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Use of Industrial Wastewater for *Chlorella* sp. Culturing to obtain Commercially Important Compounds

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Abstract

In the present study, *Chlorella* sp. was grown in volume-graded industrial wastewater and showed increased growth in 10% and 30% of wastewater but the maximum growth was shown in 100% industrial wastewater. Among nitrogen sources, maximum growth was observed in urea (6.80×10^5 cells/ml) while the least was determined in NH_4Cl (1.40×10^5 cells/ml). The maximum no. of algal cells (2.50×10^5) was determined in 0% NaCl while the minimum cells were determined in 2% NaCl (0.002×10^5). The optimum algal growth was determined at pH 7 (7.0×10^5 cells/ml) but at pH 9 the alga showed significant growth (5.9×10^5 cells/ml). The alga showed resistance towards erythromycin and chloramphenicol but was sensitive against ampicillin and gentamicin. Alga showed high growth in the presence of Cd and Pb ($5\mu\text{g}$) while less growth was determined in the presence of Hg, Cu, and Cr ($5\mu\text{g}$). Different compounds including cyclotetrasiloxane, octamethyle-, cyclopentasiloxane, decamethyle-, cyclohexasiloxane, dodecamethyle-, cycloheptasiloxane, tetradecamethyle- and benzeneethaneamine were isolated and analyzed by GC-MS analysis.. Siloxanes have great significance in industrial products, especially in cosmetics and textile industries. Besides biofuel production alga has great potential to be used for various industrial products.

KEYWORDS

Chlorella sp., industrial wastewater, GC-MS analysis, siloxanes

INTRODUCTION

Microalgae have immense economic and industrial potential [1] as valuable sources for pharmaceuticals, carotenoids, health foods, fine chemicals, dyes, most importantly biofuels, and many others [2]. Microalgae may have an efficient ability to solve emerging environmental problems including the greenhouse effect, waste treatments, and especially wastewater treatment. Moreover, microalgae can fix

carbon dioxide (CO_2) by photosynthetic process, and it can efficiently remove excess nutrients at a minimal cost [3]. In addition, microalgae use different organic compounds as carbon sources and can use various forms of nitrogen to accumulate heavy metal ions [4]. As a result of all these algal characteristics, several workers have studied microalgae as an alternative feasible and reliable solution to environmental problems [5]. Microalgae

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are the primary producers, and undoubtedly serve as a food source, for a large number of aquatic organisms [6].

Microalgae have ability to grow efficiently in environments which require minimal freshwater input as compared to the biofuel based on plant crops. The land which is not suitable for growth of other plant crops, microalgae can utilize this land and make the process potentially feasible with regard to preserve freshwater resources [7], wastewater has the capacity to sustainable growth of algal feedstock i.e. a remarkable interest exists to purify industrial wastewater by growing microalgae.

In wastewater, growth of microalgae can be affected by various variables including temperature and pH, availability of O₂, CO₂, and light and amount of essential nutrients i.e. N, P, and organic carbon. The growth of microalgae was increased considerably in sewage water under long light period conditions and following CO₂ addition while algal biomass was decreased with increased temperature [8]. Likewise, the ability of various algal species to tolerate a specific wastewater environment will be changeable. Chlorophytic microalgae have been shown to be very effective accumulating nutrients from the industrial wastewater [9]. Among algal species, *Chlorella* sp., are widely used for the purification of industrial wastewater and its ability to remove P, N, and chemical oxygen demand (COD) with retention times from 10 h to 42 days has been documented [10].

A variety of useful compounds can be isolated from algae such as siloxanes. The siloxane name is derived from its components including a silicon, oxygen, and alkane compound. Siloxanes play an important role in our daily routine lives i.e. they wash our hands, brush our teeth, clean

our clothes, help us lose weight, drive us to work, and even used in printing our newspapers [11]. The most important usage of siloxanes is in the industry of cosmetics where they add useful characteristics i.e. increased absorption, enhanced skin feel, reduction in greasiness, that silky shiny look, and many more. Siloxanes are also frequently used in many kinds of domestic products, such as detergents, conditioners, shampoos, deodorants, gels, creams, water repellents and other products [12].

MATERIALS AND METHOD

Sample Collection and Isolation of *Chlorella* sp.

Chlorella sp. was isolated from industrial wastewater sample collected from Sheikhupura road (Pakistan). *Chlorella* sp. was identified on the basis of its morphological characteristics using the method described in “Standard methods for the examination of water and wastewater” [13]. Pure culture of *Chlorella* sp. was obtained by subsequent culturing in BG 11 medium by providing light in 8/16 h L/D cycle through fluorescent lamps at temperature 30 ±2°C [23].

Growth of Alga in Different Physical and Chemical Conditions

Attributes of *Chlorella* sp. were monitored on the basis of different experiments (physical and chemical) using BG 11 as an algal growth medium. For all works, 1% inoculum was given from 7 days-old algal cultures.

Utilization of Different Carbon Substrates under Light and Dark Conditions

The algal growth response was evaluated in the BG11 medium by supplementing it with different carbon sources (such as sucrose and sodium acetate at the concentration of 0.01 g/ml) along with unamended BG11 medium as control under light and dark conditions. The samples were incubated for

10 days under fluorescent light. Algal growth was determined in terms of cell count using Neubauer chamber.

Utilization of Nitrogen Sources

To determine the most preferred nitrogen source by *Chlorella* sp. it was treated with various nitrogen sources such as sodium nitrate, urea, and ammonium chloride at the concentration of 250 µg/ml. Algal growth was determined after 10 days of incubation.

Determination of Algal Tolerance to NaCl

Halotolerance of *Chlorella* sp. was determined by treating it with graded concentrations of NaCl salt (0-5%). The algal growth medium, BG11 medium, was mixed with different concentration of NaCl such as 0%, 1%, 2%, 3%, 4%, and 5%. Cultures were incubated for 10 days and growth was evaluated by cell count using Neubauer chamber.

Determination of Algal Tolerance to pH

To check the optimum pH for algal growth, alga was grown at different pH regimes such as 5, 7, and 9 and final growth was determined after 10 days of incubation. The algal growth at each pH was determined in terms of cell count using a Neubauer chamber.

Determination of Algal Ability to Grow on Wastewater

The BG11 medium was amended with wastewater, e.g. 10% (10ml/90ml), 30% (30ml/70ml) and 100% (100% of wastewater). Wastewater was collected from industrial area of Kot Lakhpat, Lahore (Pakistan). The pH was maintained at 7.0. Wastewater was added to BG11 medium after centrifugation at 6000 rpm for 10 min. Wastewater without centrifugation was also used for algal growth and incubated for 15 days. Growth was measured in terms of cell count using Neubauer chamber.

Antibiotic Sensitivity

Antibiotic sensitivity was measured by disk diffusion method in BG11 medium amended with agar. Different antibiotic disks i.e. 10µg ampicillin, 10µg gentamicin, 15µg erythromycin, and 30µg chlorempenicol were used. Petriplates were incubated for 7 days under fluorescent light.

Resistance of Heavy Metals by Alga

Five microgram of each metal i.e. mercury (Hg), copper (Cu), chromium (Cr), lead (Pb), and cadmium (Cd), was added to 100ml of algal culture. Metal uptake was determined in terms of cell count after 6 days. To check the ultimate uptake of heavy metals by *Chlorella* sp., metal concentration was increased by adding 5µg of each metal twice the 3-day interval. Now total metal concentration has become 15 µg. Metal uptake was determined in terms of cell count using a Neubauer chamber.

Organic Compounds Isolation from Algal Cells

For this purpose, the following steps were performed.

(a) Algal harvesting

Algal culture was taken in the Falcon tube and centrifuged at 6,000 rpm for 15 min. Algal pellets were obtained and dried in the oven at 80 °C for 1 h.

(b) Cell disruption

For algal cell disruption, an aliquot of dry cell biomass of about 0.5g was taken and blended with 100 ml of distilled water. The mixture was disrupted using two different methods: autoclaving at 121°C with 15 lb for 15 min and microwave using a microwave oven (about 100 °C) for 5 min.

(c) Fatty compound extraction

The organic compounds were extracted by mixing samples with chloroform-methanol (1:1 v/v) in proportion of 1:1 with slight modification according to procedure

described in Bligh and Dyer's method (Lee and Lee, 2002). The mixtures were then transformed into a separatory funnel and shaken well for 5 min. The separatory funnel was kept undisturbed for 10 days. After 10 days two layers were appeared. The organic compound layer was separated from separatory funnel and solvent evaporation was done by a rotary evaporator (Heidolph).

Gas Chromatography–Mass Spectrophotometry

The composition of organic compound was determined by Gas chromatography. Fifty milligram of sample were taken into the capped test tubes. One milliliter of saturated KOH-CH₃OH solution was added to the sample to saponify it at 75°C for about 10 min. Then for the methanolysis of the sample, 5% HCl in methanol was added to the sample at 75°C for about 10 min. Two phases formed. The phase containing the organic compound was separated by adding 2 ml of distilled water and then recovered. The pattern of peaks of the injected sample into the Gas chromatographic analyzer was observed.

RESULTS AND DISCUSSION

Utilization of Organic Carbon Sources

In the present study, *Chlorella* sp. has been grown in different carbon substances which

showed its ability to use a variety of carbon sources. Such type of ability has been observed in many green algae. A typical microalga has 50% carbon and 10% nitrogen content. Some algae, like *Chlorella*, can take up both organic and inorganic forms of carbon [14]. In the current investigation, *Chlorella* sp. was provided with different carbon sources, and the growth rate was determined under light and dark conditions. Sucrose has been proven to be a better carbon source as compared to acetate because acetate does not play an important role in algal metabolic activities. Under light conditions, the growth was good and showed a high mixotrophic growth rate but under dark conditions (Fig. 1). The algal cells under photoheterotrophic conditions were maximum as compared to the chemoheterotrophic conditions (Table 1). The algal growth with regards to cell number was BG11 > sucrose > citrate in light conditions while it was sucrose > citrate > BG 11 in the dark. These organic substances were inhibitory to photosynthesis to some extent. In many other algae mixotrophy had been reported e.g. *Chlorella vulgaris* and *Chlamydomonas acidophila* [15].

Table 1. Algal growth in the presence of sucrose and sodium acetate contains BG11 medium under light and dark conditions

Treatment	Growth parameter	BG11 medium (Control)	BG11 medium with	
			Sucrose	Sodium acetate
Light	Cell count (cells/ml)	7.62×10 ⁵ + + + +	0.7×10 ⁵ + +	0.4×10 ⁵ + +
Dark	Cell count (cells/ml)	–	0.3×10 ⁵ +	0.2×10 ⁵ +

Key: + = Positive growth - = Negative growth

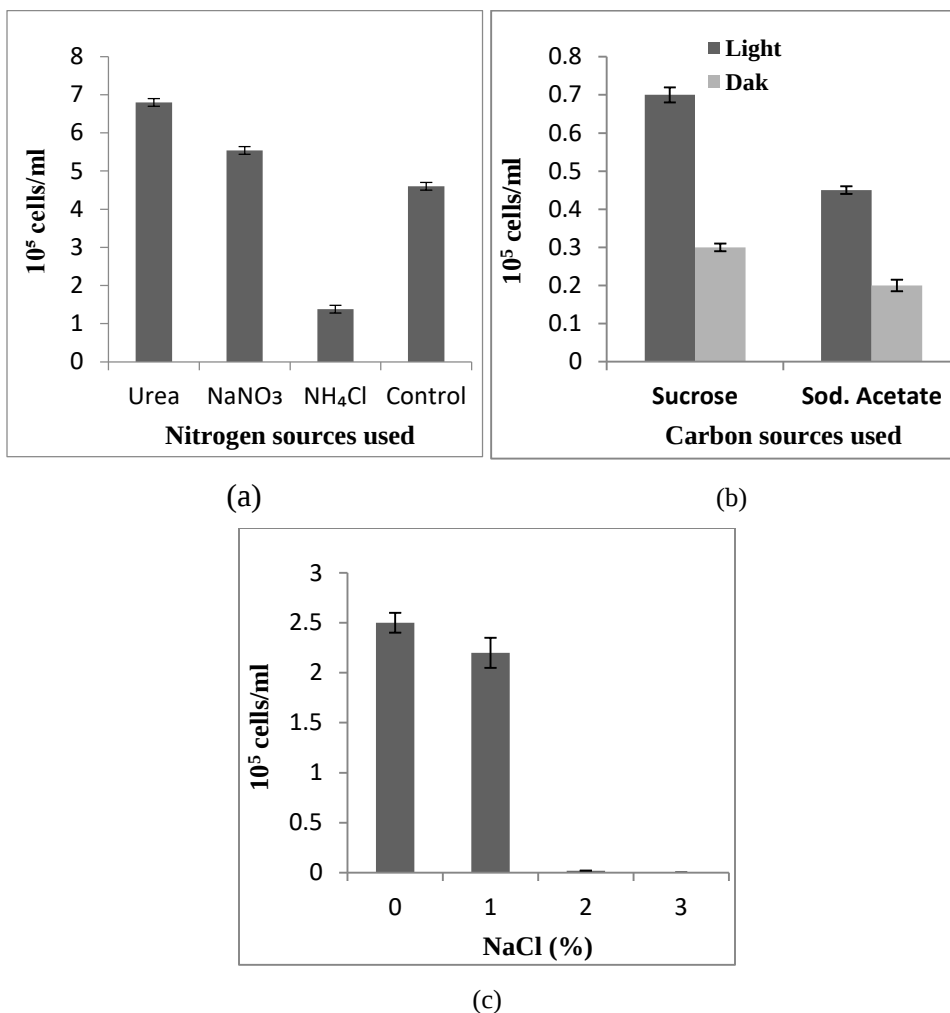


Figure 1 (a) Algal growth in BG11 medium containing sucrose and sodium acetate under light and dark conditions; (b) Algal growth (No. of cells/ml) in the presence of urea, sodium nitrate, and ammonium chloride in BG11 medium; (c) Algal growth (No. of cells/ml) in BG11 medium containing sodium chloride concentrations

Utilization of Nitrogen Sources

Nitrogen sources, e.g. urea, NaNO_3 and NH_4Cl were used and significantly enhanced the growth of *Chlorella* sp. Maximum growth, i.e. most of cells was observed in urea. On the other hand, sodium nitrate (NaNO_3) also supported the growth of alga but less as compared to urea. However, the least growth supported was

determined in the presence of ammonium chloride (NH_4Cl). In BG11 medium contains no nitrogen source showed minimum growth of the alga. The culture greenish intensity clearly indicates the growth supported by the nitrogen source given in the medium. The algal growth rate was in the following order urea > NaNO_3 > NH_4Cl > BG11 (Fig. 2).

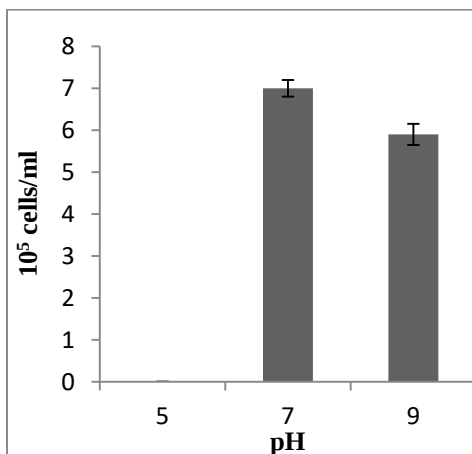


Figure 2. Algal growth (No. of cells/ml) in BG11 medium at different pH

The variation in nitrogen contents depends on algal species and physiological conditions. Usually, the content of nitrogen increases when nutrient availability and algal growth rate are increased and can be ranged from 4 to 9% (dry weight) in algal cells. Our studies showed that *Chlorella* sp. used maximum nitrogen from urea and NaNO_3 and showed maximum growth as compared to the control. Low growth was observed when NH_4Cl was used because nitrogen was not freely available to algal cells. It was also observed that algae are better adaptable to utilize alternative nitrogen sources under limiting nitrogen environments [14]. It is also reported that accumulation of reduced and oxidized nitrogen form can be segregated with regard to time due to association with certain phytoplankton groups [16].

Halotolerance

Chlorella sp. showed halotolerance up to 2% NaCl but its optimum growth was determined at 0% NaCl. It also showed good growth at 1% NaCl but no growth at 3% NaCl was observed as depicted with culturing flasks. The maximum no. of algal cells determined in 0% NaCl was 2.50×10^5

cells/ml while the minimum cells (0.002×10^5) were determined in 2% NaCl (Fig. 3). Algae can withstand high salt concentration to some extent. The observations showed that *Chlorella* sp. showed good growth at 1% NaCl but at high salt concentrations such as 2% NaCl, the growth rate decreased rapidly because high salt concentration changes the metabolic activities of algal cells. It was observed that not only algal growth was affected but the synthesis of protein and lipids was also affected in salt-stressed alga [17].

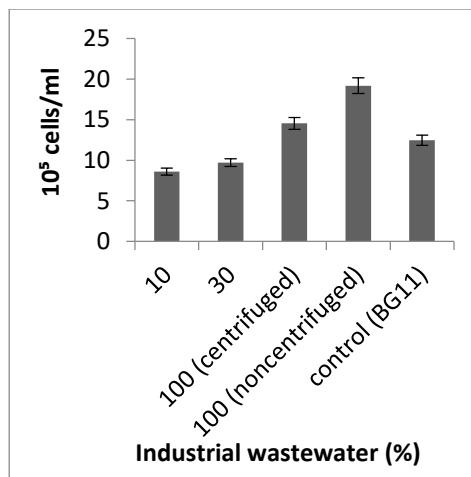


Figure 3. Algal growth (No. of cells/ml) in 10, 30, 100 % (centrifuged), and 100 % (non-centrifuged) industrial wastewater and BG11 medium (Control)

pH Tolerance

Chlorella sp. was grown at pH i.e. 5, 7, and 9. No growth was observed at pH 5. This indicates that acidic pH (in this case 5) has inhibitory effect on the algal growth. The optimum growth of alga was determined at pH 7.0. Fairly good growth was also observed at pH 9 which indicates the ability of alga to grow on alkaline medium (Fig. 4).

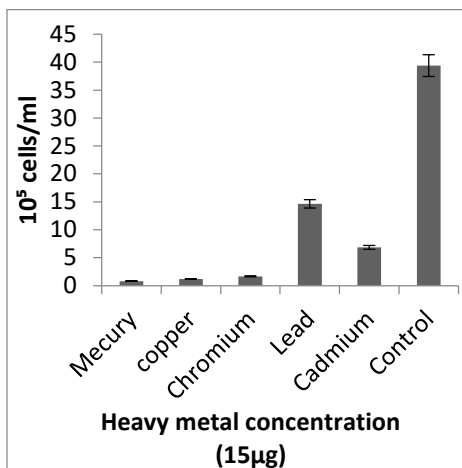


Figure 4. Heavy metal ions resistance by alga in the presence of different heavy metals after 3 days

Several studies have shown that pH may be important in regulating the algal growth rate [18]. Many reports suggested that *Chlorella* sp. could grow at varying pH. In the present study, *Chlorella* sp. showed optimum growth at pH 7 but no growth was observed at pH 5 suggesting that acidic conditions seldom support algal growth [19]. On the other hand, *Chlorella* sp.

showed good growth at pH 9 suggesting that it can grow at alkaline conditions but not too high pH. As pH increases, CO₂ availability decreases and may become the limiting factor to photosynthesis and phytoplankton growth [2]. Besides, salt concentration and pH, microalgae responses varied when exposed to any hazardous environmental change, for example lipid composition is changed to maintain the membrane fluidity of the cell [20].

Growth of *Chlorella* sp. on Industrial Wastewater

Significant algal growth was determined in BG11 medium amended with industrial wastewater. At 10% wastewater, good growth was estimated and a small increase in growth was determined when wastewater volume was increased to 30% but growth was still less as compared to the growth in BG11 medium. In 100% filtered wastewater algal growth was higher than unamended BG11 medium. On the other hand, the algal growth in 100% unfiltered wastewater was much higher than unamended BG11 medium (Fig. 5).

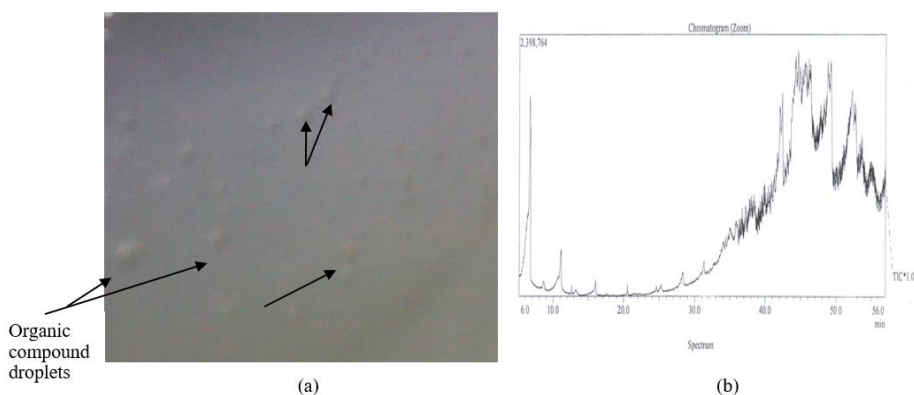


Figure 5. (a) Organic compound droplets (black arrows). (b) Gas chromatography-mass spectrometry

In the present investigation, diluted industrial wastewater (10% and 30%) supported the growth of *Chlorella* sp. On the other hand, centrifuged industrial wastewater (100%) supported better growth of *Chlorella* sp. than BG11 medium. However, non-centrifuged industrial wastewater (100%) surprisingly enhanced the growth of *Chlorella* sp. which was more than any other diluted, centrifuged wastewater, and BG11 medium. Because it had been contained dissolved organic matter rich in carbohydrates and proteins [21]. However, the quality of industrial wastewater varies from one source to another and is changing from time to time. Another work reported by [22] with 85–90% carpet industry wastewater along with a small proportion (10–15%) of municipal sewage wastewater ascertained that both marine and fresh water algae showed significant growth in industrial wastewater. It was studied that wastewater could contain valuable nutrients including phosphorus and nitrogen for algal growth [23]. Many other studies also showed that nutrient removal efficiency depends upon the level of nutrients in wastewater and also the extent of nutrients used by algae for growth [19].

Antibiotic Sensitivity

Chlorella sp. showed resistance towards erythromycin (15µg) and chloramphenicol (30µg) i.e. no hollow zone was observed around the antibiotics used. However, it showed sensitivity towards ampicillin (25µg) and gentamicin (10µg). The zone of inhibition size around ampiciline and gentamicine was 1 and 1.7cm, respectively.

Algal Metal Resistance

Maximum growth was observed in BG11 medium in which no metal was added. Algae showed efficient growth in the presence of 5µg Cd and Pb. On the other hand low growth was observed in the

presence of 5µg Hg, Cu, and Cr. When metal concentration was increased to 10 µg, the no. of algal cells were decreased in the presence of Hg, Cu, Cr, and Cd. On the other hand, significant increase in algal growth in the presence of Pb was determined. Further, when metal concentration was increased up to 15 µg, the algal cells were decreased sequentially as compared to the 10 µg of heavy metals. Culture without any metal showed the highest number of algal cells.

Chlorella sp. showed resistance to heavy metal ions at the concentration of 15µg/100ml. At 15µg, low growth was determined in *Chlorella* sp. culture containing Hg, Cu, and Cr while cell count was high in the presence of Cd and Pb. In the presence of Pb, cell count was high but cell size became small. Alga tolerates such heavy metal concentrations because it adsorbs the metal ions on its cell surface or absorbs them in its body rather than using them in its metabolism [24]. Very high concentrations of heavy metals can affect algal cell growth and cell size. Different algal species showed different tendency towards different concentrations of a variety of heavy metals [25].

Gas Chromatography-mass Spectrophotometry

Organic compounds were extracted to perform GC-MS analysis to determine the exact composition of these compounds. Different peaks showed the presence of different compounds in *Chlorella* sp.. The isolated compounds were cyclotetrasiloxane, octamethyle-, cyclopentasiloxane, decamethyle-, cyclohexasiloxane, dodecamethyle-, cycloheptasiloxane, tetradecamethyle- and benzenethaneamine.

Organic compounds were extracted from *Chlorella* sp. using microwave and

autoclaved method. Microwave method is proved better to extract lipids as small lipid droplets. For *C. vulgaris*, the microwave oven method and autoclaving methods revealed the highest efficiency. For animal fats and vegetables oils, a method using microwaves has already been reported for lipid extraction. It has been studied that the microwave oven method was easy, simple and most efficient method for tested microalgae [23].

Various compounds of different composition were analyzed by GC-MS analysis. Many researchers have opined that the composition of fatty acids and other organic compounds of the algae are related to different environment factors. The isolated compounds from *Chlorella* sp. were siloxanes like cyclotetrasiloxane, octamethyle-, cyclopentasiloxane, decamethyle-, cyclohexasiloxane, dodecamethyle-, cycloheptasiloxane, tetradecamethyle- and other organic compounds like benzenethaneamine. These compounds have great importance in industries like the cosmetics industry, textile industry, and rubber industry. In addition to all these siloxanes are used as paint additives, plastic product additives, and in shoe polish. Siloxanes are also used in food industry, as an oil alternative to produce low-calorie food products, i.e. salad dressings, mayonnaise, and potato chips [26].

CONCLUSION

The *Chlorella* sp. was grown in volume-graded industrial wastewater and highest growth was obtained in 100% industrial wastewater. In sucrose and sodium acetate presence, growth was observed in a dark environment but it was increased in the presence of light suggesting that both carbon sources were not inhibitory to the photosynthetic process. Among nitrogen

sources, maximum growth was observed in urea while the least was determined in NH_4Cl . The maximum no. of algal cells determined in 0% NaCl was 2.50×10^5 cells/ml while the minimum cells were determined in 2% NaCl (0.002×10^5). The optimum algal growth was determined at pH 7 but at pH 9 the alga showed the fairly good growth. *Chlorella* sp. showed resistance towards erythromycin and chloramphenicol but was sensitive against ampicillin and gentamicin. Alga showed efficient growth in the presence of Cd and Pb ($5\mu\text{g}$) while less growth was determined in the presence of Hg, Cu, and Cr ($5\mu\text{g}$). Various compounds including cyclotetrasiloxane, octamethyle-, cyclopentasiloxane, decamethyle-, cyclohexasiloxane, dodecamethyle-, cycloheptasiloxane, tetradecamethyle- and benzenethaneamine were analyzed through GC-MS analysis which can be used in the formation of different industrial materials which have great impact on world's economy.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

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REFERENCES

1. Blaga AC, Zaharia C, Suteu DJP. Polysaccharides as support for microbial biomass-based adsorbents with applications in removal of heavy metals and dyes. *Polymers*. 2021;13(17):2893. <https://doi.org/10.3390/polym13172893>
2. Das SK, Ghosh GK, Avasthe RJBC. Conversion of crop, weed and tree biomass into biochar for heavy metal

- removal and wastewater treatment. Biomass Conversion. 2021;1-14. <https://doi.org/10.1007/s13399-021-01759-3>
3. Sharma P, Pandey AK, Udayan A, Kumar S. Role of microbial community and metal-binding proteins in phytoremediation of heavy metals from industrial wastewater. Bioresour Technol. 2021;326:124750. <https://doi.org/10.1016/j.biortech.2020.124750>
 4. Rue GP, Darling JP, Graham E, Tfaily MM, McKnight DM. Dynamic changes in dissolved organic matter composition in a Mountain Lake under ice cover and relationships to changes in nutrient cycling and phytoplankton community composition. Aquat Sci. 2020;82:1-16. <https://doi.org/10.1007/s00027-020-00714-8>
 5. Cutajar J, Sterba-Boatwright B, Wetz MS. Coastal residential canals harbor distinct water quality conditions and phytoplankton community composition. Coastal Shelf Sci. 2024;296:108595. <https://doi.org/10.1016/j.ecss.2023.108595>
 6. Bandyopadhyay D, Biswas H. Impacts of variable nutrient stoichiometry (N, Si and P) on a coastal phytoplankton community from the SW Bay of Bengal, India. Eur J Phycol. 2021;56(3):273-288. <https://doi.org/10.1080/09670262.2021.1946824>
 7. Butts TJ, Moody EK, Wilkinson GMJ. Contribution of zooplankton nutrient recycling and effects on phytoplankton size structure in a hypereutrophic reservoir. J Plankton Res. 2022;44(6):839-853. <https://doi.org/10.1093/plankt/fbab045>
 8. Low SS, Bong KX, Mubashir M, et al. Microalgae cultivation in palm oil mill effluent (POME) treatment and biofuel production. Sustainability. 2021;13(6):3247. <https://doi.org/10.3390/su13063247>
 9. Shahid A, Malik S, Zhu H, et al. Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. Sci Total Environ. 2020;704:135303. <https://doi.org/10.1016/j.scitotenv.2019.135303>
 10. Zainith S, Saxena G, Kishor R, Bharagava RN. Application of microalgae in industrial effluent treatment, contaminants removal, and biodiesel production: Opportunities, challenges, and prospects. In: Bioremediation for environmental sustainability. 2021:481-517.
 11. Kumar R, Ghosh AK, Pal P. Synergy of biofuel production with waste remediation along with value-added co-products recovery through microalgae cultivation: A review of membrane-integrated green approach. Sci Total Environ. 2020;698:134169. <https://doi.org/10.1016/j.scitotenv.2019.134169>
 12. Al-Jabri H, Das P, Khan S, Thaher M, AbdulQuadir MJW. Treatment of wastewaters by microalgae and the potential applications of the produced biomass—A review. Water. 2020;13(1):27. <https://doi.org/10.3390/w13010027>
 13. Lipps WC, Baxter TE, Braun-Howland EB, Association APH, Association AWW. Standard methods for the examination of water and wastewater: American public health association.

2023.
<https://doi.org/10.2105/9780875532875>
14. Zarrinmehr MJ, Farhadian O, Heyrati FP, et al. Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, *Isochrysis galbana*. Egypt J Aquat Res. 2020;46(2):153-158.
<https://doi.org/10.1016/j.ejar.2020.03.001>
15. Díaz S, Aguilera Á, de Figueras CG, et al. Heterologous Expression of the Phytochelatin Synthase CaPCS2 from *Chlamydomonas acidophila* and Its Effect on Different Stress Factors in *Escherichia coli*. Int J Environ Res Public Health. 2022;19(13):7692.
<https://doi.org/10.3390/ijerph19137692>
16. Henson SA, Cael B, Allen SR, Dutkiewicz S. Future phytoplankton diversity in a changing climate. Nat Commun. 2021;12(1):5372.
<https://doi.org/10.1038/s41467-021-25579-6>
17. Sathish T, Nazrin A, Thomas LC, Padmakumar K. Seasonal dynamics of dinoflagellates with special emphasis on potentially harmful species in a tropical estuarine system along the southwest coast of India. J Oceanography. 2022;78(5):397-408.
<https://doi.org/10.1007/s10872-021-00603-2>
18. Wang Q, Chen X, Lin L, et al. The dispersal of dinoflagellate cyst caused by international ships under repair conditions: a potential invasion risk to the Yangtze River Estuary, China. Environ Sci Pollut Res. 2023;30(36):86178-86188.
<https://doi.org/10.1007/s11356-023-12292-9>
19. Mwaurah PW, Kumar S, Kumar N, et al. Novel oil extraction technologies: Process conditions, quality parameters, and optimization. Compr Rev Food Sci Food Saf. 2020;19(1):3-20.
<https://doi.org/10.1111/1541-4337.12527>
20. Ok JH, Jeong HJ, You JH, et al. Phytoplankton bloom dynamics in incubated natural seawater: predicting bloom magnitude and timing. Front Mar Sci. 2021;8:681252.
<https://doi.org/10.3389/fmars.2021.681252>
21. Berg H, Belt S, Koole S, Van der Wulp N. Environmental preference and restoration: (How) are they related?. J Environ Psychol. 2003;23(2):23. doi: [https://doi.org/10.1016/S0272-4944\(02\)00111-1](https://doi.org/10.1016/S0272-4944(02)00111-1)
22. Guo Q, Li T, Qu Y, et al. New research development on trans fatty acids in food: Biological effects, analytical methods, formation mechanism, and mitigating measures. Prog Lipid Res. 2023;89:101199.
<https://doi.org/10.1016/j.plipres.2022.101199>
23. Kaseke T, Opara UL, Fawole OA. Fatty acid composition, bioactive phytochemicals, antioxidant properties and oxidative stability of edible fruit seed oil: Effect of preharvest and processing factors. Heliyon. 2020;6(9).
<https://doi.org/10.1016/j.heliyon.2020.e04957>
24. Alazaiza MY, Albahnasawi A, Al Maskari T, Abujazar MSS, Bashir MJ, Nassani DE, Abu Amr SSJE. Biofuel production using cultivated algae: Technologies, economics, and its environmental impacts. Energies. 2023;16(3):1316.
<https://doi.org/10.3390/en16031316>

25. Quero-Jiménez P, Felipe L, López LJ. Oil extraction and derivatization method: A review. Open Access J Sci. 2020;4:110-120.
<https://doi.org/10.13188/2572-4410.1000171>
26. Hossain MS, Mohamed SH, Khalid AMB, et al. Avenues in supercritical carbon dioxide extraction and fractionation of lipids. In: Innovative Food Processing Technologies. Elsevier; 2021:584-596.
<https://doi.org/10.1016/B978-0-12-821311-2.00045-5>