorella Vulgaris Growth Depending on Light Intensity

In experiments carried out at different light intensities (20, 60, 100 and 140 µmolphoton/m²s) for microalgae growth, *Chlorella vulgaris* biomass grown in BG11, BG11pH, C5.2 and pH 10.5 samples and chlorophyll-a pigment contents of these samples were compared. Biomass graphs were drawn for all samples depending on time and light intensity and are given in Figure 3. As the light intensity increased in all samples, the amount of biomass increased continuously for 12 days.

According to the time-dependent measurement results for 12 days, less biomass increase was measured in the C5.2 sample compared to the control sample BG11 at all light intensities. Biomass growth of BG11pH was slightly slower compared to BG11. At the end of the 12th day at 140 µmolphoton/m²s, the amounts of biomass formed in BG11pH and BG11 samples were measured in very close amounts as 1030 mg/L and 1180 mg/L, respectively. This situation indicates the negative effect of pH shock on microalgae. However, it is possible to say that pH shocking for the destruction of harmful microorganisms in the biologically treated poultry slaughterhouse wastewater is negligible in terms of microalgae growth. pH 10.5 was the sample in which the least and slow microalgae growth was detected for each light intensity among all samples, and the microalgae biomass grown was relatively small compared to C5.2. The 20 µmolphoton/m²s light intensity was insufficient for microalgae growth in all samples.

Samples containing BG11 increased their biomass from around 200 mg/L to over 1000 mg/L at 100 and 140 µmolphoton/m²s light intensities at the end of the 12th day. The biomass amount of the C5.2 sample was measured as 1000 mg/L at 100 µmolphoton/m²s and 860 mg/L at 140 µmolphoton/m²s at the end of the experiment. This is due to the difference in wastewater and artificial nutrient media content and the presence of competitive microorganisms in the biologically treated poultry slaughterhouse wastewater. As in this study, in studies in the literature, high light intensity has an inhibitory effect on algae growth after a point [3,13]. Measurement of chlorophyll-a amount depending on time and light intensity is an essential parameter for monitoring microalgae growth in samples [34]. Chlorophyll-a amounts measured for all samples at 4 different light intensities for 12 days are shown in Figure 4. The highest amount of

chlorophyll-a was obtained with the control sample, BG11. After 12 days, chlorophyll-a was measured as 4.40 and 4.19 mg/L for BG11 and BG11 pH, respectively. Increases and decreases in chlorophyll-a amounts in BG11pH and C5.2 samples indicate the effect of pH shock and algae adaptation. In samples containing biologically treated poultry slaughterhouse wastewater, the highest chlorophyll-a 3.32 mg/L for C5.2 at 140 µmolphoton/m²s; For pH 10.5, it was measured as 2.83 mg/L. Chlorophyll-a amounts due to microalgae deaths decreased in all samples at 20 µmolphoton/m²s

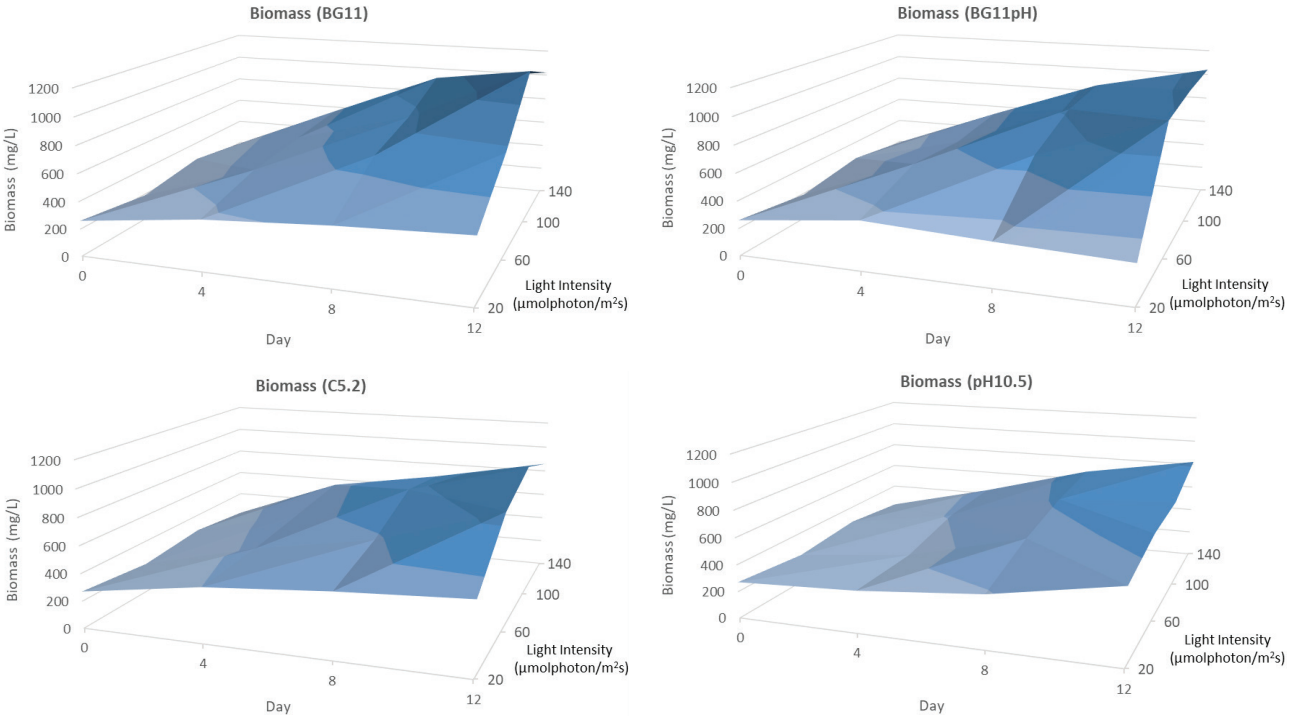


Figure 3. Biomass growth depending on light intensity.

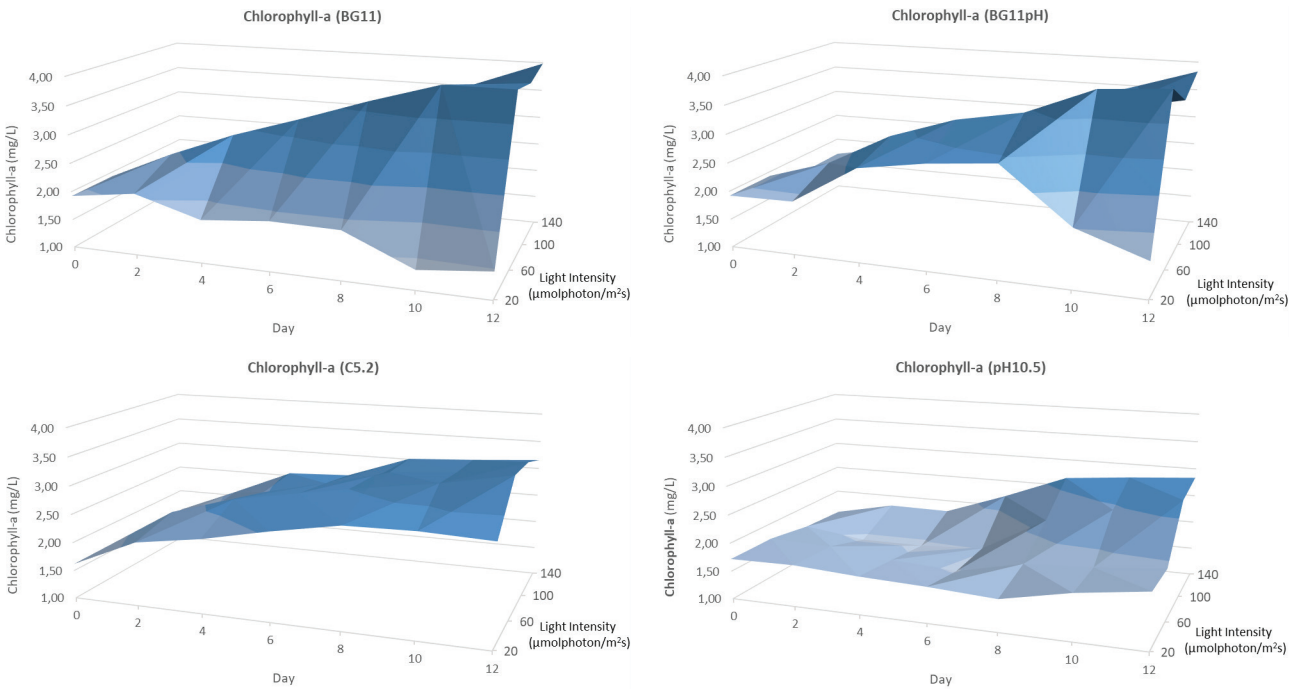


Figure 4. Chlrophyll-a growth depending on light intensity.

and were insufficient for microalgae growth under these experimental conditions.

The amount of chlorophyll-a represents viable and healthy algal cells. For this reason, it is understood by comparing the results that it is different from the total biomass amount and increases slightly. Increasing light intensity significantly increases chlorophyll-a synthesis in microalgae cells [35]. A study examining the effect of light intensity stated that chlorophyll began to degrade at high light intensities. In the same study, *Chlorella sp.* For continuous light, medium-intensity light (200 μmolphoton/m^2s) was stated to be the best for biomass increase [34].

Total Lipid and Fatty Acid Methyl Esters Formed Depending on Light Intensity

Table 2 shows that the total lipid production for all samples increased as the light intensity increased. According to the results obtained, it is clear that the pH 10.5 method is less suitable for lipid production. C5.2 sample containing the biologically treated poultry slaughterhouse wastewater developed by pH shocking method was compared with BG11 control sample, and matching results were obtained regarding lipid production. The effect of pH shocking on microalgae growth is revealed by comparing the BG11pH sample with the control sample. Comparing the total lipid ratios obtained for the BG11pH sample with the control sample BG11, it formed 1-3% less lipid at low light intensities and less than 1% at high light intensities.

The highest total lipid content in the C5.2 sample was measured with 0.1142 g at 140 μmolphoton/m^2s light intensity. The lowest total lipid amount was calculated as 0.0886 g at 20 μmolphoton/m^2s for C5.2. With the increase in light intensity, the lipid ratios of the dry biomass obtained increased. In studies in the literature, While microalgae biomass increased with increasing light intensity, lipid production increased primarily due to microalgae species and other environmental conditions [6,7], decreased in some studies [8,9], and was not affected by light intensity in some studies [10].

The lipid ratios in the dry biomass of the C5.2 sample were obtained as 19.5% and 20.5% of the dry biomass at 100 and 140 μmolphoton/m^2s light intensities, respectively. These lipid ratios obtained are slightly higher than BG11. The highest lipid content of 14.8% was obtained in the biomass developed in slaughterhouse wastewater with different microalgae species [20]. pH 10.5 has the lowest lipid content in dry biomass at all light intensities. High light intensity causes a decrease in lipid content. In a study conducted by [34] in which the effect of light intensity was examined, it was stated that chlorophyll-a began to deteriorate at high light intensities, but the lipid content of the biomass increased. In a study, it was explained that light intensities higher than 150 μmolphoton/m^2s in a sample given over a photobioreactor, depending on the microalgae species, prevent microalgae growth [5]. For this reason, it is not possible to obtain microalgae growth with the desired efficiency at very high light intensities.

Low light intensity, on the other hand, is more suitable for lipid deposition, because at high light intensity microalgae tend to proliferate rather than increase lipid [8]. For this reason, it is vital to determine the optimum light intensity for lipid production. Light intensities higher than 140 μmolphoton/m^2s were not studied in this study due to the decrease in light intensity compared to 100 μmolphoton/m^2s in fatty acid methyl esters at 140 μmolphoton/m^2s and the inhibitory property of high light intensity. Total lipid amounts and lipid ratios in dry biomass were higher for each light intensity in the C5.2 sample than in the BG11 control sample. This situation can be explained by the increased lipid ratio of *Chlorella vulgaris* under stress with pH shocking. In addition, it can be explained by the high concentration of nitrogen and phosphorus nutrient content of poultry slaughterhouse wastewater compared to BG11 nutrient medium [16,36].

FAME obtained as a result of lipid transesterification. The FAME profile obtained from Chlorella vulgaris consists of palmitic acid (C16:0), stearic acid, palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) (Table 2). As a result of the increase in light intensity, there were differences in the composition and concentration of fatty acid methyl esters.

The total amount of lipids obtained was also low in parallel with the microalgae growth, chlorophyll-a pigment analysis, and biomass measurement results at 20 μmolphoton/m^2s. Likewise, the concentration of fatty acid methyl esters and the detected FAME species are also quite low compared to the concentrations obtained at other light intensities. For this reason, low illumination was found insufficient for this study in terms of microalgae growth and the use of developed biomass as biofuel raw material. The sample, which was constantly kept at pH 10.5, gave insufficient results regarding the obtained FAME species and concentrations compared to the pH shocking method.

The highest concentrations of saturated fatty acids (C16:0 and C18:0) were obtained in the C5.2 sample at 60 μmolphoton/m^2s, and saturated fatty acids constitute 73.39% of the total fatty acids. At 140 μmolphoton/m^2s, the highest light intensity, the C5.2 sample has a higher lipid ratio than the control sample. Saturated fatty acids C16:0 and C18:0, and unsaturated fatty acids C18:2 and C18:3 formed at 100 and 140 μmolphoton/m^2s. Fatty acid methyl ester content from microalgae biomass is an important parameter for evaluating the suitability of microalgae for biodiesel production.

The presence of C16 and C18 fatty acid methyl esters is an indicator of quality biodiesel [37]. C18 fatty acids obtained from microalgae; they exist mainly in unsaturated form (C18:1, C18:2, and C18:3) [38]. C16-C18 saturated and unsaturated fatty acids obtained in this study overlap with those obtained from microalgae in the literature.

Table 2. Ratios of total lipid (g) and FAME (%) grown in total microalgae depending on light intensity

Light Intensity (μmolphoton/m²s)	Sample	Total lipid (g)	Lipid ratio in dry biomass (%)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
20	BG11	0.078	11.986	0.032					
	BG11pH	0.072	12.966	0.022					
	C5.2	0.089	16.407	0.120	0.010	0.010		0.049	
	pH10.5	0.056	13.455	0.045				0.012	
60	BG11	0.105	13.690	0.227	0.009	0.012	0.007	0.019	
	BG11pH	0.111	16.609	0.272	0.030	0.060		0.075	
	C5.2	0.103	15.785	0.208	0.021	0.043		0.070	
	pH10.5	0.056	12.790	0.047				0.010	
100	BG11	0.087	17.283	0.211		0.047		0.076	0.034
	BG11pH	0.107	16.612	0.152		0.026		0.052	0.019
	C5.2	0.109	19.517	0.184		0.033		0.074	0.023
	pH10.5	0.079	18.769	0.067		0.025		0.021	
140	BG11	0.121	18.311	0.147		0.034		0.050	0.009
	BG11pH	0.119	17.633	0.162		0.042		0.074	0.019
	C5.2	0.114	20.429	0.158		0.030		0.064	0.017
	pH10.5	0.052	9.756	0.013					

The suitability of the obtained fatty acids for biodiesel production was evaluated by the European biodiesel quality standard (EN 14214). As stated in EN 14214, the main limiting factor is considered to be the ratio of C18:2 fatty acids to total fatty acids [39]. C18:3 linolenic acid, should not exceed 12% in fatty acids; It was below this ratio at all light intensities in the C5.2 sample (Table 3). While linolenic acid (C18:3) did not form in the C5.2 sample at 20 and 60 μmolphoton/m²s light intensities, it was calculated as 7.325% and 6.320%, respectively, at 100 and 140 μmolphoton/m²s light intensities.

The obtained biodiesel is not desired that it consists of only saturated or only unsaturated fatty acids in order to establish the appropriate balance of properties such as combustion, oxidative stability, and viscosity. Ideally, quality biodiesel is expected to consist of a mixture of long-chain saturated fatty acids (SFA), short-chain mono (MUFA), and polyunsaturated (PUFA) fatty acids [1,39,40]. In this case, there is no objection to European standards to using the obtained biodiesel as fuel.

As given in Table 3, the biodiesel obtained from *Chlorella vulgaris* grown in the biologically treated water of the poultry slaughterhouse provides the necessary conditions for a quality biodiesel. Appropriate lipid ratio and FAME content for biodiesel production were obtained from the C5.2 sample at all light intensities. In terms of lipid ratio of dry biomass, fatty acid methyl ester amounts and species, the most suitable results were obtained for microalgae growth in the C5.2 sample at 100 μmolphoton/m²s light intensity.

Table 3. SFA, MUFA and PUFA ratios (%) in C5.2 sample at different light intensity

	Light intensities (μmolphoton/m²s)			
FAME	20	60	100	140
C16:0	0.12	0.208	0.184	0.158
C16:1	0.01	0.021		
C18:0	0.01	0.043	0.033	0.03
C18:2	0.049	0.07	0.074	0.064
C18:3			0.023	0.017
Total	0.189	0.342	0.314	0.269
%SFA	68.783	73.392	69.108	69.888
%MUFA	0.015	0.029	0	0
%PUFA	25.926	20.468	30.892	30.112
% C18:3	0	0	7.325	6.320

Kinetic Model Calculations

Microalgae growth kinetic model calculations were made depending on the time of microalgae developed in C5.2 sample at optimum light intensity. Similarly, the removal kinetic models were calculated for NH_4^+ ve PO_4^{3-} in the C5.2 sample at optimum light intensity.

Microalgae Growth Kinetics

The kinetic equations were calculated by Equation 1 and Equation 2 [31]. The k and R^2 values obtained from the kinetic calculations are given in Table 4.

Table 4. R^2 and k values for biomass and Chlorophyll-a obtained from kinetic calculations

Kinetic Model	Biomass		Chlorophyll-a	
	k	R^2	k	R^2
1st order	-0.1397	0.8553	-0.07	0.8647
2nd order	-0.0003	0.8499	-0.032	0.7852

According to the kinetic calculations, the increase in biomass and chlorophyll-a gave results consistent with the 1st order kinetics. As seen in Figure 5, the experimental results obtained and the theoretical results calculated according to the 1st-order kinetic model calculated for biomass increase are compatible. However, after the 8th day, the experimental concentration value of the biomass tends to stabilize. This situation is because microalgae growth has entered a stagnation phase with nutrient reduction due to nutrient depletion [41]. The microalgae growth entered the stagnation phase after the logarithmic increase phase. At the beginning of the stagnation phase, the increase in biomass tends to stabilize.

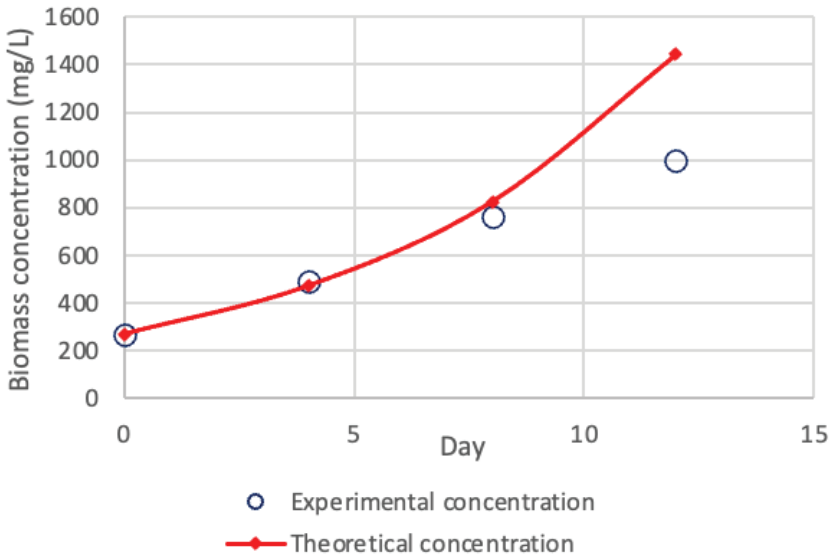


Figure 5. Comparison of theoretical and experimental biomass amounts obtained from kinetic calculations. Light intensity:100 µmolphoton/m^2s.

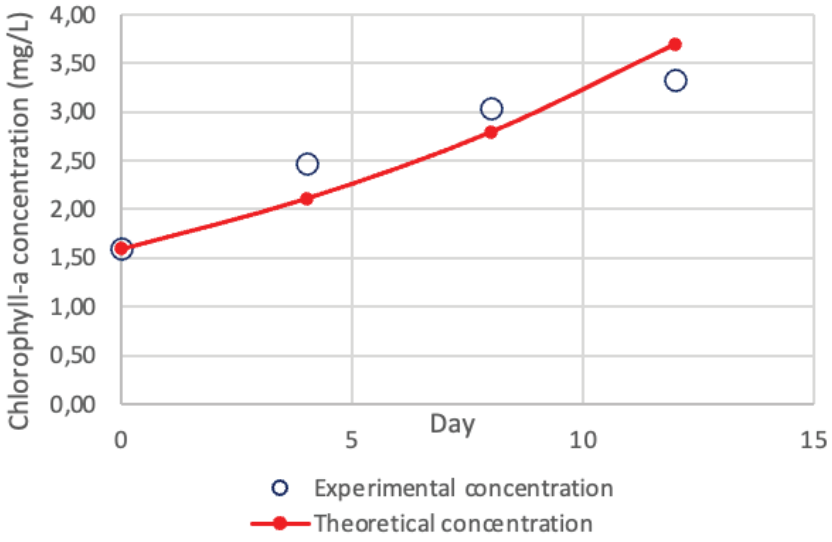


Figure 6. Comparison of theoretical and experimental chlorophyll-a amounts obtained from kinetic calculations. Light intensity:100 µmolphoton/m^2s.

Experimental and theoretical results in chlorophyll-a increase calculated according to the 1st order kinetic model are close to each other (Figure 6). On the 12th day, the experimental concentration of chlorophyll-a decreased compared to the theoretically calculated value due to the depletion of the nutrient in the medium. As for biomass, the experimental concentration of chlorophyll-a tends to stabilize at the end of the experiment. The compatibility of biomass and chlorophyll-a increases, proving the accuracy of the 1st-order kinetic models.

Nutrient Removal Kinetics from the Biologically Treated Poultry Slaughterhouse Wastewater

Kinetic model calculations were made according to reaction time and reaction rate constant. The regression coefficient values were calculated according to the kinetic model calculations. First-order and second-order kinetic models were examined in the kinetic calculations for removing PO_4^{3-} and NH_4^+ from the biologically treated poultry slaughterhouse wastewater by microalgae. The k and R^2 values were calculated for the investigated kinetic models. The kinetic equations were calculated by Equation 1 and Equation 2 [31]. The k and R^2 values obtained from the kinetic models are given in Table 5.

Calculated k and R^2 values from the kinetic models for the removal of PO_4^{3-} and NH_4^+ have been given in Table 5. According to the table, the most suitable model for PO_4^{3-} and NH_4^+ has been fit to the 1st-order kinetics with the R^2 values of 0.9634 for PO_4^{3-} and 0.8516 for NH_4^+, respectively. R^2 values close to 1 indicate the reliability and predictability of the nutrient removal of the process. In a study by Abirama et al. 2021, the biokinetic model of ammonium and phosphate removal from meat processing unit wastewater by Botrycoccus sp. microalgae was investigated. The kinetics of ammonium and phosphate removal of Botrycoccus sp. microalgae from wastewater were found to be 99.03 % and 99.93 %, respectively [42].

Table 5. R^2 and k values for the removal of PO_4^{3-} and NH_4^+ obtained from kinetic calculations

Kinetic model	PO_4^{3-}		NH_4^+	
	k	R^2	k	R^2
1st order	0.5383	0.9634	0.3911	0.8516
2nd order	54.853	0.5533	0.1693	0.5512

Moreover, k values have been figured out for kinetic models, and the first order model, which was the most appropriate, have been calculated as 0.5383 for PO_4^{3-} and 0.3911 for NH_4^+. Obtained experimental data and calculated theoretical results using the 1st order kinetic model for the removal of PO_4^{3-} and NH_4^+ have nearly overlapped, as seen in Figure 7 and Figure 8.

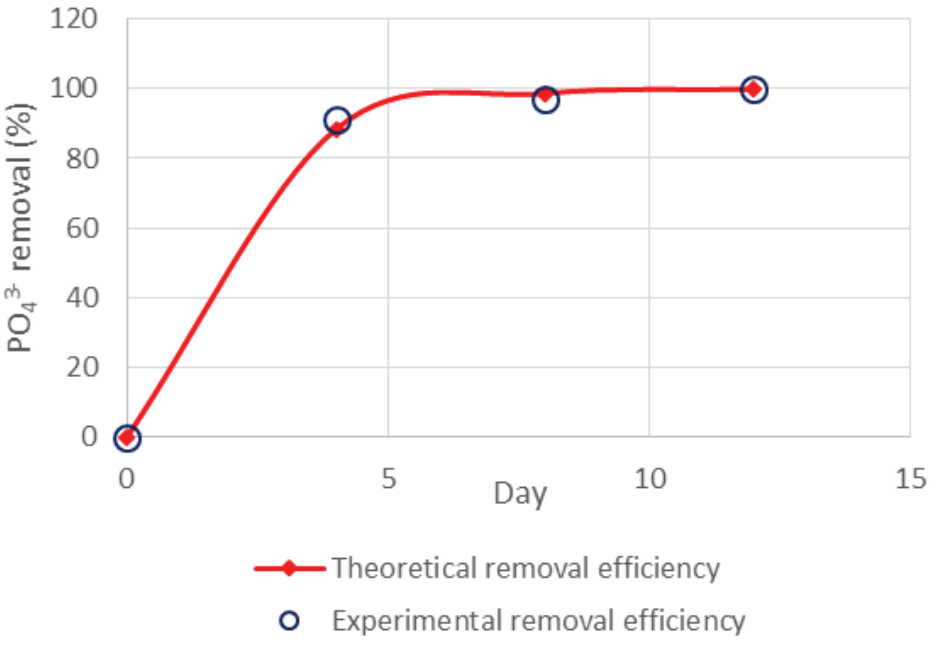
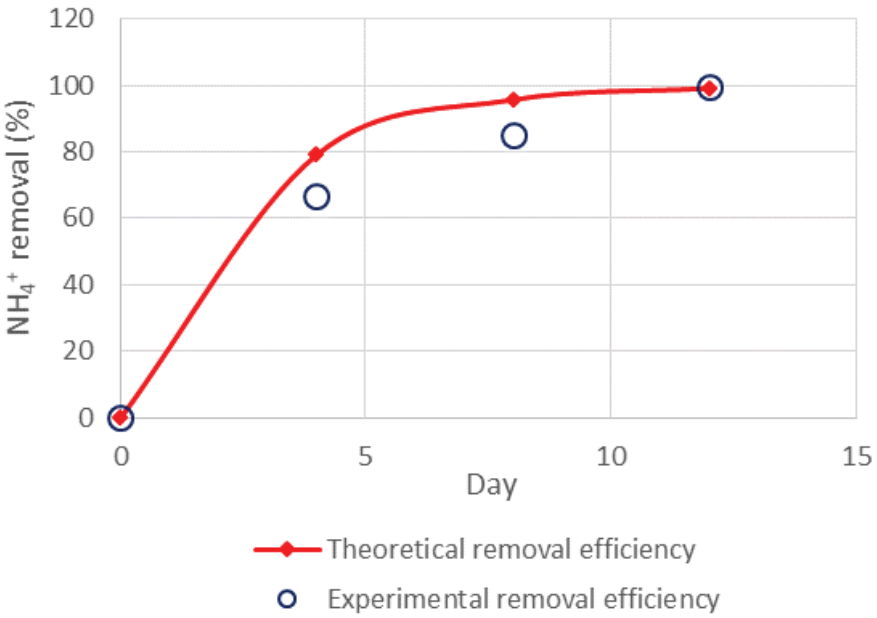


Figure 7. Experimental data and theoretical results using the 1st order kinetic model for the removal of PO_4^{3-}. Light intensity:100 μmolphoton/m^2s.



Figurel 8. Experimental data and theoretical results using the 1st order kinetic model for the removal of NH_4^+. Light intensity: 100 µmolphoton/m^2s.

CONCLUSION

This study aimed to investigate the advanced treatment of biologically treated poultry slaughterhouse wastewater and to find the optimum light intensity for developing microalgae culture. Although biologically treated wastewater contains suitable nutrients for microalgal growth, it is not possible due to rotifers and bacteria in wastewater. For this reason, suitability for microalgal growth and advanced treatment of the biologically treated poultry slaughterhouse wastewater were investigated by changing the pH of the nutrient medium with two different methods. The harmful organisms were destroyed by the alkaline pH method (pH 10.5), but fixing the pH at 10.5 restricted microalgal growth, as seen from the biomass, chlorophyll-a, total lipid and fatty acid methyl ester results. However, since the ammonium and phosphate removal rates from the wastewater are pretty high, it has been found suitable for using biologically treated wastewater with *Chlorella vulgaris* at pH 10.5 for advanced treatment of poultry slaughterhouse wastewater. The growth of *Chlorella vulgaris* in the biologically treated poultry slaughterhouse wastewater by pH shocking method gave similar results with the BG11 artificial medium in terms of biomass and lipid formation. High removal efficiencies (up to 100%) have been obtained at all light intensities for nutrient removal from the biologically treated poultry slaughterhouse wastewater with the C5.2 sample. Nutrient removal rates also increased with the light intensity. According to the study results, *Chlorella vulgaris* developed at 100 µmolphoton/m^2s light intensity is more suitable for biodiesel production in terms of fatty acid methyl ester formation and high lipid ratio in dry biomass. The most important contribution of this study to the literature is the development of *Chlorella vulgaris* culture with pH shocking method with wastewater taken after biological treatment at 100 µmolphoton/m^2s light intensity. Biomass grown in the biologically treated poultry slaughterhouse wastewater with pH shocking method, C5.2, has a higher total lipid amount and lipid ratio in dry biomass than biomass grown in BG11 artificial nutrient medium. The lipid ratio of dry biomass obtained at high light intensities was calculated as 18% for BG11 and 20% for C5.2. According to the results, higher lipid and fatty acid methyl ester were obtained with the establishment of stress conditions. According to European biodiesel quality standards, the obtained fatty acid methyl ester types are also suitable for biodiesel production (EN 14214). When approached in terms of advanced biological treatment and development of microalgae culture ideal for biodiesel production, large-scale application is possible in line with the information obtained in this study. The advantage of implementing this system on a large scale is that pH adjustments are low cost and easy to apply. Microalgae cultivation in large tanks has been done worldwide for many years. Similar designs can be made in addition to the existing biological treatment plants.

AUTHORSHIP CONTRIBUTIONS

Gamze KATIRCIOĞLU SINMAZ (PhD): Conceptualization, formal analysis, validation, investigation, writing - original draft preparation. İsmail Ayhan ŞENGİL (Prof. Dr.) Supervision, methodology, writing - review & editing.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

REFERENCES

[1] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306. [CrossRef]

[2] Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol 2005;86:1059–1070. [CrossRef]

[3] Ananthi V, Brindhadevi K, Pugazhendhi A, Arun A. Impact of abiotic factors on biodiesel production by microalgae. Fuel 2021;284:118962. [CrossRef]

[4] Chang HX, Huang Y, Fu Q, Liao Q, Zhu X. Kinetic characteristics and modeling of microalgae Chlorella vulgaris growth and CO2 biofixation considering the coupled effects of light intensity and dissolved inorganic carbon. Bioresour Technol 2016;206:231–238. [CrossRef]

[5] Ogbonna JC, Tanaka H. Light requirement and photosynthetic cell cultivation - Development of processes for efficient light utilization in photobioreactors. J Appl Phycol 2000;12:207–218. [CrossRef]

[6] Seo SH, Ha JS, Yoo C, Srivastava A, Ahn CY, Cho DH, et al. Light intensity as major factor to maximize biomass and lipid productivity of Ettlia sp. in CO2-controlled photoautotrophic chemostat. Bioresour Technol 2017;244:621–628. [CrossRef]

[7] Nzayisenga JC, Farge X, Groll SL, Sellstedt A. Effects of light intensity on growth and lipid production in microalgae grown in wastewater. Biotechnol Biofuels 2020;13:1–8. [CrossRef]

[8] Cheirsilp B, Torpee S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. Bioresour Technol 2012;110:510–516. [CrossRef]

[9] George B, Pancha I, Desai C, Chokshi K, Paliwal C, Ghosh T, et al. Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae Ankistrodesmus falcatus - A potential strain for biofuel production. Bioresour Technol 2014;171:367–374. [CrossRef]

[10] Pancha I, Chokshi K, Mishra S. Enhanced biofuel production potential with nutritional stress amelioration through optimization of carbon source and light intensity in Scenedesmus sp. CCNM 1077. Bioresour Technol 2015;179:565–572. [CrossRef]

[11] Kligerman DC, Bouwer EJ. Prospects for biodiesel production from algae-based wastewater treatment in Brazil: A review. Renew Sustain Energy Rev 2015;52:1834–1846. [CrossRef]

[12] Wang M, Keeley R, Zalivina N, Halfhide T, Scott K, Zhang Q, et al. Advances in algal-prokaryotic wastewater treatment: A review of nitrogen transformations, reactor configurations and molecular tools. J Environ Manage 2018;217:845–857. [CrossRef]

[13] Yan C, Luo X, Zheng Z. Effects of various LED light qualities and light intensity supply strategies on purification of slurry from anaerobic digestion process by Chlorella vulgaris. Int Biodeterior Biodegrad 2013;79:81–87. [CrossRef]

[14] Cheng T, Wei CH, Leiknes TO. Polishing of anaerobic secondary effluent by Chlorella vulgaris under low light intensity. Bioresour Technol 2017;241:360–368. [CrossRef]

[15] Yang J, Li X, Hu H, Zhang X, Yu Y, Chen Y. Growth and lipid accumulation properties of a freshwater microalga, Chlorella ellipsoidea YJ1, in domestic secondary effluents. Appl Energy 2011;88:3295–3299. [CrossRef]

[16] Cho S, Luong TT, Lee D, Oh Y-K, Lee T. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. Bioresour Technol 2011;102:8639–8645. [CrossRef]

[17] Kim D, Kim EK, Koh HG, Kim K, Han JI, Chang YK. Selective removal of rotifers in microalgae cultivation using hydrodynamic cavitation. Algal Res 2017;28:24–29. [CrossRef]

[18] Katırcıoğlu Sınmaz G, Şengil İA. Microalgae culture development in biologically treated water of poultry slaughterhouse. In: Proc 16th Int Conf Clean Energy; 2018. p. 9–11.

[19] Katırcıoğlu Sınmaz G, Erden B, Şengil İA. Cultivation of Chlorella vulgaris in alkaline condition for biodiesel feedstock after biological treatment of poultry slaughterhouse wastewater. Int J Environ Sci Technol 2022;20:3237-3246. [CrossRef]

[20] Hernández D, Riaño B, Coca M, Solana M, Bertucco A, García-González MC. Microalgae cultivation in high rate algal ponds using slaughterhouse wastewater for biofuel applications. Chem Eng J 2016;285:449–458. [CrossRef]
[21] Azam R, Kothari R, Singh HM, Ahmad S, Ashokkumar V, Tyagi VV. Production of algal biomass for its biochemical profile using slaughterhouse wastewater for treatment under axenic conditions. Bioresour Technol 2020;306:123116. [CrossRef]
[22] Taşkan E. Performance of mixed algae for treatment of slaughterhouse wastewater and microbial community analysis. Environ Sci Pollut Res 2016;23:20474–20482. [CrossRef]
[23] Terán Hilares RA, Garcia Bustos KA, Sanchez Vera FP, Colina Andrade GJ, Pacheco Tanaka DA. Acid precipitation followed by microalgae (Chlorella vulgaris) cultivation as a new approach for poultry slaughterhouse wastewater treatment. Bioresour Technol 2021;335:125284. [CrossRef]
[24] Andersen RA. Algal culturing techniques. 1st ed. New York: Academic Press; 2005.
[25] APHA. Standard methods for the examination of water and wastewater. 19th ed. American Public Health Association Inc.; New York; 1995.
[26] EPA. ESS Method 150.1: Chlorophyll – Spectrophotometric. Environmental Sciences Section Inorganic Chemistry Unit; 1991.
[27] Lee I, Park JY, Choi SA, Oh YK, Han JI. Hydrothermal nitric acid treatment for effectual lipid extraction from wet microalgae biomass. Bioresour Technol 2014;172:138–142. [CrossRef]
[28] Bligh E, Dyer W. Rapid method of total lipid extraction. J Biochem Physiol 1959;37:911–917. [CrossRef]
[29] Soares AT, da Costa DC, Silva BF, Lopes RG, Derner RB, Antoniosi Filho NR. Comparative analysis of the fatty acid composition of microalgae obtained by different oil extraction methods and direct biomass transesterification. Bioenergy Res 2014;7:1035–1044. [CrossRef]
[30] Tanattı P, Şengil A, Özdemir A. Treatment of biodiesel wastewater by solvent extraction: Evaluation of kinetic and thermodynamic data. Environ Eng Manag J 2018;17:2657–2665. [CrossRef]
[31] Lam MK, Yusoff MI, Uemura Y, Lim JW, Khoo CG, Lee KT, Ong HC. Cultivation of Chlorella vulgaris using nutrients source from domestic wastewater for biodiesel production: Growth condition and kinetic studies. Renew Energy 2017;103:197–207. [CrossRef]
[32] Sydney EB, da Silva TE, Tokarski A, Novak AC, de Carvalho JC, Woiciecohwski AL, et al. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. Appl Energy 2011;88:3291–3294. [CrossRef]
[33] Solmaz A, Işik M. Microalgae production with microalgal submerged membrane photo bioreactor (Msmpbr) and examining the nutrient removal yield. Sigma J Eng Nat Sci 2017;8:255–261.
[34] He Q, Yang H, Wu L, Hu C. Effect of light intensity on physiological changes, carbon allocation and neutral lipid accumulation in oleaginous microalgae. Bioresour Technol 2015;191:219–228. [CrossRef]
[35] Gim GH, Ryu J, Kim MJ, Kim II P, Kim SW. Effects of carbon source and light intensity on the growth and total lipid production of three microalgae under different culture conditions. J Ind Microbiol Biotechnol 2016;43:605–616. [CrossRef]
[36] Chinnasamy S, Bhatnagar A, Hunt RW, Das KC. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technol 2010;101:3097–3105. [CrossRef]
[37] Gao F, Yang HL, Li C, Peng YY, Lu MM, Jin WH, et al. Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae Chlorella sp. Bioresour Technol 2019;282:118–124. [CrossRef]
[38] Deshmukh S, Kumar R, Bala K. Microalgae biodiesel: A review on oil extraction, fatty acid composition, properties and effect on engine performance and emissions. Fuel Process Technol 2019;191:232–247. [CrossRef]
[39] Mohd-Sahib AA, Lim JW, Lam MK, Uemura Y, Isa MH, Ho CD, et al. Lipid for biodiesel production from attached growth Chlorella vulgaris biomass cultivating in fluidized bed bioreactor packed with polyurethane foam material. Bioresour Technol 2017;239:127–136. [CrossRef]
[40] Komolafe O, Velasquez Orta SB, Monje-Ramirez IY, Noguez I, Harvey AP, Orta Ledesma MT. Biodiesel production from indigenous microalgae grown in wastewater. Bioresour Technol 2014;154:297–304. [CrossRef]
[41] Çirik S, Gökpınar Ş. Plankton bilgisi ve kültürü. 5th ed. İzmir: Ege Üniversitesi Yayınları; 2008. [Turkish]
[42] Abirama V, Mohamed RMSR, Al-Gheethi A, Abdul Malek M, Kassim AHM. Meat processing wastewater phycoremediation by Botryococcus sp.: A biokinetic study and a techno-economic analysis. Sep Sci Technol 2021;56:577–591. [CrossRef]