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Cultivation of blue green algae (Arthrospira platensis Gomont, 1892) in wastewater for biodiesel production

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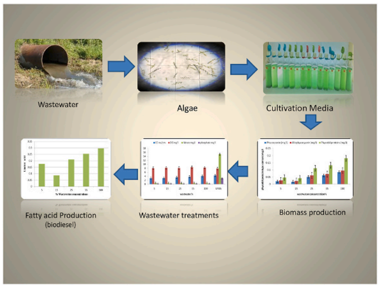
HIGHLIGHTS

- *Arthrospira platensis* has ability to produce five fatty acids by cultivation in wastewater as alternative media.
- The value of nitrate, phosphate were reduced and the dissolved oxygen was increased in 35% concentration.
- Increase of biomass, total protein, chlorophyll *a* and decreased for carbohydrate content with increasing concentration of wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

The production of biodiesel has become an important issue in the effort to reduce gas emissions due to the climate change crisis; therefore, algae have widely used to produce biodiesel for energy sustainability. The present study represented an effort to assess the ability of the alga *Arthrospira platensis* to produce fatty acids involved in biofuel (diesel) by cultivation in Zarrouk media enriched with different municipal wastewater concentrations. Wastewater was used in different concentrations (5, 15, 25, 35 and 100% [control]). Five fatty acids from the alga were determined and included in the present study. These were inoleic acid, palmitic acid, oleic acid, gamma-linolenic acid, and docosahexaenoic acid. Impact of different cultivation conditions were studied in terms of observed changes in growth rate, doubling time, total carbohydrate, total protein, chlorophyll *a*, carotenoids, phycocyanin, allophycocyanin, and phycobiliproteins. Results showed an increase in the values of growth rate, total protein content, chlorophyll *a*, and levels of carotenoids at all treatments except for carbohydrate content, which decreased with an increasing concentration of wastewater. The high value of doubling time (11.605 days) was recorded at treatment 5%. Fatty acids yields were increased at treatment 5% and 15%. The highest concentrations of fatty acids were 3.108 mg/g for oleic acid, gamma-linolenic acid (28.401 mg/g), docosahexaenoic acid (41.707 mg/g), palmitic acid (1.305 mg/g), and linoleic acid (0.296 mg/g). Moreover, the range of phycocyanin (0.017–0.084 mg/l), allophycocyanin (0.023–0.095 mg/l), and phycobiliproteins

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(0.041–0.180 mg/l) were obtained in treatment with 15–100%, respectively. Cultivation with municipal wastewater reduced the values of nitrate, phosphate, and electrical conductivity as well as increased dissolved oxygen. Maximum electrical conductivity was recorded in untreated wastewater with algae, while the highest level of dissolved oxygen was noted at 35% concentration. The use of the household wastewater is more environmentally friendly as an alternative of the traditional cultivation techniques used for long-term for biofuel production.

Author contributions statement

Conceptualization, direction, validation, writing, peer review, and editing were contributions of all authorships. All authors have read and approved the writing's final draft. Cultivation of blue green algae (Arthrospira platensis Gomont, 1892) in wastewater for biodiesel production.

1. Introduction

Algae have major roles in different ecosystems and differ in their habitat, size, life forms, physiology, reproduction, and constitution of chemical compounds (Richmond, 2004). Heavy metallic pollutants in the environment have severe side-effects and can lead to chronic poisoning of humans (Zhang and Karimi-Maleh, 2023a,b). Surface contamination and underground water, because of organic compounds, have led to notable issues and the need to develop effective methods to treat polluted water (Karimi-Maleh et al., 2023). Microalgae are significant as a source of several chemicals used in industrial activities and a source of carbon compounds according to Das et al. (2011). Khan et al. (2018) explained the role of algae in renewable energy production via biodiesel production and mentioned the requirement to improve their ability for increased growth by genetic engineering and stimulating high production of oil (Katiyar et al., 2017; Hassan et al., 2015).Researchers have reported that it is crucial to use substitutional media to lower the cost of producing algal biomass (Pittman et al., 2011; Salman et al., 2023). Metal ions are one of the main and dangerous materials in water samples and one of the main causes of environmental problems (Zhang and Karimi-Maleh, 2023a,b), therefore, wastewater is important to use from both conomic and ecological perspectives to achieve effective treatment of wastewater and biodiesel production (Hwang et al., 2016) explained that researchers have investigated the ability of microalgae to produce biodiesel by using wastewater as culture media. The alga *Chlorella* sp. was isolated from a maturation pond in Kwa-Zulu Natal (South Africa) as part study to test their ability to produce biodiesel in heterotrophic growth and compared with the growth in photoautotrophic condition in the bioreactor. The heterotrophic condition produced high Most lipid content. (Viswanath et al., 2012).

Ananadhi and Stanley (2012) revealed that the absent of sulfur or aromatics compounds in biofuel encouraged the researchers to produce biodiesel, which is considered as a renewable energy source and also helping to reduce the impact of the emission of undesirable gases and unburned materials. Most importantly, biodiesel is biodegradable, nontoxic, renewable, and environmentally benign (Hassan et al., 2013; El-Sheekh et al., 2022).The sluggish nature of the cathodic oxygen reduction reaction (ORR) and the expensive price of the precious metal-based nanocatalysts are the biggest obstacles to the practical applications of cutting-edge technologies, including metal-air batteries and fuel cells (Karimi-Maleh et al., 2022).

Many studied have revealed that microalgae can be considered as potential biodiesel feedstock, including such exampleas as *Chlorella vulgaris* (Hassan et al., 2013) and *Dictyochloropsis splendida* (Shanab and Ali, 2022).

Nascimento et al. (2013) revealed that algal species differ in their content of oil and the latter is about 30% among different algal groups. *Nannochloris* sp. has a high oil content (56%) and the same is true for.

Chlorella sp. (53%), *Neochloris oleoabundans* (65%) and *Schizochytrium* sp. (80%). It was noticed that algae with a higher oil content have a slower growth rate in contrast with algae with a low oil content (Hassan et al., 2013; Shanab and Ali, 2022).

Algae are involved in different industrial activates besides in bioenergy purpose throughout increasing their biomass (Zaki et al., 2021; Gonzalez-Bautista and Laroche, 2021). Several researchers have reported that the diesel production in a large scale is not yet improved, and its economic values was highly coast besides the environmental importance, also they classified algal cultivation as an open/closed system (Singh et al., 2014; Wobbe and Remacle, 2015; Hassan et al., 2014).

The growing of algal species in wastewater encouraged researchers to cultivate algae for biodiesel experiments due to their nutrient content and to reduce nutrients in the treatment plants of different industrial ofor domestic activities as well as preventing eutrophication and mitigating CO_2 emissions (Pittman et al., 2011; Gouveia, 2011). Algae are used to improvement of wastewater quality in many studies (Hwang et al., 2016). The ability of *Phormidium*, *Spirulina*, *Chlorella*, and *Scendesmus* was tested for their improvement of wastewater quality and effects on the requirements of other organisms and as biofertilizers (Praveenkumar et al., 2014).

Arthrospira platensis is an alga belonging to the family Cyanophyceae. It is very important in different industrial purposes such as food and biofuel production (Hassan et al., 2022). This ability is related to its carbohydrate (polysaccharide) protein profile, fatty acid content, and function as a glycogen repository (Gonzalez-Bautista and Laroche, 2021; Serrà et al., 2020). *Arthrospira platensis*is is crucial for the generation of biodiesel and the nutrient requirements of other aquatic organisms, according to Zaki et al. (2021).

The manufacture of biodiesel has become an important issue in an effort to reduce gas emission due to the climate change crisis; therefore, algae have been widely used to produce the biodiesel for sustainability. The current research attemped to assess the ability of the alga *Arthrospira platensis* to produce biodiesel and to optimize the growth conditions by mixing it with municipal wastewater. Also, it how wastewater affected the alga's biochemical makeup as a source of nutrition was assessed.

2. Materials and methods

2.1. Culturing of algae

The alga *A. platensis* strain was purchased from Algal research and Supply (US San Diego, USA). The culture was activated according to the instructions provided. The alga was inoculated in a Zarrouk medium (Table 1, Supplementary data) according to Walter et al. (2011). A 10% (inoculation/media volume) was used for inoculation in Erlenmeyer flasks. The incubation conditions were 32 ± 1 °C, pH 9, $135 \mu E m^2 s^{-1}$(cool white fluorescent lamps) and photoperiod cycle light/dark (12:12 h), with daily shaking by hand.

2.2. Experimental design

Bio-parameters determined included growth rate, doubling time, total carbohydrate, total protein, chlorophyll (chlorophyll *a*, *b*, and total chlorophyll), and other pigments (carotenoids, phycocyanin, allophycocyanin, and phycobiliproteins) in addition to their effects on biodiesel production, which include palmitic acid, oleic acid, linoleic acid, gamma-linolenic acid and docosahexaenoic acid. A municipal wastewater was used in different concentrations for cultivation.

Wastewater samples were taken from a wastewater treatment plant in Al-Hilla City. The sample was centrifuged (4000 rpm for 20 min), then filtered by Whatman filters (No. 4, 20–25 μm) and autoclaved (for 60 min at 1 bar and 121 °C). Four different concentrations of wastewater were prepared as 5%, 15%, 25% and 35% with distilled water (DW) in 1.0 L volumetric flasks. Each 1.0 L flask was filled with effluent wastewater (50, 150, 250 and 350 ml) before being filled with DW to the proper level. Wastewater (100%) was used as control. The pH was adjusted to around 9 before autoclaving the medium at 121 °C. Afterwards, these wastewater concentrations were used for the cultivation of the algal strain.

Wastewater before and after the treatment was analyzed and the some physico-chemical parameters such as electrical conductivity (EC), dissolved oxygen (DO), phosphate and nitrate content were determined.

2.3. *Characterization of municipal wastewater*

EC and DO Determination of the wastewater were carried out using a portable multipara meter analyzer.

Estimation of Phosphate and Nitrate.

Phosphate determined by (PO_4Profi Test kit, Holland), also Nitrate was estimated by using (JBL nitrate test set NO_3kit, Germany).

2.3.1. *Biological parameters*

The density of algae measured through the spectrophotometry (UV–Vis spectrophotometer) and the optical density estimated at 560 nm and calculating the cell density (cells/ml) according to Saranraj et al. (2013). Alga growth rate (K) and doubling time (G) were calculated using the Fogg and Thake (1987) equations:

$$K = 3.322 * (\log OD_t - \log OD_0) / t$$

$$G = 0.301 / K$$

OD_0: optical density before the experiment started.

OD_t: optical density after (t) day.

According to Dubois et al. (1956), total carbohydrate was determined. The material was centrifuged (cooling centrifuge at the rate of 5000 r/min for 30 min, 4C°), then 1.0 of 5% phenol and 5 ml of 96% sulphuric acid were added to each tube and the latter shaken thoroughly. Next, after shaking violently for 10 min, the material was placed in a water bath at 25–30 °C for 20 min. Spectrophotometry at 490 nm was used to determine carbohydrate concentration. The standard graph created by Nordin et al. (2014) was used determine the total amount of carbohydrates contained.

Total protein was measured according to Bradford (1976). After extraction of protein from algae by using a cooling centrifuge (5000 r/min for 30 min, 4C°), and the collected supernatant was used for subsequent processes of protein determination. The collected extract sample treated with 5 ml of Bradford dye reagent. The absorbance was measured at 595 nm after mixing the sample and use a standard curve of bovine serum albumin prepared according to Tijjani-Oshungboye (2011).

The estimation of chlorophyll was measured spectrophotometer at 663 nm and 645 nm according to Arnon (1948). The following equations were used for estimation of Chlorophyll:

$$\text{Chlorophyll } a \text{ (mg / l)} = (12.7 \times A663) - (2.698 \times A645)$$

Other pigments were measured spectrophotometry according to Jensen (1978) for carotenoids at 450 nm and Bennett and Bogorad (1973) for phycobiliproteins at 652, 615, and 562 nm. The following equations were used for calculating the pigments (Devanathan and Ramanthan, 2012):

$$C = A450 * V * f * 10 / 2500 \quad \text{for Carotenoids}$$

C = Total amount of Carotenoids (mg/ml).

V = Volume of extract (ml), f = Dilution factor

$$Phococyanin(PC) = 0D615 - 0.474(0D652)/5.34$$

$$Allophycocyanin(APC) = 0D652 - 0.208(0D615) / 5.09 \ (5)$$

$$\text{Phycobiliproteins} = \text{PC} + \text{APC}$$

2.4. *Production of biodiesel*

2.4.1. *Transesterification*

Centrifugation was used to separate alga's cells from the media for 10 min at 7500 rpm. For further study, biomass was dried at 55 °C for 2 h, ground in a mortar, and kept at −4 °C (Uslu et al., 2011). A 0.1 g taken form algal sample for dry freezing processes followed method of Lewis et al. (2000).

Dry freezing biomass (0.1 g) was placed in 10 ml Teflon-capped Pyrex tubes and 8 ml of fresh reaction solution (hydrochloric acid/chloroform/methanol, 4:4:40v/v/v) was added (Lewis et al., 2000) The suspension of biomass placed at 90 °C for 1 h for trans esterification immediately after mixing and then kept at room temperature before adding 3 ml of a hexane/chloroform, 4:1v/v. The fatty acid methyl esters underwent two extraction steps. Gas chromatography was used to examine the combined supernatants.

2.4.2. *Composition of fatty acids*

A gas chromatography (GC) from Shimadzu Instruments, Japan, was used to evaluate the washed fatty acid methyl esters. This instrument has a flame ionization detector and an SP-2480 column and helium (carrier gas). The detector was 330 °C whereas the injector port was 280 °C. By infusing 1 L of the sample, the column temperature was raised from 150 to 300 °C at a rate 10 °C per minute. By comparing the retention durations (RT) of the sample peak with those of the reference fatty acids (Uma et al., 2016), the fatty acid methyl esters of the test alga were determined.

2.5. *Analysis of statistics*

The statistical package for social sciences (SPSS) program, version 26

Table (1)
Effect of different wastewater concentrations onpalmitic acid, oleic acid, linoleic acid, gamma-linolenic acid and docosahexaenoic acid in *A. platensis* *Significant differences ($p < 0.05$) betweencontrol and all treatments except 35% in palmitic acid.

Wastewater Concentrations %	Fatty acids concentration					Total fatty acids	Fatty acids induction (%)
	Palmitic acid	Oleic acid	Linoleic acid	γ-linolenic acid	Docosa-hexaen-oic acid		
100(control)	0.318	0.234	0.175	1.884	3.992	6.603	
35	0.116	0.522	0.086	4.569	10.667	15.96	58.627
25	0.549	1.362	0.210	11.335	26.871	40.327	83.626
15	1.059	1.719	0.254	19.781	37.502	60.315	89.052
5	1.305	3.108	0.296	28.401	41.707	74.817	91.174
LSD value	0.202*	0.279*	0.034*	2.615*	1.982*		

Data was used to analyze the data using a randomized block design ANOVA and least significant difference (LSD) was utilized to examine the significant difference between means at $p < 0.05$. SD-bar added to figures by three replicates.

3. Results

3.1. Growth rate and doubling time

The highest growth rate (0.079) was measured in the culture with the undiluted wastewater (100% concentration) and the lowest growth rate 0.025, which equated to 68.354% growth inhibition was measured in the wastewater with a concentration of 5%. The growth rate value in 15%, 25% and 35% were 0.036, 0.042 and 0.052, respectively. (Table 2 in Supplementary data), Fig. 1).

The longest doubling time (G) was 11.605 days at 5% treatment, while the shortest was 3.797 days in the 100% treatment. Doubling time in the 15, 25, and 35% treatments were 8.257, 7.053 and 5.782 days, respectively (Table 2 Fig. 2).

3.2. Carbohydrate content

As shown in (Table 3(Supplementary data), Fig. 3), increased wastewater concentration caused a decrease in total carbohydrate content. The concentration at which the lowest carbohydrate content (19.313 mg/l) and the maximum carbohydrate content (36.568 mg/l) were reported at 5% (see Fig. 4).

It is clear from (Table 3 in Supplementary data), Fig. 4) that increasing wastewater concentration caused a significant increase in total protein content. In 100% (control) the maximum protein content of 32.375 mg/l was observed, and in 5%, the lowest protein content of 10.276 mg/l.

3.3. Photosynthesis pigments content

3.3.1. Chlorophyll a content

Spirulina platensis exhibited 0.751, 0.919, 1.052 and 1.175 mg/l decreases in chlorophyll *a* content in 5, 15, 25 and 35% wastewater concentrations, respectively, compared with 1.527 mg/l in 100% (Table 5 (Supplementary data) , Fig. 5).

3.3.2. Carotenoids content

Total carotenoid content decreased to 0.0046, 0.0041, 0.0048 and 0.0051 in 5, 15, 25 and 35% concentrations, respectively, compared to 100% that contains0.0056 mg/l (Table 6(Supplementary data), Fig. 6).

3.3.3. Phycocyanin, allophycocyanin and phycobiliproteins

The content of phycocyanin, allophycocyanin, and phycobiliproteins in *S. platensis* decreased significantly when wastewater concentration decreased (Table 7 (Supplementary data), Fig. 7). Phycocyanin content decreased to 0.020, 0.017, 0.051 and 0.061 mg/l, while allophycocyanin content decreased to 0.026, 0.023, 0.061 and 0.071 mg/l. Moreover, phycobiliproteins decreased to 0.046, 0.041, 0.112 and 0.132 mg/l in 5, 15, 25 and 35% of wastewater, respectively, as compared with 100% wastewater concentration that contained 0.084 mg/l (phycocyanin), 0.095 mg/l (allophycocyanin) and 0.180 mg/l (phycobiliproteins).

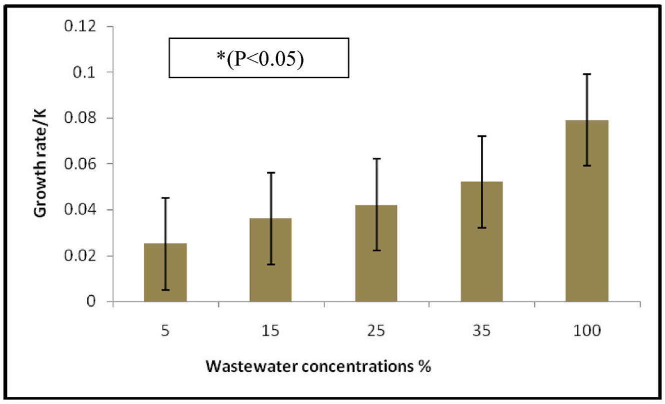

Fig. 1. Growth rate of *A. platensis* in different wastewater concentrations.

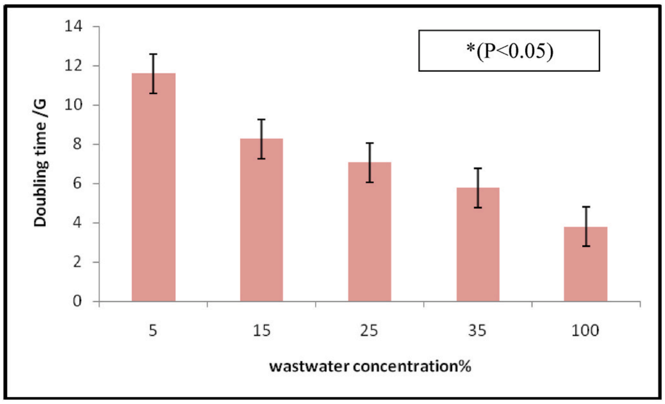

Fig. 2. Doubling time of *A. Platensis* at different wastewater concentrations.

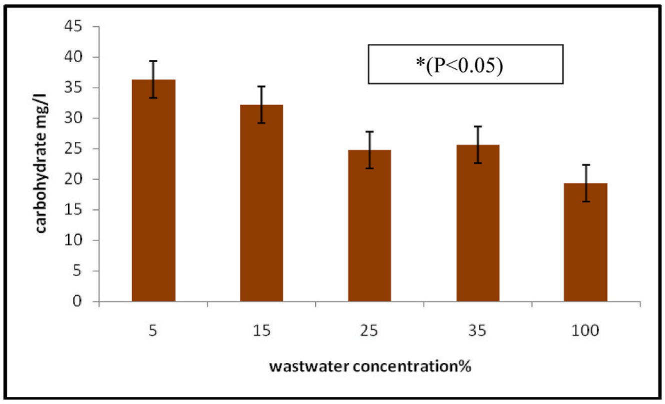

Fig. 3. Carbohydrate content of *A. platensis* at different wastewater concentrations.

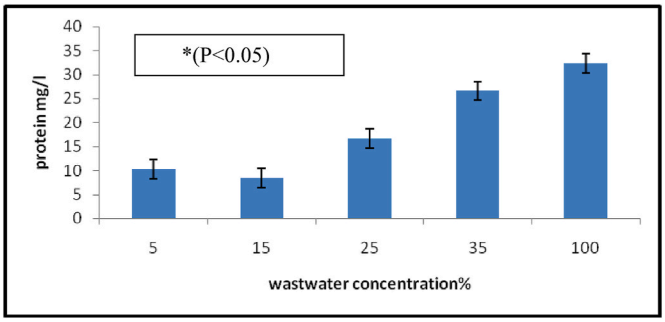

Fig. 4. Protein content of *A. platensis* at different wastewater concentrations.

3.4. Effect of municipal wastewater concentrations on biodiesel production of A. platensis

Except for palmitic acid and oleic acid, which increased by 35% and 5%, respectively, when wastewater concentration fell, all five fatty

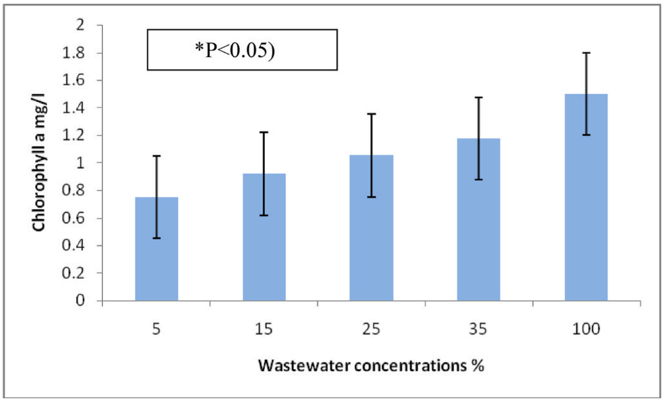

Fig. 5. Chlorophyll *a* content of *A. platensis* at different wastewater concentrations.

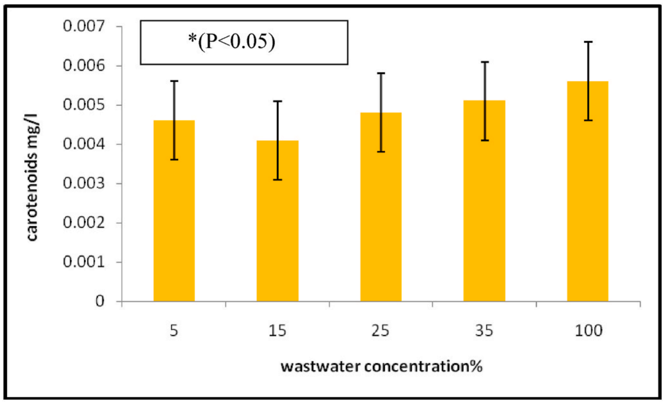

Fig. 6. Carotenoids content of *A. platensis* at different wastewater concentrations.

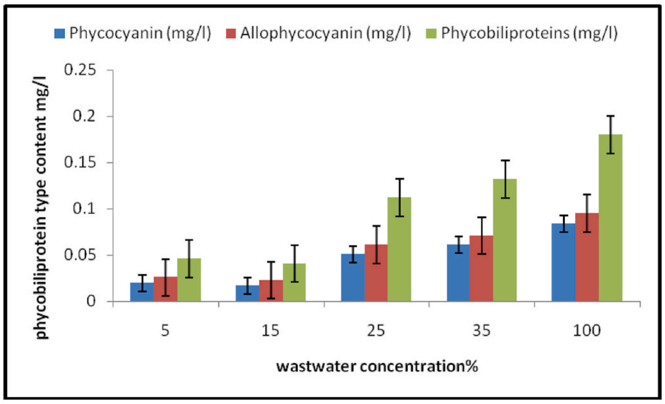

Fig. 7. Phycocyanin, allophycocyanin, and phycobiliproteins content of *A. platensis* at different wastewater concentrations.

acids, as well as the high induction rate of the total fatty acids were noted.

Linoleic acid, gamma-linolenic acid, and docosahexaenoic acid) increased when wastewater concentration decreased except for palmitic acid and linoleic acid in 35%, as well as the high induction rate of the total fatty acids were recorded in 5% treatment (Table 1). As compared to 0.318 mg/g (palmitic acid),0.234 mg/g (oleic acid), 0.175 mg/g (linoleic acid), 1.884 mg/g (gamma-linolenic acid) and 3.992 mg/g (docosahexaenoic acid) in 100% (control), the maximum concentration of palmitic acid, oleic acid, linoleic acid, gamma-linolenic acid, and docosahexaenoic acid were 1.305, 3.108, 0.296, 28.401 and 41.707 mg/g, respectively in 5%

3.5. *Characterization of municipal wastewater*

The characteristics of wastewater are shown in table (8) (Supplementary data).

Fig. (8). Electrical conductivity (4.37 ms·cm^{-1}) decreased, while dissolved oxygen (7.86 mg/l) increased with increasing wastewater concentration. Furthermore, the total nitrate and phosphate reduced from an initial value of about 15 mg/l and 3 mg/l in untreated municipal wastewater (UMW) to 0.5 mg/l and 0.03 mg/l which corresponds to 96.66% and 99% removal efficiency, respectively.

4. Discussion

4.1. *Municipal wastewater effects*

4.1.2. *Growth rate and doubling time*

For isolated algae, the varying wastewater dilutions results in distinct biomass growth. When wastewater concentrations increased, the growth rate value climbed and the doubling time decreased. This might be because the biochemical makeup and nutrient concentration of the medium, which also affected the growth of microalgae, were different in the medium's composition and concentration (Mitra et al., 2012). The primary nutrients for microalgal growth are nitrogen and phosphorus, which can be obtained from variety of wastewater produced by diverse sources (Becker, 2004). Additional research demonstrated the impact of nitrogen on various metabolic processes in algae, including a reduction in the photochemical efficiency and activity of the reaction centers of the alga *P. cruentum* (Zhao et al., 2017) and increasing *A. platensis* biomass (Can et al., 2017).

Similar outcomes from additional investigations into *Anabaena doliolum* have been reported when cultivated in domestic wastewater. Ji et al. (2014) investigated the typical specific growth rate of the alga *Chlorella vulgaris* grown in wastewater diluted with monosodium glutamate wastewater (MSGW). The Alga grew more quickly in each diluted MSGW sample than it did in BG11 media. The growth rates increased as the concentration of MSGW to increased, leading to a 100-fold dilution. In the 25-, 50- and 100-fold diluted MSGW, the growth rates were 0.716, 0.705 and 0.697 d^{-1}, respectively. This result showed that there were sufficient nutrients in the 100-fold diluted MSGW to support vigorous algal growth. The growth rates curve additionally showed that the MSGW had no negative effect on algal development.

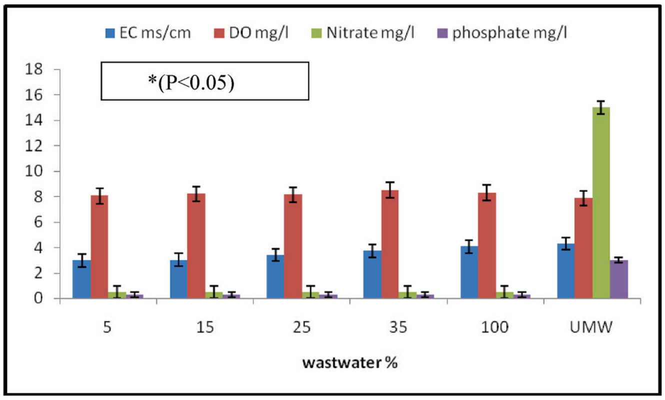

Fig. 8. Electric conductivity (EC), dissolved oxygen (DO), nitrate and phosphate concentrations in different wastewater concentrations.

4.1.2. Carbohydrate and protein content

The results showed an increase in the carbohydrate content of *A. platensis* when wastewater concentrations were decreased but the value of protein was decreased. As a result, Venckus et al. (2017) and the data obtained in the present study agreed. Venckus et al. (2017) cultivated *Chlorella vulgaris* in municipal wastewater also reported the ability of the alga *Chlorella vulgaris* to remove nitrogen and phosphorus wastewater plant in Vilnius City, Lithuania, where the experiments showed high removed percentage reach 93% for total nitrogen and 87% for total phosphorus. Moreover, research revealed that algae grown in nitrogen-rich environments accumulated more proteins and fewer carbohydrates in their biomass. According to Chinnasamy et al. (2010) in nitrogen-limited condition, carbon fixation during photosynthesis preferred the pathway leading to the production of carbohydrate rather than protein. Moreover, Uslu et al. (2011) favored this concept. The harvested biomass of *A. platensis* contain ranged from 67.4 to 5.6% protein while lipid ranged from 5.78 to 17.05% in the control treatment and the percentages in a nitrogen-limiting medium were 50% (protein) and 100% (lipid).

A study showed the effect of algal cultivation (*C. vulgaris*) in mechanically treated wastewaters (Li et al., 2016). The study showed the impact of low and high nitrogen supplies on some biochemical aspects of algae. A highest carbohydrate content (577 mg g^{-1} d. Wet) was recorded for cultivation in 10% mechanically treated wastewater. Whereas a highest value for protein content was 432 mg g^{-1} d when cultivated in undiluted mechanically treated wastewaters. Therefore, nitrogen status has a large role in shifting the metabolic pathway of carbohydrates or lipids instead of proteins.

Li et al. (2019) found *Porphyridium purpureumSCS-02* responded in a different manner to different concentrations of nitrogen throughout the alga protein and carbohydrate content. A high biomass of alga with an increase of protein and fatty acids content in high nitrogen supply, while a low nitrogen supply increases the carbohydrate, arachidonic acid content, and exopolysaccharide, while the total lipid was did not change with nitrogen in the same context..

4.1.3. Photosynthesis pigments

Photosynthetic pigments including chlorophyll *a*, phycocyanin, allophycocyanin and phycobiliproteins, and carotenoids were reduced by decreasing wastewater concentration The decreasing in pigment content caused by decline in photosynthetic activity and degradation of photosynthetic pigments in cells deficient in N (Da-Silva Ferreira and SantAnna, 2017). The fact that chlorophyll molecular has four nitrogen atoms in its structure makes it very challenging for the cell organelles to synthesis chlorophyll in the absence of nitrogen may be the cause of the large decrease in chlorophyll ring nitrogen shortage (Da-Silva Ferreira and SantAnna, 2017). While the observed increases in carotenoids in high nitrogen environment on physically N free substances could be related to the need for N in the synthesis of pigment-protein complexes in the thylakoid membrane (Takaichi, 2011). Additionally, at 0.04 M $NaNO_3$, *S. platensis* growing with high nitrogen levels revealed maximum protein (732.60 μ g/mg) and total phycocyanin (173.02 μg/mg) concentrations. This might be because amino acids, which make up proteins and other biological elements such as phycocyanin and for whose production nitrogen is necessary (Kand and Nagarajan, 2013).

These findings corroborate those of He et al. (2013), who observed a positive association between pigments content and levels of NH^{+4}-N and discovered that pigments reached their maximum levels (0.2%–0.5% of the microalgal biomass) at highly elevated levels of NH^{+4}-N (210 mg/l). El-Baky (2003) also noted that phycocyanin pigments and soluble protein content increased with nitrogen concentration, whereas this led to an increase in phycocyanin pigments and soluble protein content but a decrease occurred with increasing nitrogen concentration due to the destruction of the entire chloroplast. In addition, the high nitrogen treatment had greater concentration of the total carotenoid and chlorophyll *a* than the medium or low nitrogen treatment (6554–8250 and 2975–3109 mg/g, respectively) (Tossavainen et al., 2019). Another study by Guiheneuf and Stengel (2015) found that nitrogen limitation caused phycobiliprotein concentration to dramstically decrease from 1.7% DW (day 0) to almost 0% DW (day 10), and from 1.4% DW (day 9) to 0.6% DW (day 17) when a nitrogen deficiency was present.

In addition highest concentration of chlorophyll and carotenoids were recorded in 100% wastewater treated with BNM (peak values of 3.421 and 1.047 on day 16), followed by 75% wastewater treated with BNM (peak values of 3.310 and 0.943 on day 16), 50% wastewater treated with BNM (peak values of 2.968 and 0.767 on day 16), 25% wastewater treated with BNM (peak values of 2.519 and 0.603 on day16), and the control (peak value 2.386 and 0.513 on day 16). The level of industrial processed water (ICW) was also observed to positively correlate with the pigment content (chlorophylls and carotenoids) of *Chlorella pyrenoidosa* and *Chlorella vulgaris*, with higher concentrations of industrial processed water in growth media increasing the pigment content (Safafar et al., 2016).

4.2. Biodiesel production of *S. platensis*

The primary substances used by algae for energy storage are lipids and carbohydrates. The process of increasing lipid and starch accumulation in algae has involved nutrient stress. Some algae produce significant concentrations of triacylglycerols (TAG), which are lipids, when nitrogen levels are low. According to Gong et al. (2013), increased lipid production required a longer time of nutritional restriction. Algal fatty acid concentration has frequently been demonstrated to rise when the growing conditions are nitrogen-limited (Fields et al., 2014), and cultures with high P levels can regulate the carbon transfer from starch to lipid production (Zhu et al., 2015).

Since earlier researchers had discovered that the lipid content of algal cells was induced by the stress of nitrogen deprivation, the use of wastewater was advantageous to produce algal lipids (Lewis et al., 2000). Olguiin et al. (2001) also described this dependence; they cultured *S. platensis* to compare the amounts of lipid in two different culture media (Zarrouk's and nitrogen deficient) and found that the nitrogen deficient medium produced a higher amount of lipids (28.6%) in the total dry biomass than was the case for Zarrouk's media.

Another study by Khozin-Goldberg and Cohen (2006) hypothesized that starvation would increase the amount of lipids in *Monodus subterraneus*. Hassan et al. (2013) also demonstrated that nitrogen starvation (reduction from 8 g/l to 0 g/l) can result in increasing lipids content in *Chlorella vulgaris* and *Nitzchia palea*as as well as an increasing the production of oleic acid and stearic acid, both of which are crucial for the production of biodiesel.

Fatty acids are composed mainly of palmitic, oleic, linoleic acid, gamma-linolenic acid, and docosahexaenoic acid. Low municipal wastewater concentrations have a significant percentage of fatty acids. In accordance with these results, Bertoldi et al. (2006) determined the fatty acid profile of the microalga *Chlorella vulgaris* cultivated with different dilutions of hydroponic wastewater (HW). When compared to HW and HW25 cultivation. The HW25 cultivation percentage of saturated fatty acids revealed a discernible difference. It demonstrated that microalgae grown in HW25 had a lower saturated fatty acid content. However, despite having the largest concentration of unsaturated fatty acids and polyunsaturated fatty acids, it was the only one that did not exhibit any discernible variations, except for HW.

By diluting the wastewater effluent with synthetic media or DW, the growth of microalgae, lipid productivity, and nutrient removal all improved. In all of the experimental steps, both saturated and unsaturated fatty acids were produced, and wastewater that had been diluted by more than 50% allowed the growth of microalgae with fewer saturated fatty acids, which could then be converted into biodiesel with a high conversion efficiency.

4.3. Composition of the wastewater concentrations

4.3.1. Nitrogen and phosphorus

Arthrospira platensis reduced nitrate and phosphate concentrations. This might be connected to the capacity of the algal cell to uptake nitrogen and phosphorus from growth media (Georgianna and Mayfield, 2012). In the absence of inorganic orthophosphate, microorganisms, especially algae, will uptake organic phosphorus which will then be converted to orthophosphate at the cell surface via the enzyme phosphatase (Larsdotter, 2006; Al-sareji et al., 2023).

Singh et al. (2017) revealed the high ability of the alga *Chlorella* to remove the total nitrogen and total phosphorous by using urban wastewater (UWW).

In autoclaved and non-autoclaved samples, the total phosphorous was reduced by 79.0 and 80.9%, respectively, and algal treatment removed more than 93% of NH_4–N and 89% of total nitrogen (Li et al., 2011). According to an experiment done by Lodi et al. (2003) using *A. platensis* biomass to lower the concentrations of nitrogen and phosphorus in wastewater, all of the eliminated nitrate was used for biomass development (biotic removal), but phosphate didn't seem to be removed (Dalrymple et al., 2013).

4.3.2. Electrical conductivity and dissolved oxygen

Physicochemical parameters of treated and untreated wastewater were estimated. In general, the amount of dissolved salts is related to conductivity (Iyasele and Idiata, 2015). In agreement with this study, Kulkarni et al. (2016) cultured *S. platensis* on different concentrations of dairy effluent (control before treatment), 1, 2.5, 5, 7.5 and 10%) and reported extremely effective COD/Phosphate/EC removal in all concentrations. All concentration of *S. platensis* reduced conductivity. The maximum reduction following *S. platensis* treatment was 55.71% in dairy effluent with a 7.5% concentration, whereas the minimum reduction was 54.59% in the set. In addition, all algal treatments decreased electric conductivity values over the course of the initial phase to stationary phase.

Additionally, the estimated ammonia, nitrate, phosphorous, and BOD parameters appear to be decreased, whereas DO appeared to significantly rise in the tenth day. Consequently, microalgae are useful for treating wastewater (Shekhawat et al., 2012). Due to the high BOD value, this prevented the water from absorbing dissolved oxygen and caused anaerobiosis, which killed the aquatic organisms. The minimum concentration of dissolved oxygen was 0.2, while the maximum was 18 mg/l, and rose during the course of both experiments as whole and individual batches. In addition, there was a strong correlation between nitrogen removal and dissolved oxygen (Gentili and Fick, 2017).

5. Conclusions

The current work might serve as confirmation for a wastewater treatment method which produces algal fatty acid for possible use as feedstock for biodiesel. Overall, it can be said that using the household wastewater as nutrient media for microalgae growing is more environmentally friendly and has a lower impact than using the traditional cultivation techniques for long-term use in biodiesel and biofuel production. Also, cultivation of *A. platensis* in municipal wastewater reduced EC, nitrate, and phosphate concentrations as well as increased DO.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2023.139107.

References

Al-sareji, O.J., Meiczinger, M., Salman, J.M., Al-Juboori, R.A., Hashim, K.S., Somogyi, V., Jakab, M., 2023. Ketoprofen and aspirin removal by laccase immobilized on date stones. Chemosphere 311, 137133. https://doi.org/10.1016/j.chemosphere.2022.137133, 2023.

Ananadhi, P.M.R., Stanley, S.A., 2012. Microalgae as an oil producer for biofuel applications. Res. J. Recent Sci. 1 (3), 57–62.

Arnon, D.I., 1948. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 (1), 1.

Becker, W., 2004. Microalgae in human and animal nutrition. In: Handbook of Microalgal Culture: Biotechnology and Applied Phycology, 312.

Bennett, A., Bogorad, L., 1973. Complementary chromatic adaptation in a filamentous blue-green alga. J. Cell Biol. 58 (2), 419–435.

Bertoldi, F.C., Sant'Anna, E., da Costa Braga, M.V., Oliveira, J.L.B., 2006. Lipids, fatty acids composition and carotenoids of *Chlorella vulgaris* cultivated in hydroponic wastewater. Grasas Aceites 57 (3), 270–274.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254.

Can, S.S., Koru, E., Cirik, S., 2017. Effect of temperature and nitrogen concentration on the growth and lipid content of *Spirulina platensis* and biodiesel production. Aquacult. Int. 25 (4), 1485–1493.

Chinnasamy, S., Bhatnagar, A., Claxton, R., Das, K.C., 2010. Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresour. Technol. 101 (17), 6751–6760.

Dalrymple, O.K., Halfhide, T., Udom, I., Gilles, B., Wolan, J., Zhang, Q., Ergas, S., 2013. Wastewater use in algae production for generation of renewable resources: a review and preliminary results. Aquat. Biosyst. 9 (1), 2.

Das, P., Aziz, S.S., Obbard, J.P., 2011. Two phase microalgae growth in the open system for enhanced lipid productivity. Renew. Energy 36 (9), 2524–2528.

Da-Silva Ferreira, V., SantAnna, C., 2017. Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. World J. Microbiol. Biotechnol. 33 (1), 20.

Devanathan, J., Ramanathan, N., 2012. Pigment production from *Spirulina platensis* using seawater supplemented with dry poultry manure. J. Algal Biomass Utiliz. 3 (4), 66–73.

Dubois, M., Gilles, K.A., Hamiton, J.K., Robers, P.A., andSmith, F., 1956. Colorimetric method for determination of sugar and substances. Anal. Chem. 28 (3), 350–356.

El-Baky, H.H.A., 2003. Over production of phycocyanin pigment in blue green alga *Spirulina* sp. and it's inhibitory effect on growth of ehrlich ascites carcinoma cells. J. Med. Sci. 3 (4), 314–324.

El-Sheekh, M.M., Salman, J.M., Grmasha, R.A., Abdul-Adel, E., Saleh, M.M., Al-sareji, O.J., 2022. Influence of Fe+2 on the biomass, pigments, and essential fatty acids of Arthrospira platensis. In: Biomass Conversion and Biorefinery. Springer. https://doi.org/10.1007/s13399-022-02470-9.

Fields, M.W., Hise, A., Lohman, E.J., Bell, T., Gardner, R.D., Corredor, L., Moll, K., Peyton, B.M., Characklis, G.W., Gerlach, R., 2014. Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Appl. Microbiol. Biotechnol. 98 (11), 4805–4816.

Fogg, G.E., Thake, B., 1987. Algal Culture and Phytoplankton Ecology, third ed. University of Wisconsin Press, p. 267.

Gentili, F.G., Fick, J., 2017. Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants. J. Appl. Phycol. 29 (1), 255–262.

Georgianna, D.R., Mayfield, S.P., 2012. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature 488 (7411), 329.

Gong, Y., Guo, X., Wan, X., Liang, Z., Jiang, M., 2013. Triacylglycerol accumulation and change in fatty acid content of four marine oleaginous microalgae under nutrient limitation and at different culture ages. J. Basic Microbiol. 53 (1), 29–36.

Gonzalez Bautista, E., Laroche, C., 2021. *Arthrospira platensis* as a feasible feedstock for bioethanol production. Appl. Sci. 11 (15), 6756.

Gouveia, L., 2011. Microalgae as a feedstock for biofuels. In: Microalgae as a Feedstock for Biofuels. Springer, Berlin, Heidelberg, pp. 1–69.

Guiheneuf, F., Stengel, D.B., 2015. Towards the biorefinery concept: interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. Algal Res. 10, 152–163.

Hassan, F.M., Aljbory, I.F., Kassim, T.I., 2013. An attempt to stimulate lipids for biodiesel production from locally isolated microalgae in Iraq. Baghdad Sci. J. 10 (1), 97–108.

Hassan, F.M., Mahdi, W.M., Al-Haideri, H.H., Kamil, D.W., 2022. Identification of new species record of Cyanophyceae in Diyala River, Iraq based on 16S rRNA sequence data. Biodiversitas J. Biolog. Divers. 23 (10).

Hassan, F.M., Hayder, N.H., Hammadi, S.S.F., 2015. Enhancement of biodiesel production from local isolates of microalgae. Mesop. environ. j. 1 (3), 66–81.

Hassan, F.M., Salman, J.M., Abdulameer, S., 2014. Seasonal variation of environmental properties and phytoplankton community in Al- hussainya river, holly karbala – Iraq, mesop. Environ. J 1 (1), 56–82.

He, P.J., Mao, B., Shen, C.M., Shao, L.M., Lee, D.J., Chang, J.S., 2013. Cultivation of *Chlorella vulgaris* on wastewater containing high levels of ammonia for biodiesel production. Bioresour. Technol. 129, 177–181.

Hwang, J.H., Church, J., Lee, S.J., Park, J., Lee, W.H., 2016. Use of microalgae for advanced wastewater treatment and sustainable bioenergy generation. Environ. Eng. Sci. 33 (11), 882–897.

Iyasele, J.U., Idiata, D.J., 2015. Investigation of the relationship between electrical conductivity and total dissolved solids for mono-valent, di-valent and tri-valent metal compounds. J. Eng. Res. Rev. 3 (1), 40–48.

Jensen, A., 1978. Chlorophylls and carotenoids. In: Hellebust, J.A., Craigie, J.S. (Eds.), Handbook of Phycological Methods, Physiological and Biochemical Methods. Cambridge University Press, Cambridge, p. 5970.

Ji, Y., Hu, W., Li, X., Ma, G., Song, M., Pei, H., 2014. Mixotrophic growth and biochemical analysis of *Chlorella vulgaris* cultivated with diluted monosodium glutamate wastewater. Bioresour. Technol. 152, 471–476.

Kand, S., Nagarajan, P., 2013. Effect of different nitrogen concentrations on the biomass and biochemical consituents of *Spirulina platensis* [Geitler]. Asian J. Bio. Sci. 8 (2), 245–247.

Katiyar, R., Gurjar, B.R., Biswas, S., Pruthi, V., Kumar, N., Kumar, P., 2017. Microalgae: an emerging source of energy based bio-products and a solution for environmental issues. Renew. Sustain. Energy Rev. 72, 1083–1093.

Karimi-Maleh, H., Darabi, R., Karimi, F., Karaman, C., Shahidi, S.A., Zare, N., Baghayeri, M., Fu, L., Rostamnia, L.S., Rouhi, J., Rajendran, S., 2023. State-of-art advances on removal, degradation and electrochemical monitoring of 4-aminophenol pollutants in real samples: a review. Environ. Res. 222, 115338 https://doi.org/10.1016/j.envres.2023.115338.

Karimi-Maleh, H., Karaman, C., Karaman, O., et al., 2022. Nanochemistry approach for the fabrication of Fe and N co-decorated biomass-derived activated carbon frameworks: a promising oxygen reduction reaction electrocatalyst in neutral media. J. Nanostruct. Chem. 12, 429–439. https://doi.org/10.1007/s40097-022-00492-3.

Khan, M.I., Shin, J.H., Kim, J.D., 2018. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb. Cell Factories 17 (1), 36.

Khozin-Goldberg, I., Cohen, Z., 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water *Eustigmatophyte monodussubterraneus*. Phytochemistry 67 (7), 696–701.

Kulkarni, S.D., Auti, T., Saraf, S., 2016. Bioremediation study of dairy effluent by using *Spirulina platensis*. Res. J. Life Sci. Bioinform. Pharm. Chem. Sci 1, 317.

Larsdotter, K., 2006. Wastewater treatment with microalgae-a literature review. Vatten 62 (1), 31.

Lewis, T., Nichols, P.D., McMeekin, T.A., 2000. Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. J. Microbiol. Methods 43 (2), 107–116.

Li, T., Xu, J., Gao, B., Xiang, W., Li, A., Zhang, C., 2016. Morphology, growth, biochemical composition and photosynthetic performance of *Chlorella vulgaris* (Trebouxiophyceae) under low and high nitrogen supplies. Algal Res. 16, 481–491.

Li, T., Xu, J., Wu, H., Jiang, P., Chen, Z., Xiang, W., 2019. Growth and biochemical composition of *Porphyridium purpureum* SCS-02 under different nitrogen concentrations. Mar. Drugs 17 (2), 124.

Li, Y., Chen, Y.F., Chen, P., Min, M., Zhou, W., Martinez, B., Zhu, J., andRuan, R., 2011. Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour. Technol. 102 (8), 5138–5144.

Lodi, A., Binaghi, L., Solisio, C., Converti, A., Del Borghi, M., 2003. Nitrate and phosphate removal by *Spirulina platensis*. J. Ind. Microbiol. Biotechnol. 30 (11), 656–660.

Mitra, D., van Leeuwen, J.H., Lamsal, B., 2012. Heterotrophic/mixotrophic cultivation of oleaginous *Chlorella vulgaris* on industrial co-products. Algal Res. 1 (1), 40–48.

Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., Pereira, S.A., Druzian, J.I., de Souza, C.O., et al., 2013. Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. Bioenergy Res. 6 (1), 1–13.

Nordin, N., Yusof, N., Samsudin, S., 2014. Microalgae biomass production and nitrate removal from landfill leachate. In: Proceeding of International Conference on Research, Implementation and Education of Mathematics and Sciences, pp. 73–82.

Olguin, E.J., Galicia, S., Angulo-Guerrero, O., Hernández, E., 2001. The effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina* sp. (*Arthrospira*) grown on digested pig waste. Bioresour. Technol. 77 (1), 19–24.

Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresour. Technol. 102 (1), 17–25.

Praveenkumar, R., Kim, B., Choi, E., Lee, K., Cho, S., Hyun, J.S., Park, J.Y., Lee, Y.C., Lee, H.U., Lee, J.S., Oh, Y.K., 2014. Mixotrophic cultivation of oleaginous *Chlorella* sp. KR-1 mediated by actual coal-fired flue gas for biodiesel production. Bioproc. Biosyst. Eng. 37 (10), 2083–2094.

Richmond, A. (Ed.), 2004. Handbook of Microalgal Culture: Biotechnology and Applied Phycology, 577. Blackwell Science, Oxford.

Safafar, H., Hass, M., Møller, P., Holdt, S., Jacobsen, C., 2016. High-EPA biomass from *Nannochloropsis salina* cultivated in a flat-panel photo-bioreactor on a process water-enriched growth medium. Mar. Drugs 14 (8), 144.

Salman, J.M., Grmasha, R.A., Stenger-Kovács, C., Lengyel, E., Al-sareji, O.J., AL-Cheban, A.M.A.A.R., Meiczinger, M., 2023. Influence of magnesium concentrations on the biomass and biochemical variations in the freshwater algae, *Chlorella vulgaris*. Heliyon 9, e13072. https://doi.org/10.1016/j.heliyon.2023.e13072, 2023.

Saranraj, P., Stella, D., Usharani, G., Sivasakthi, S., 2013. Effective recycling of lignite fly ash for the laboratory cultivation of blue green algae-*Spirulina platensis*. Int. J. Microbiol. Res. 4 (3), 219–226.

Serrà, A., Artal, R., García-Amorós, J., Gómez, E., Philippe, L., 2020. Circular zero-residue process using microalgae for efficient water decontamination, biofuel production, and carbon dioxide fixation. Chem. Eng. J. 388, 124278.

Shanab, S.M.M., Ali, H.E.A., 2022. Impact of culture media composition, nutrients stress and gamma radiation on biomass and lipid of the green microalga, *Dictyochloropsis splendida* as a potential feedstock for biodiesel production. Baghdad Sci. J. 19 (1) https://doi.org/10.21123/bsj.2022.19.1.0043, 0043-0043.

Shekhawat, D.S., Bhatnagar, A., Bhatnagar, M., Panwar, J., 2012. Potential of treated dairy waste water for the cultivation of algae and waste water treatment by algae. Universal J. Environ. Res. Tech. 2 (1), 101–104.

Singh, B., Guldhe, A., Rawat, I., Bux, F., 2014. Towards a sustainable approach for development of biodiesel from plant and microalgae. Renew. Sustain. Energy Rev. 29, 216–245.

Singh, R., Birru, R., Sibi, G., 2017. Nutrient removal efficiencies of *Chlorella vulgaris* from urban wastewater for reduced eutrophication. J. Environ. Protect. 8 (1), 1.

Takaichi, S., 2011. Carotenoids in algae: distributions, biosyntheses and functions. Mar. Drugs 9 (6), 1101–1118.

Tijjani-Oshungboye, K., 2011. Microalgae Biomass as Fermentation Feedstock (Doctoral Dissertation. Rhodes University, pp. 1–85.

Tossavainen, M., Ilyass, U., Ollilainen, V., Valkonen, K., Ojala, A., Romantschuk, M., 2019. Influence of long term nitrogen limitation on lipid, protein and pigment production of *Euglena gracilis* in photoheterotrophic cultures. PeerJ 7, e6624.

Uma, V.S.S., Dineshbabu, G., Deviram, G., Arulananth, D., Uma, L., Prabaharan, D., 2016. Rapid, cost effective, enhanced lipid extraction from marine cyanobacteria in A biodiesel perspective. Int. J. Adv. Biotechnol. Res. 7 (3), 976–2612.

Uslu, L., Içik, O., Koç, K., Göksan, T., 2011. The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. Afr. J. Biotechnol. 10 (3), 386–389.

Venckus, P., Kostkevičienė, J., Bendikienė, V., 2017. Green algae *Chlorella vulgaris* cultivation in municipal wastewater and biomass composition. J. Environ. Eng. Landsc. Manag. 25 (1), 56–63.

Viswanath, B., Bux, F., 2012. Biodiesel production potential of wastewater microalgae *Chlorella* sp. under photoautotrophic and heterotrophic growth conditions. Br. J. Eng. Technol. 1 (1), 251–264.

Walter, A., Carvalho, J.C.D., Soccol, V.T., Faria, A.B.B.D., Ghiggi, V., Soccol, C.R., 2011. Study of phycocyanin production from *Spirulina platensis* under different light spectra. Braz. Arch. Biol. Technol. 54 (4), 675–682.

Wobbe, L., Remacle, C., 2015. Improving the sunlight-to-biomass conversion efficiency in microalgal biofactories. J. Biotechnol. 201, 28–42.

Zaki, M.A., Ashour, M., Heneash, A.M., Mabrouk, M.M., Alprol, A.E., Khairy, H.M., Elshobary, M.E., 2021. Potential Applications of native cyanobacterium isolate (*Arthrospira platensis* NIOF17/003) for biodiesel production and utilization of its byproduct in marine rotifer (Brachionusplicatilis) production. Sustainability 13 (4), 1769.

Zhang, Z., Karimi-Maleh, H., 2023a. Label-free electrochemical aptasensor based on gold nanoparticles/titanium carbide MXene for lead detection with its reduction peak as index signal. Adv. Compos. Hybrid Mater. 6 (68) https://doi.org/10.1007/s42114-023-00652-1.

Zhang, Z., Karimi-Maleh, H., 2023b. In situ synthesis of label-free electrochemical aptasensor-based sandwich-like AuNPs/PPy/Ti3C2Tx for ultrasensitive detection of lead ions as hazardous pollutants in environmental fluids. Chemosphere 324, 138302. https://doi.org/10.1016/j.chemosphere.2023.138302.

Zhao, L.S., Li, K., Wang, Q.M., Song, X.Y., Su, H.N., Xie, B.B., Zhang, X.Y., Huang, F., Chen, X.L., Zhou, B.C., Zhang, Y.Z., 2017. Nitrogen starvation impacts the photosynthetic performance of *Porphyridium cruentum* as revealed by chlorophyll a fluorescence. Sci. Rep. 7 (1), 8542.

Zhu, S., Wang, Y., Xu, J., Shang, C., Wang, Z., Xu, J., Yuan, Z., 2015. Luxury uptake of phosphorus changes the accumulation of starch and lipid in *Chlorella* sp. under nitrogen depletion. Bioresour. Technol. 198, 165–171.