Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 53, No. 3 September 2024
pages (310-320)

sciendo

ISSN 1730-413X
eISSN 1897-3191

Lipid content and wastewater treatment potential of *Chlorella vulgaris* and *Scenedesmus obliquus* isolated from Uzuncayır Dam Lake

by

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DOI: **https://doi.org/10.26881/oahs-2024.3.09**
Category: **Original research paper**
Received: **April 13, 2022**
Accepted: **December 22, 2023**

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Abstract

Production of microalgae for biodiesel, one of the alternative renewable energy sources, is costly due to nutrient media. Industrial and domestic wastewater contains nitrogen, phosphorus and other nutrients that are the primary food source for algae. Releasing this environmentally harmful effluent into receiving bodies of water, such as the sea or a freshwater reservoir, without prior treatment causes serious problems. Combining microalgae cultivation with wastewater treatment is a promising strategy for improving wastewater and reducing the cost of nutrient media required for algae production. In this study, wastewater obtained from a wastewater treatment facility was diluted with clean water to 0.25%, 50%, and 75% concentrations, and *Chlorella vulgaris* and *Scenedesmus obliquus* were cultured in these nutrient media for 20 days. As a result, microalgae increased their biomass and lipid content, while consuming nitrite, nitrate, phosphate, and ammonium from the wastewater as nutrients.

Key words: *Chlorella vulgaris*, *Scenedesmus obliquus*, wastewater treatment, lipid, fatty acids

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online at www.oandhs.ug.edu.pl

1. Introduction

Today, the strategic importance of freshwater is universally accepted, and issues related to sustainable water management are constantly the subject of scientific meetings, social media, and even political agendas around the world. Water resources face many threats, both quantitative and qualitative. Industrialization and rapid economic development also increase these threats. In particular, inefficient use of water resources is a problem in almost all countries. Agricultural, domestic and industrial pollution strongly affects water resources. Such waters contain large amounts of nitrite, nitrate, ammonium and phosphate (Eltem 2001). Untreated water has caused problems for years in many receiving environments such as seas, lakes, and rivers (Martínez et al. 2000).

Various traditional methods for wastewater treatment have been used over the years, but they are both very costly and insufficiently effective. Many studies have been conducted on some new techniques called green technology wastewater treatment methods to solve such problems (Satpal 2016). Over the past 50 years, biological wastewater treatment systems, in which microalgae play a leading role, have come to the fore. Many researchers have agreed that microalgae-based wastewater treatment is as effective as conventional systems (Sen et al. 2013).

One of the most remarkable uses of algae, which has many applications such as health, cosmetics, and food, is their role in biological purification. Treating wastewater with algae is more advantageous than traditional methods, because conventional methods require aeration of wastewater for aerobic bacteria, and aeration involves additional costs. However, there is a symbiotic relationship between algae and bacteria in waters rich in organic matter. While algae support aerobic bacterial oxidation by producing free oxygen during photosynthesis, they also use carbon dioxide and nutrients released during oxidation to produce new biomass (Eltem 2008). For this reason, growing microalgae in wastewater, controlling pollution with algae, and generating energy from algal biomass is a method that has been continuously researched and developed. In addition, since algae consume large amounts of carbon dioxide to produce oxygen and glucose through photosynthesis, a microalgae-based treatment system is a solution for environmental problems such as global warming, ozone hole increase, and climate change (Ahmed Al Darmaki et al. 2012). Microalgae can remove heavy metals, organic and inorganic toxic substances, as well as nutrients from wastewater by using sunlight and CO_2.

While doing all this, they synthesize oxygen, which helps biodegrade aerobic bacteria in wastewater. In other words, while bacteria synthesize the CO_2 necessary for algal growth, the algae produce the oxygen necessary for the bacteria (Sekaran et al. 2013).

Scarponi et al. (2021) tested the growth of *S. obliquus* and *C. vulgaris* on 1/10 diluted digestate after pretreatment of wastewater by centrifugation and filtration. According to the obtained results, the biomass increase of *C. vulgaris* was better than that of *S. obliquus*. However, the ammonia removal by both species was more than 90% under mixotrophic conditions.

Fadeyi et al. (2016) cultured *C. vulgaris* and *S. obliquus* in BG11 medium containing various proportions of N and in wastewater containing N in the same amount. They observed that biomass yield increased as the amount of N in the nutrient medium increased.

In another study (Ahmad et al. 2013), lipids obtained by producing *C. vulgaris* in wastewater was converted to biodiesel. It was reported that the biodiesel obtained from *C. vulgaris* was of good quality for use in vehicles.

Algae-based integrated wastewater treatment systems are gaining importance every year due to the sustainable production of lipids. This system appears to be a method that reduces environmental concerns, given that while algae extract nutrients from wastewater, they also use CO_2 from factory chimneys or CO_2 from sea and land vehicles, which is another pollutant for the environment (Shanmugam et al. 2020).

Many successful studies have been carried out on microalgae-based wastewater treatment. For example, one involves delivering 70% of COD by using *Chlorella* in municipal wastewater (Min et al. 2011). Another study reported that more than 90 nutrients in wastewater, including nitrogen and phosphorus, are taken up by algae (Khaldi et al. 2017). In addition, some studies have reported that 50% of the energy used can be reduced by algae-based treatment compared to standard treatment technologies (Passos et al. 2013; Passos, Ferrer 2014).

Algae are one of the oldest photosynthetic life forms that are found in a wide variety of habitats such as rivers, oceans, ponds, rocks, soil, tree bark, and have recently been investigated for biodiesel production (Barsanti, Gualtieri 2022). This is due to the fact that algae also attract attention for their high lipid content, such as carbohydrates, proteins, and many other valuable metabolites. This high lipid content has made algae a potential source of biodiesel (Shanmugam et al. 2020).

However, large amounts of biomass are needed to produce biodiesel from algae, and the cost of chemicals required to produce this dense biomass is also high. Wastewater already contains the chemicals necessary for algae growth, and various studies have proven that algae grow better in wastewater than in seawater or freshwater (Yadav et al. 2021). Although it varies by species, about 50% of the total weight of microalgae is carbon, which they obtain from CO_2. Other inorganic nutrients required for effective algae growth are nitrogen and phosphorus (Pradhan et al. 2019). Inorganic compounds such as nitrogen and phosphorus, which are crucial for algae, are already abundant in wastewater. For this reason, wastewater is an inexpensive, environmentally friendly, rich, and efficient source for obtaining biodiesel from algae (Seyhaneyildiz Can et al. 2015).

Wastewater, the amount of which is increasing every day due to the growing population, is being used to produce algae to convert it into biodiesel instead of discharging it into receiving environments, which will be a very efficient approach for the environment as a whole. To date, many researchers have conducted multiple studies on this topic. However, previous studies have not provided information on whether there is any change in the fatty acid content of the algae produced in wastewater depending on the dilution of the wastewater. In this study, *Chlorella vulgaris* and *Scenedesmus obliquus* were cultured in effluent supplied from the Tunceli wastewater treatment plant. At the end of the study, the growth performance of algae produced in wastewater with different dilution ratios was examined, while their capacity to treat wastewater was also evaluated and changes in the lipid content of the algae were determined.

2. Materials and methods

2.1. Strain and culture conditions

The algae (*C. vulgaris* and *S. obliquus*) used in the study were isolated from the Uzunçayır Dam Lake in Tunceli, Turkey. The species were identified morphologically by light microscopy. The main diagnostic features such as cell shape, length, width, arrangement, cell details, shape and length of spines were used to identify the species (Komarek and Ruzicka 1969; Komarek and Fott 1983; Soeder and Hegewald 1989). To isolate algal samples, distillation was used first, followed by the agar plate method (Bold 1942). The wastewater used in the study was collected from a pond at the Tunceli municipality wastewater treatment plant, where the pre-treatment settling had been conducted. The wastewater was first autoclaved at 121°C and 1 atm vapor pressure for 15 min, and then filtered through a coarse filter. In this way, all microorganisms in the wastewater were eliminated. The nutrient media were then prepared by diluting the wastewater with 0%, 25%, 50%, and 75% distilled water. The basic culture medium used for the control group of *C. vulgaris* in this experiment was SWE (soil water extract), consisting of 0.25 g l^{-1} $NaNO_3$, 0.025 g l^{-1} $CaCl_2 \cdot 2H_2O$, 0.075 g l^{-1} $MgSO_4 \cdot 7H_2O$, 0.075 g l^{-1} K_2HPO_4, 0.175 g l^{-1} KH_2PO_4, 0.025 g l^{-1} NaCl, 40 ml of soil water, and 940 ml of distilled water (Bischoff and Bold 1963). The basic culture medium used for the control group of *S. obliquus* in this experiment was Bold 3N, consisting of 0.75 g l^{-1} $NaNO_3$, 0.025 g l^{-1} $CaCl_2 \cdot 2H_2O$, 0.075 g l^{-1} $MgSO_4 \cdot 7H_2O$, 0.075 g l^{-1} K_2HPO_4, 0.175 g l^{-1} KH_2PO_4, 0.025 g l^{-1} NaCl, 6 ml P-IV metal solution, consisting of 0.75 g l^{-1} $Na_2EDTA \cdot 2H_2O$, 0.097 g l^{-1} $FeCl_3 \cdot 6H_2O$, 0.041 g l^{-1} $MnCl_2 \cdot 4H_2O$, 0.005 g l^{-1} $ZnCl_2$, 0.002 g l^{-1} $CoCl_2 \cdot 6H_2O$, 0.004 g l^{-1} $Na_2MoO_4 \cdot 2H_2O$, 1 ml of vitamin B12 (1 ml l^{-1}) solution, and 1000 ml of distilled water (Starr and Zeikus 1993). The algae were inoculated into the prepared culture media at a ratio of 1:10 and cultured for 20 days in a 14/12 h light/dark period in the laboratory with a light intensity of 33.6 μmol photon m^{-2} s^{-1}. Aeration was continuous and the temperature was kept constant at 26°C.

2.2. Biomass monitoring

Daily optical densities of the algae cultured for 20 days were measured in a UV spectrophotometer (SP-300 nano) at a wavelength of 680 nm (Griffiths et al. 2011).

2.3. Total lipid determination

Filtered algae were mixed with hexane and treated in an ultrasonic bath (SK3200HP model Ultrasonic Power 135W Kudos, China, 4.5 L, internal dimensions: 30 × 15 × 10 cm) at 53 kHz and 30 W/L ultrasonic intensity for 25 min for lipid extraction. After ultrasonication, the hexane/algae mixture was filtered using filter paper, and the algal pulp was separated from the algal lipid and hexane mixture. Hexane in the supernatant separated from the algae pulp was evaporated using an evaporator (4000 eco/G1 model Heidolph, Germany) set at 60°C. The procedure was repeated three times until all the lipid was extracted, and the weight of total lipid was measured gravimetrically. The lipid extraction procedure was adapted and modified from the methods proposed by Lee et al.1998 and Hadrich et al. 2018. In this study, the procedure was optimized using hexane and an ultrasonic bath.

2.4. Esterification process

Lipid extracts of *C. vulgaris* and *S. obliquus* were converted to methyl ester forms before GC analysis. Approximately 40 mg of lipid samples were dissolved in 2 ml of n-heptane, and then 2 ml of KOH solution (2 M, prepared in methanol) was added. The mixture was vigorously shaken for 1 minute using a vortex. After the formation of clear phases, 1 ml of the upper layer was transferred to a GC vial. Then 1 μl of esterified lipid samples were injected directly into the GC system (Telci et al. 2014).

2.5. GC-FID Analysis

The fatty acid composition of oils extracted from *C. vulgaris* and *S. obliquus* was analyzed using an Agilent G26/30B instrument equipped with a TR-Fame column (30 m × 0.32 mm, 0.25 μm film thickness) along with a flame ionization detector (FID-6850 A). The temperatures of the detector and injection site were both set at 250°C. Helium was used as the carrier gas with a flow rate of 1 ml min^{-1} in split mode with a split ratio of 50:1. The initial oven temperature was set at 100°C and gradually increased to 180°C at a rate of 5°C min^{-1}, where it was maintained for 10 min. The temperature was then further increased to 250°C at a rate of 3°C min^{-1} and maintained for 20.7 min. Peaks were identified by comparing their retention times with those of authentic FAME mix standards (Supelco 37 Component FAME Mix, CRM47885, Lot No. LRAC9768, Sigma-Aldrich). Relative percentages of peak areas for different compounds were determined based on the FID data.

2.6. Wastewater analysis

The algae-free wastewater was analyzed to determine the treatment potential of algae. A Hach DR900 photometer and ammonium, nitrite, nitrate, and phosphate test kits (Hach) were used for this process. As a result of the analysis, the wastewater cleaning potential of the algae was measured and recorded.

2.7. Statistical analysis

Optical densities and the amount and content of extracted lipids were compared using one-way ANOVA and Duncan's multiple comparison method. All tests were performed in triplicate. The level of significant difference was $p < 0.05$.

3. Results and discussion

3.1. Algal biomass

The daily optical densities of 0.035 for initial *C. vulgaris and* 0.015 for *S. obliquus* are shown in Figure 1 and Figure 2, respectively.

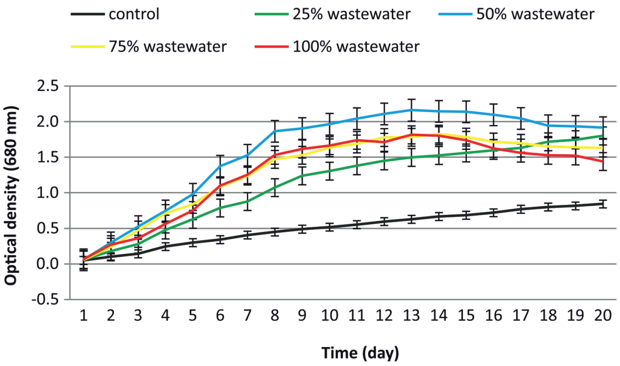

Figure 1

Daily optical density of *C. vulgaris*.

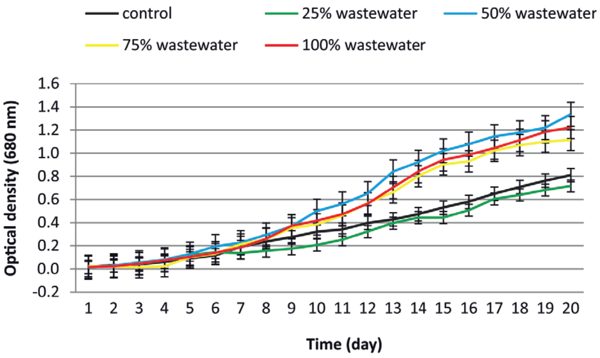

Figure 2

Daily optical density of *S. obliquus*.

As shown in Figure 1, the optical density of *C. vulgaris* measured at 680 nm reached 2.16 on day 18, and the biomass velocity began to decrease after day 18. Previous studies have shown that algae grow faster for a certain period, and then the growth slows down and stops (Martínez et al. 2000; Jiang et al. 2011). Nutritional parameters such as carbon, nitrogen, phosphorous sources, mineral salts in the environment, and physiological parameters such as light, ventilation, temperature, and pH can affect the growth rate and biochemical composition of algal cells. Disruption of even one of these factors during algae culture limits their growth (Chai et al. 2021; Fernandez-Marchante et al. 2018; Aburai et al. 2020; Schnurr, Allen 2015; Adalioglu, Caliskan 2020).

In the experiment conducted with *S. obliquus*, on the other hand, the stationary phase and death phase were not observed within 20 days of the logarithmic phases, and the stationary phase lasted from day 27. Despite using the same wastewater, the reason why *S. obliquus* transitioned to the stationary phase after *C. vulgaris* can be interpreted as the slower growth of *S. obliquus* algae. This result is also evident from the data shown in Figure 2.

It was found that the best biomass increase for both *C. vulgaris* and *S. obliquus* occurred in the group containing 50% wastewater. In one experiment, *S. obliquus* was cultured in three different nutrient media. Of these media, biomass does not grow well in the environments with the highest and lowest nitrogen content (Darki et al. 2017). In this study, while biomass growth increased slowly in a nutrient medium containing 100%, 75%, and 25% wastewater, the increase in biomass was higher in the nutrient medium containing 50% wastewater. In a study with *Botryococcus braunii*, it was determined that the best group in terms of biomass efficiency was the algae cultured in a 100% wastewater environment, and this difference may vary depending on species-specific characteristics and wastewater content (Seyhaneyildiz Can et al. 2015). In addition, when the results of the two algae are compared with the control group, wastewater is more effective in terms of biomass efficiency than the specially prepared culture medium.

3.2. Algal lipids

The total amounts of lipids obtained from the algae *C. vulgaris* and *S. obliquus* produced in wastewater diluted at different ratios are given in Figure 3 and Figure 4.

Microalgae continue to photosynthesize and increase their biomass as long as there is light, CO_2, and nutrients in the environment. However, if the amount of nutrients decreases, they tend to accumulate lipids (Courchesne et al. 2009). Previous studies have reported that biomass growth decreased and the amount of lipids increased inversely proportional to the reduction of nutrients in the environment (Tedesco, Duerr 1989; Griffiths et al. 2009; Chen et al. 2012; Seyhaneyildiz Can et al. 2021). In this study, gradually diluted wastewater was used for algae production. The results showed that the biomass growth of algae produced in wastewater with high dilution ratios was low, while the lipid ratios were high. As shown in Table 1, the accumulation of lipids in *C. vulgaris* was higher in the control group, followed by the group with 25% wastewater content. Although lipid production is higher for *C. vulgaris* in the control group, the lipid ratio of algae grown in a 25% wastewater environment is also sufficient. Since the present study addressed wastewater treatment and aimed to determine its potential for conversion to biodiesel by using the wastewater as a nutrient medium for algae, the results obtained were satisfactory. For *S. obliquus*, the optimum lipid accumulation was observed in the group containing 25% wastewater. When we compared the biomass and lipid content of both groups, we calculated using the equation (Seyhaneyildiz Can et al. 2021) below that the most efficient group in terms of both biomass and lipid accumulation was the environment containing 50% wastewater:

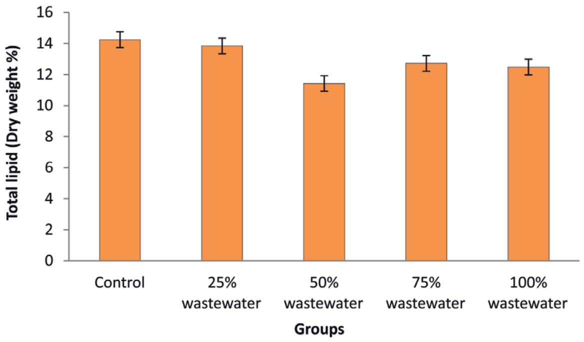

Figure 3

Total amount of lipids produced by the groups of *C. vulgaris*.

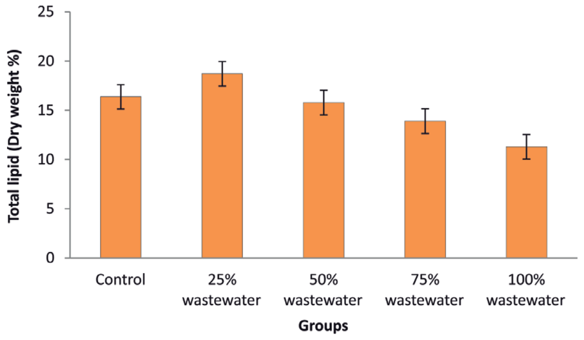

Figure 4

Total amount of lipids produced by the groups of *S. obliquus*.

Table 1

Statistical data on optical density and total lipid ratio of *C. vulgaris* and *S. obliquus* in wastewater diluted at different ratios.

	C. vulgaris		*S. obliquus*	
Groups	OD_{680nm}	Total lipids (%DW)	OD_{680nm}	Total lipids (%DW)
Control	0.4880^{c} ± 0.2318	14.2467^{a} ± 0.2517	0.3525^{b} ± 0.2544	16.3667^{b} ± 0.5262
25% WW	1.1394^{b} ± 0.5580	13.8467^{b} ± 0.3055	0.3051^{b} ± 0.2288	18.7100^{a} ± 0.0100
50% WW	**1.5870^{a} ± 0.7388**	11.4233^{e} ± 0.2082	**0.5917^{a} ± 0.4547**	15.7800^{c} ± 0.2646
75% WW	1.3214^{b} ± 0.5542	12.7167^{c} ± 0.2548	0.5071^{a} ± 0.4037	13.8900^{d} ± 0.2646
100% WW	1.2879^{b} ± 0.5848	12.4767^{d} ± 0.1528	0.5350^{a} ± 0.4274	11.2900^{e} ± 0.1000

The letters ^{a,b,c,d} indicate the statistical significance of the differences in the same column ($p < 0.05$).

$$Lipid\ amount = \frac{\mathrm{OD}_{680} \times lipid(\%DW)}{100}$$

3.3. Fatty acids

Previous studies have shown that the amount and quality of fatty acids are affected by many factors such as salinity, temperature, pH, and nutrient levels (Juneja et al. 2013; Zhila et al. 2011; Nomura et al. 2013). Due to unsuitable conditions such as salinity, temperature, pH, and nutrient, most algae tend to produce and accumulate neutral lipids to control intracellular stress (Hu et al. 2008; El-Kassas 2013). To ensure efficient and fit-for-purpose production, it is necessary to know under what conditions the production of specific fatty acids is stimulated and to adjust environmental conditions accordingly. In particular, it is important to trigger the production of fatty acids required for biodiesel production. Esters of six fatty acids are commonly used in the production of biodiesel. These are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) and small amounts of palmitoleic acid (C16:1) (Chuck et al. 2009). The main purpose of this study was to determine whether fatty acids suitable for biodiesel would increase consistently with changes in fatty acids of algae depending on the dilution ratio.

In this study, the content of palmitic acid (C16:0) and oleic acid (C18:1) in *C. vulgaris* grown in four different media (including the control group) increased, while linoleic acid (C18:2) and linolenic acid (18:3) decreased (Table 2). No significant changes were observed in other fatty acids (Table 2). Table 3 listing the fatty acids of *S. obliquus* grown under the same conditions shows that oleic acid increased in the environment containing 25% wastewater, where the nutrient medium decreased (Table 3). Breuer et al. (2013) reported that oleic acid predominated in nitrogen starvation conditions. Our work shows concurrence with the study by Breuer et al. (2013). Although the fatty acid content and composition of the algae vary depending on the nutrient environment, the results show that the two species contain sufficient amounts of the fatty acids required for biodiesel (Tables 2 and 3). The results indicate that the content of fatty acids required for biodiesel, especially oleic acid, is higher in both groups in the diluted wastewater. According to these results, we can conclude that algae produced in diluted wastewater will be more suitable for biodiesel.

3.4. Wastewater treatment

The rapid population growth and industrialization have led to excessive water pollution. To protect water resources and reduce eutrophication, it is necessary to reduce the amount of effluent containing chemical or biological pollutants that cause water contamination. There are several studies on the potential of microalgae culture in reducing these pollutants (Choi 2016; Zhou et al. 2018; Villar-Navarro et al. 2018; Ullah et al. 2023). Microalgae can treat both domestic and industrial wastewater. They have great potential to remove nitrogen, phosphorus, nitrate, silica, iron, magnesium, and many other harmful chemicals found in wastewater and to increase their biomass during the removal process. Municipal wastewater has been used to culture microalgae in many studies. Studies have shown that while microalgae increase their biomass, they also clean the wastewater of many pollutants, such as nitrate phosphate (Ye et al. 2020; Qu et al. 2020). Scarponi et al. (2021) reported that in their study of *C. vulgaris* and *S. obliquus*, algae removed more than 96% of ammonia from wastewater under mixotrophic

Table 2

Fatty acid profile of *C. vulgaris* in wastewater diluted at different ratios.

Fatty acid (common name)	IUPAC	Control	100% wastewater	75% wastewater	50% wastewater	25% wastewater
C14:0 (myristic)	tetradecanoic acid	9.63	9.65	9.62	**9.78**	9.71
C16:0 (palmitic)	hexadecanoic acid	24.93	21.42	24.78	26.61	**27.09**
C16:1 (palmitoleic)	(9Z)-hexadec-9-enoic acid	3.75	3.65	3.73	3.69	3.46
C16:2 (palmitolinoleic)	(9Z,12Z)-hexadeca-9,12-dienoic acid	10.25	**11.26**	10.28	10.27	10.23
C16:3 (hexadecatrienoic)	(4E,7E,10E)-hexadeca-4,7,10-trienoic acid	9.67	**9.85**	9.42	9.18	9.02
C18:0 (stearic)	octadecanoic acid	**1.25**	1.12	1.08	1.03	1.05
C18:1 (oleic acid)	(9Z)-octadec-9-enoic acid	11.75	13.96	13.96	14.08	**14.29**
C18:2 (linoleic)	(9Z,12Z)-octadeca-9,12-dienoic acid	**12.4**	12.36	11.03	10.96	9.98
C18:3 (linolenic)	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	15.72	**15.76**	15.22	14.35	14.21
Total		**99.35**	**99.03**	**99.12**	**99.95**	**99.04**

Table 3

Fatty acid profile of *S. obliquus* in wastewater diluted at different ratios.

Fatty acid (common name)	**IUPAC**	**Control**	**100% wastewater**	**75% wastewater**	**50% wastewater**	**25% wastewater**
C14:0 myristic)	tetradecanoic acid	0.96	0.95	0.95	**0.97**	**0.97**
C16:0 (palmitic)	hexadecanoic acid	**22.73**	18.95	17.92	16.17	15.37
C16:1 (palmitoleic)	(9Z)-hexadec-9-enoic acid	3.51	**3.56**	3.21	3.52	2.71
C17:0 (margaric)	heptadecanoic acid	**0.44**	0.41	**0.44**	0.41	0.28
C17:1 (margaroleic)	(9Z)-heptadecenoic acid	**0.98**	0.84	0.71	0.96	0.12
C18:0 (stearic)	octadecanoic acid	3.72	3.12	3.97	3.75	2.99
C18:1 (oleic)	(9Z)-octadec-9-enoic acid	46	50.21	49.97	51.10	**52.29**
C18:2 (linoleic)	(9Z,12Z)-octadeca-9,12-dienoic acid	12.4	12.53	13.12	13.21	**15.29**
C18:3 (linolenic)	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	**6.9**	5.98	6.29	5.41	5.23
C20:0 (arachidic)	Icosanoic acid	0.59	0.55	0.61	**2.78**	2.71
C20:1 (gadoleic)	(9Z)-Icos-9-enoic acid	0.38	0.38	0.38	0.35	0.37
C22:0 (behenic)	docosanoic acid	**1.38**	1.03	0.95	0.33	0.32
Total		**99.55**	**98.51**	**98.52**	**98.96**	**98.65**

conditions. Franchino et al. (2013) reported that *C. vulgaris* and *S. obliquus* provided ammonia removal rates of 99.9% and 83.7%, respectively, over 20 days in wastewater diluted at a ratio of 1/10. Industrial or sewage effluent is rich in nutrients, and the accumulation of pollutants such as nitrogen and phosphorus significantly affects both freshwater and marine ecosystems. These contaminants must be removed before water can be safely discharged into the environment and be suitable for human consumption. Microalgae have great potential to perform their cellular functions and increase their biomass with these nutrients in wastewater. Water pollution is not only an environmental problem. In addition, it is estimated that it will cause a shortage of drinking water in a short period of time. Therefore, wastewater treatment is essential to recover water quality and reduce water scarcity (Karimi-Maleh et al. 2020). A Hach DR900 photometer, as well as ammonium, nitrite, nitrate, and phosphate test kits (Hach) were used to analyze wastewater treatment potentials of the microalgae *C. vulgaris* and *S. obliquus* cultured in wastewater obtained from the Tunceli municipality wastewater treatment plant. At the beginning of the performed analysis, the ratios of nitrite, nitrate, phosphate, and ammonia in the nutrient medium containing 100% wastewater were 0.38, 15.2, 1.43, 10.4 mg l^{-1}, respectively. The removal rate of pollutants from the wastewater by the algae at the end of the 20-day algae production period was high. Figures 5 and 6 show the wastewater removal rates obtained in this study, that is, the percentage of nitrite, nitrate, phosphate, and ammonia removed by the algae. *C. vulgaris* cultured in a nutrient medium with 25% wastewater removed 96.84%, 89%, 96.97%, and 99.23% of nitrite, nitrate, ammonium, and phosphate, respectively. *S. obliquus* cultured in a nutrient medium with 25% wastewater removed 74%, 81.57%, 95.10%, and 96.62% of nitrite, nitrate, ammonium, and phosphate, respectively.

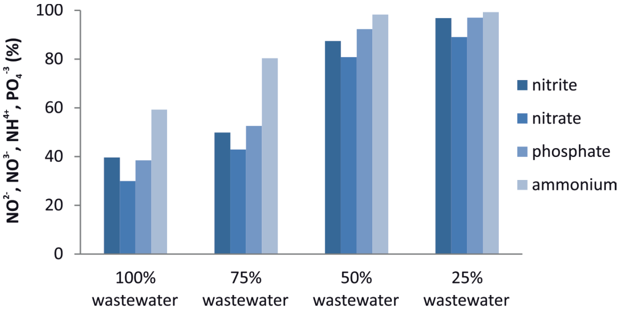

Figure 5

Percentage uptake of nitrite, nitrate, phosphate and ammonium from wastewater by *C. vulgaris*.

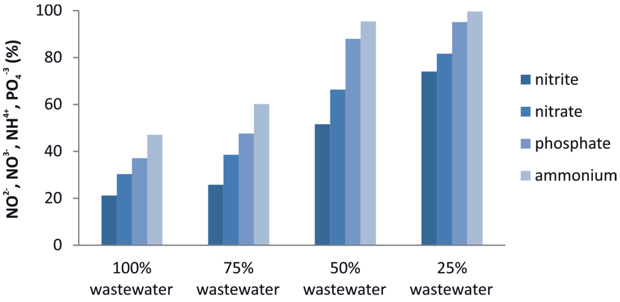

Figure 6

Percentage uptake of nitrite, nitrate, phosphate and ammonium from wastewater by *S. obliquus*.

The history of intensive culture of microalgae in wastewater treatment goes back 75 years. Palmer (1969) compiled a list of microalgae tolerant of organic pollutants, reported by 165 researchers (Palmer 1969). The list included 60 genera and 80 species. The eight most tolerant genera were *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula*, and *Stigeoclonium* (Abdel-Raouf et al. 2012).

In this study, we also used the microalgae *C. vulgaris* and *S. obliquus*, which we were able to isolate from the Uzunçayır Dam Lake and which we considered tolerant based on previous studies. At the end of the study, we observed that *C. vulgaris* and *S. obliquus* increased their biomass during wastewater treatment.

4. Conclusion

The potential of algae in the production of biofuels from renewable energy sources has recently come to the fore. However, the main problem with this approach is that the chemicals required during algae production are expensive and make production unprofitable. Algae have many uses today, just as they did in ancient times. They are widely used, for example, in food, cosmetics, and medicine. However, it is inappropriate to use algae produced in wastewater for these purposes. For this reason, the evaluation of algae used in wastewater treatment for biodiesel production is very important from an economic and ecological point of view. In this study, we used *C. vulgaris* and *S. obliquus* isolated from the Uzunçayır Dam Lake as experimental species and wastewater obtained from a wastewater treatment plant, which is discharged into the Uzunçayır Dam Lake after treatment as a nutrient medium. In the study, both *C. vulgaris* and *S. obliquus* proliferated successfully in 50% wastewater medium, and their biomass (OD680) was 1.5870a ± 0.7388 and 0.5917a ± 0.4547, respectively. The crucial question in this study was to discover whether the lipid content and composition changed in wastewater diluted at different ratios. In our study, we observed that the lipid content increased as the amount of wastewater in the nutrient medium decreased. Therefore, the lipid content was higher in the group with 25% wastewater. However, considering the biomass yield and lipid content, we observed that the most efficient group for biodiesel production was the nutrient medium containing 50% wastewater. Another objective of the study was to determine whether the composition of fatty acids in algae produced in wastewater diluted at different ratios changed and whether there were fatty acids suitable for biodiesel. At the end of the study, different ratios of wastewater did not alter the profile of fatty acids produced. Although some fatty acids were present in different amounts in different groups, no significant difference was determined. Only the behenic acid content in *S. obliquus* was higher in the control, 100%, and 75% wastewater groups compared to the other groups. However, behenic acid was omitted because it is not one of the essential fatty acids for biodiesel. Nevertheless, both algae produced fatty acids suitable for biodiesel in all experimental groups. In brief, the algae yielded adequate lipid content and composition for biodiesel by treating the wastewater.

Acknowledgements

This study is part of the project "Research on lipid content and wastewater treatment potential of algae in Uzuncayır Dam Lake." We would like to thank Munzur University.

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