

Bioremediation of Domestic Wastewater Using *Chlorella Sorokiniana* Microalgae and Techno-Economic Feasibility Assessment in Arid Desert Regions

Abdellatif Rahmani^{a*}, Ahmed Tabchouche^b, Abdelkader Hassani^c, Bengrina M. hamza^d and Lahcène Djafer^e

^{a,b}, Univ. Ouargla, Fac. des sciences appliquées, Lab. dynamique interaction et réactivités des systèmes BP 511, Route de Ghardaïa, Ouargla 30000, Algeria

^cDepartment of Chemistry, University of Ouargla, P.O. Box 511, 30000, Ouargla, Algeria

^dEconomics of organizations and environment laboratory, Ouargla, Algeria

^eLaboratoire Eau Environnement, Université Hassiba Ben Bouali, BP 151, 02000 Chlef, Alger

*Corresponding author: dr.rahmani.univ.ouargla@gmail.com

tabchouchahmed@yahoo.fr

hassani.ak@gmail.com

bengrinamohamedhamza@gmail.com

ldjafer@yahoo.fr

Received: 17/06/2023;

Accepted: 22/11/2023

Abstract

This study aimed to investigate the viability of using *Chlorella sorokiniana* microalgae for bioremediation of domestic wastewater and the production of algal biomass that may be used in several applications, as well as the viability of producing microalgae economically in arid regions.

Due to its temperate climate, abundant water supply, and large flat areas, Southern Algeria was an ideal location for growing microalgae. Due to the availability of this species in southern Algeria. In an open pond-style Rasway, 45 litres of domestic wastewater were used to cultivate microalgae. The microalgae *Chlorella sorokiniana* were shown to be quite effective at removing COD, $\text{NH}_4^+ - \text{N}$, TN, and TP by 89.90%, 100%, 97.65%, and 98.91%, respectively. By the end of the experiment, the maximum biomass yield, which was reached after 15 days, was $1.79 \pm 0.01 \text{ g L}^{-1}$. Furthermore, 149 tonnes of microalgae per hectare were harvested annually; the crop's value per hectare is put at 64500 euros.

According to this assessment, the project was feasible, especially if it was larger than ten hectares. The findings showed that *Chlorella sorokiniana* microalgae may be used to treat wastewater and create algal biomass at the same time, which can then be processed and converted into biofuels, natural fertiliser, or animal and fish feed.

Keywords: bioremediation; wastewater treatment; Open pond; *Chlorella sorokiniana*; techno-economic feasibility.

Tob Regul Sci. TM 2023 ;9(2): 1394-1404

DOI: doi.org/10.18001/TRS.9.2.86

Introduction:

Energy in general and the global warming threat in particular have grown to be crucial problems that humanity must deal with in the twenty-first century.

Microalgae was an integrated and promising method for producing biodiesel from microalgae and wastewater treatment [1].

The need for fresh water and energy resources was rising as the world's urban population expanded. The environment was seriously threatened by the large amounts of domestic municipal wastewater that people emitted.

Growing microalgae was a good way to combat environmental issues like ozone depletion, climate change, and global warming because the process of photosynthesis used up a lot of carbon dioxide [2].

Algal biomass was useful for producing biofuels among many other applications [3]. However, the lipid concentrations of microalgae grown on wastewater vary greatly, and lipid extraction technologies are currently in development [4].

Numerous researches have mentioned that microalgae are extremely effective at removing metals and nutrients from different sources of wastewater in order to comply with international standards [5-6].

Due to the low initial cost and utilisation of a free energy source, sun irradiation, microalgae were grown in open ponds [7].

Among the various green microalgae, *C. pyrenoidosa* was frequently utilised for metal adsorption [8], production of biomass for bioenergy utilisation such as biofuel and lipid, and treatment of wastewater [9].

In the overall process of producing biodiesel, the extraction of lipids from microalga biomass was a crucial stage. Microalgae's cell walls are made up of a wide range of materials, including cellulose, chitin, murein, protein, silica, and CaCO_3 .

This research examined the viability of employing *Chlorella sorokiniana* microalgae for bioremediation of domestic wastewater and the production of algal biomass with numerous potential applications.

Materials and Methods

Wastewater and the test site

The research was carried out at the Kasdi Merbah University in Ouargla, Algeria (latitude: $32^{\circ}53'1.44''$; longitude: $6^{\circ}07'22''$). The steps of the experiment were carried out outside in the autumn sun. The daytime temperature was between 19 and 33 °C, and the nighttime temperature was between 8 and 16 °C. The isolation time frame was roughly 11 hours and 15 minutes every day (from 7:15 a.m. to 6:30 p.m.). The maximum irradiation was approximately 1200 W/m². In this investigation, municipal wastewater was collected from station Said Otba in Ouargla, Southern Algeria. Wastewater was filtered to remove suspended matter. A pump was used to maintain constant stirring. .

Biomass growth for inoculation

Chlorella sorokiniana biomass propagation

The identified *Chlorella* strain was cultured in the Dynamique for biomass production. Interaction et Réactivité des Systèmes (DIRS) laboratory at the Faculty of Applied Sciences at Ouargla Algeria University. Using a compound light microscope, single colonies were inspected for purity before being cultured in 10 mL of Bold's Basal Medium (BBM) under the following conditions:

Light intensity $120\text{--}150\text{ photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with mixing at 350 rpm throughout a 24-hour period at $22 \pm 1\text{ }^{\circ}\text{C}$.

After flowering, the culture was examined for any signs of contamination before being used to seed 100 mL of BBM. After that, the culture was ramped up to 500 mL BBM. The pH was equal to 6.8.

The Bold's Basal Medium (BBM) contained KH_2PO_4 8.75 g/500 mL 10 mL and $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ 12.5 g/500 mL 1 mL $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$. NaNO_3 125 g/500 mL 1 mL, $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ 10 g/L 1 mL, K_2HPO_4 37.5 g/500 mL 1 mL, KOH 6.2 g/L, NaCl 12.5 g/500 mL 1 mL, $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ 4.98 g/L, and concentrated H_2SO_4 are all included. 1 mL/L of the trace metal solution view below 1 mL, H_3BO_3 5.75 g/500 mL, 0.7 mL, and 1 mL of the F/2 Vitamin Solution are required.

Analytical methods

The solarimeter (SL200, instrument, France) was used to measure the sunlight irradiance (W/m^2). A multiparameter analyser (Consort C3020, Belgium) was used to measure conductivity (mS/cm), pH, and dissolved oxygen (DO, mg/l). In order measure the growth of the microalgae, a UV 2300 Spectrophotometer was used to measure the optical density at 680 nm ($\text{OD}_{680\text{nm}}$) on a daily basis. The $\text{OD}_{680\text{nm}}$ associated to suspended solids (SS) in wastewater, on the other hand, was deducted from the overall OD 680 nm (with biomass). By subtracting the biomass in the control experiments from the overall biomass, The algal biomass's final concentration was calculated. The total volatile was calculated using algae biomass [10].

Following DIN 38,049-4, COD was evaluated using cuvette test kits LCK 514 (100–2000 mg COD/L).

LCK303 ammonium-N ($\text{NH}_4 + \text{-N}$) kits (2.0–47.0 mg/L $\text{NH}_4 + \text{-N}$) and nitrate-N ($\text{NO}_3\text{-N}$) kits were utilised in accordance with DIN38406-E5-1 standards.

LCK 339 (0.23–13.50 mg/L $\text{NO}_3\text{-N}$) was utilised in accordance with DIN 38,402-1 A51 standards, and total phosphorus (TP) was accomplished in accordance with ISO 6878-1-1986 standards, DIN 38,405 D11-4, and test kit LCK 349 ranges of $\text{PO}_4\text{-P}$ (2–20 mg TP/L) [11].

The absorbance was measured using a spectrophotometer DR 2800 (Hach Lange, Germany). For COD, however, all samples were heated for 2 hours at $148\text{ }^{\circ}\text{C}$.

Before being put in the spectrophotometer, the sample was examined for 60 minutes at 100°C for TP measurement. An atomic absorption spectrophotometer (AAS Varian, Walnut Creek, CA). was used to determine the heavy metal content.

Where Si and Sf are the concentrations of nutrient/pollutant in wastewater before and after algae cultivation.

Data analysis

The tests were conducted in triplicate, and GraphPad Prism version 6 was used to create the graphs.

Results

Outdoor culture in open raceway ponds

A galvanised open raceway pond measuring 100 cm long, 45 cm wide, and 20 cm deep was used for the outdoor experiments.

A 10% algal suspension (v/v) of the working volume (50 L) was used to inoculate open raceway.

The starting cell concentrations in the inoculum were 7.7×10^6 cells/ml.

Experiments were run for 15 days under external conditions. A stirring system was used to achieve the culture medium's mixing and recirculation.

Characterization of Wastewater before and after algae treatment

The characteristics of the wastewater were analysed in order to identify the concentrations of nutrient.

The physico-chemical parameters of the wastewater are given in Table 1.

Table.1 Wastewater characteristic before/after algae

Characteristic	Raw ww	Algae treated ww	%
PH	7.70	9.51	/
Conductivity(mS/cm)	7.85	11.36	/
Salinity (g/L)	4.25	6.60	/
DO (mg/L)	1.29	9.15	/
COD (mg/L)	500	50.5	89.90%,
NH ₄ ⁺ -N (mg/L)	40.8	0.1	100%
NO ₃ ⁻ -N (mg/L)	1.20	14.65	/
TP (mg/L)	3.89	0.05	98.71%,
TN (mg/L)	42.43	0.08	99.81%

$$\text{Removal (\%)} = \left(\frac{Si - Sf}{Si} \right) \times 100 \quad (1)$$

Microalgae Growth

The results of biomass production are shown in Figure 1.

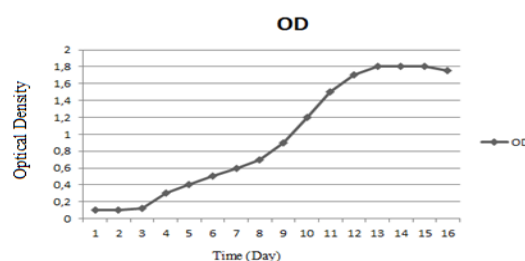


Figure.1. Variation of the optical density during the culture period

The average biomass productivity showed a high yield of 1.79 ± 0.01 g/L. Some studies in the climatic conditions indicate similar results 1.71 ± 0.04 g/L (Dahmani et al. 2016). 1.15 ± 0.07 g/L (Rahmani et al. 2022).

Elimination of nutrient

After cultivating the algae, COD and nutrient removal levels were used to assess the potential of the isolated microalgae.

The physicochemical characteristics of raw WW were presented in Table 1. The Results showed that NH_4^+-N was efficiently eliminated with a removal rate of 98.99%. NH_4^+-N was preferentially used over other forms of nitrogen and was completely consumed near the end of the experiment.

The process involved oxidation to NO_2^--N and then oxidation of NO_2^--N to NO_3^--N , with NO_3^--N being consumed after the ammonia N level approaches zero.

Discussion

According to [13], the wastewater utilised in the experiment had nutrient concentrations that ranged from a medium to strong level. Concentration of phosphorus was found sufficient to support algal growth. It was discovered that the phosphorus concentration was adequate to support algal growth. The wastewater had a pH of 7.70, which was roughly neutral.

Microalgae growth was directly affected by the availability of nutrients, light, temperature, and the initial inoculation density [14].

The microalgae culture would see a shift in nutritional profile when the microalgae were moved from the Bold's Basal Medium (BBM) to the municipal wastewater.

As a result, the microalgae's growth rate was slow for the first three days as they adapted to the wastewater. After the lag period, there was a period of rapid growth for the following 4–7 days, followed by an exponential phase lasting from day 8 to day 13, during which the culture density reached the highest level.

The high pH values at that time period are an indication of intense photosynthetic processes during the exponential phase (Figure 1). In the 14–15 days of the experiment, the culture gradually reached stationary phase. This indicated that the number of microalgae that were born and those that died was equal. And after the sixteenth day, it starts to decrease.

Chlorella and *Scenedesmus* species have been the subject of much research into wastewater treatment [15–17]. The findings showed that cultivation algae in wastewater with sufficient levels of nitrogen and phosphorus could lower the cost of biomass production by removing the need for any external nutrients.

The higher removal rate of ammonium was explained by the less energy required for its uptake [14]. From treated wastewater, *Chlorella* and *Scenedesmus* species may remove 80% to virtually 100 % of the ammonium [18].

However, the effectiveness of the treatment varied depending on the type of wastewater and the climate conditions [19].

The outcomes of this study demonstrated that microalgae cultivation was suitable for the efficient nutrient removal from wastewater. Chemical oxygen demand (COD), as demonstrated by Bhatnagar et al. [20], was employed to measure the amount of organic chemicals present in wastewater.

The COD removal rate was 89.90%. The reduction of COD indicated that microalgae could utilize organic carbon as a source of energy and a substrate for cell growth besides carbon dioxide CO_2 [14]. The wastewater used in the experiment contained bacteria.

Microalgae and the wastewater's indigenous bacteria, which consumed microalgae, have a synergistic and integrative connection. Oxygen (O_2) was created by microalgae during photosynthesis, while carbon dioxide (CO_2) was emitted when bacteria break down organic material in wastewater. This leads to improved COD elimination by bacteria and greater algal growth [21].

The ability of microalgae to absorb inorganic nitrogen and phosphorus was well known. The N/P ratio and starting nutrient concentration are regarded as important parameters influencing algal development and nutrient uptake efficiency.

It was suggested that the ideal N/P ratio for algal development was between 6.8 and 10. The N/P ratio in raw WW (10.52) in the current investigation was more than the ideal ratio, indicating P limitation. Raw WW had a PH of 7.70, which subsequently increased to 9.51. This was primarily due to photosynthetic activity. Because microalgae use CO_2 , the pH raised from acidic to alkaline when CO_2 levels fall.

Figure 2 showed that the dissolved oxygen DO increased during the experiment.

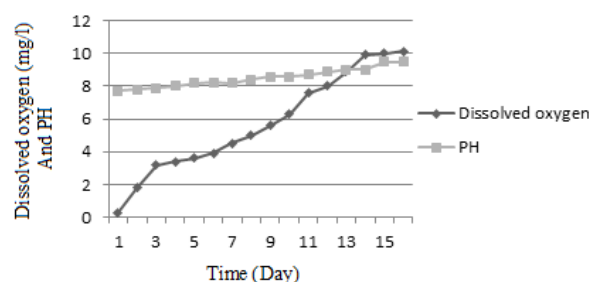


Figure.2 Dissolved oxygen and pH fluctuated during the culture stage.

On the first day, the lowest possible level of dissolved oxygen was 1.29 mg/L. This was due to organisms like bacteria depleting the dissolved oxygen in sewage. During the culture stage, the concentration gradually increased to an approximate concentration of 9.15 mg/L. According to the findings, dissolved oxygen (DO) increased with the proliferation of microalgae. Microalgae photosynthesis was used to release dissolved oxygen in the culture medium.

The Results showed that NH_4^+-N was efficiently eliminated with a removal rate of 98.99%. Completely consumed towards the end of the experiment, NH_4^+-N is preferentially utilized rather than other forms of nitrogen.

The process involves oxidation to NO_2^--N followed by oxidation of NO_2^--N to NO_3^--N , which will be consumed once the ammonia N reaches zero.

The higher removal rate of ammonium is explained by the less energy required for its uptake [23]. *Chlorella* and *Scenedesmus* species are able to remove 80% to almost 100% of ammonium from treated wastewater [24].

Nevertheless, the efficiency of the treatment changes according to wastewater type as well as climate conditions [25].

The changes of TN and TP were significantly. The final TP removal efficiencies were 98.71% and TN 99.81%

The results of this experiment showed that microalgae culture is adequate for the effective removal of nutrients from wastewater.

This indicates [22] that microalgae need phosphorus and nitrogen to grow, and that the presence of bacteria contributes to consuming these two elements. The TP in both RW and AW was reduced.

The final TP removal efficiencies in RW and AW were 83% and 69% and TP (RW 81%, AW 25%). using *chlorella pyrenoidosa*.

A study of the technical and economic feasibility of biological treatment

In this work, the costs of a *sorokiniana* microalgae production process for municipal wastewater treatment and biomass production in an arid desert environment were determined using a technical-economic evaluation. If the average value was the price of a kilogramme of microalgae on the global market, which was 5 euros [12].

The profit is calculated with the following relation:

$$\text{Return} = \text{Income} - \text{Cost} \quad (2)$$

Financial aspects

The following costs were studied:

Investment costs (civil engineering and electromechanical equipment) and operating costs (staff, energy, and maintenance) and annual cost price.

The lifespan of the basins was 25 years; the lifetime of the equipment was 15 years,

The average evaporation rate over the year was 35%. The yield decreased by 50% in four months of the year.

Maintenance expenses can be computed using the investment costs for building the station.

The percentages used are 0.5% per year for civil engineering and 1% per year for electromechanical equipment. The findings of this study's techno-economic analysis are reported in Tables 2, 3, and 4.

Microalgae cultivation

In this project, we used a 1 hectare area to cultivate microalgae; one open pond has a capacity of (3050.2) m³ and was supplied with a pump to circulate the culture. Photovoltaic panels generated electricity during the day and the electrical network at night. Table 2 contained the data.

Table. 2 Incomes

Incomes	(€) euros
Microalgae mass price/ha/year	69 500
Price of treated water per/ha/year	10 000
Total	795 00

Table .3: Fixed costs (FC).

Item	Item cost (€) euros	% of FC
Land	free	
The cost of basins	20000	19.29
The cost of pumps	4000	3.85
The greenhouse	20000	19.29
Solar panels (monocrystalline type)	7000	6.76
Electricity	1000	0.96
Equipment	20000	19.29
Prefabricated building (laboratory).	10000	9.64
Cost of workers per year	19200	18.51
Insurance	500	0.48
Contingency	2000	1.93
Total	103 700	

Table .4 Variable costs (VC)

Item	Item cost (€) euros	% of VC
Maintenance and repair	728,5	26.67
Transport	2000,0	73.33
Total	2 728,5	

Harvest

Harvesting was accomplished in two ways:

The first method involved using special porous bags and pressing them to create a microalgae paste.

The second method was an electrochemical method (électroflotation) that employed a photocell. When the density of microalgae was high, we applied the first approach first, and then switch to the second method when the density was low [11].

Drying

Expose the paste to the sun to dry it so that the final product was (in the form of powder).

We estimate the algal mass to be 139 tan/ha/year, which was equivalent to 695000 euros/ha/year.

Conclusion

The results showed that *Chlorella sorokiniana* can be grown successfully in desert regions on wastewater in open pond systems using direct sunlight. Algal treatments had better nutrient removal efficiency (100% NH_4^+-N , 89.90% COD, and 98.71% TP; 99.81% TN). The average biomass productivity was high, with a yield of 1.79 ± 0.01 g/L.. We can harvest 139 tonnes of microalgae per acre per year. This crop was projected to be worth 695000 euros per hectare per year. The project costs 106 428,5 euros per hectare, and the revenue per year per hectare was 695000 euros, which means that after a year and four months, the expenses are recovered, and we solely reap the profits.

Acknowledgement

I am truly thankful and incept to the technical staff of the Faculty of Applied Sciences at Ouargla Algeria University.

References

- [1] Penghui, W. , Z.Lei , C.Haixing , X.Guoli, and L. Mengxue. 2022. " Investigation of hydrogen peroxide-driven transcriptional stress on the biomass growth of *Chlorella pyrenoidosa*". *Algal Research* 1(68): 1-25 <https://doi.org/10.1016/j.algal.2022.102897>
- [2] Al Darmaki,A., L. Govindrajan, T.Sahar, A.Sara, A.Tahir , and A.Zainab. .2012. "Cultivation and Characterization of Microalgae for Wastewater Treatment". *Proceedings of the World Congress on Engineering* 1(1): 1-4
- [3] Lam, M.K., Khoo, C.G., and Lee, K.T. 2019 "Scale-up and commercialization of algal cultivation and biofuels production". *Biofuels from Algae (Second Edition)*.1(1): 475–506. <https://doi.org/10.1590/1678-4324-2019160816>.
- [4] Woertz, I., A. Feffer, T. Lundquist, and Y. Nelson. 2009. "Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Biofuel Feedstock." *Journal of Environmental Engineering* 135 (11): 1115–1122. [doi:10.1061/\(ASCE\)EE.1943-7870.0000129](https://doi.org/10.1061/(ASCE)EE.1943-7870.0000129).
- [5] Santiago, A.F., M.L.Calijuri, P.P.Assemany, M.D.C.Calijuri, and A.J.Delgado dos Reis. 2013. "Algal Biomass Production and Wastewater Treatment in High Rate Algal Ponds Receiving Disinfected Effluent." *Environmental Technology* 34 (13-16): 1877–1885.[doi:10.1080/09593330.2013.812670](https://doi.org/10.1080/09593330.2013.812670)
- [6] Pacheco,M.M., M. Hoeltz, M.S.A. Moraes, and R.S.C. Schneider. 2015. "Microalgae: cultivation techniques and wastewater phycoremediation." *Journal of Environmental Science and Health. Part A, Toxic/hazardous Substances & Environmental Engineering* 50 (6): 585–601. [doi:10.1080/10934529.2015.994951](https://doi.org/10.1080/10934529.2015.994951).
- [7] Winckelmann,D. F.Bleeke, B. Thomas, C. Elle, and G.Klok. 2015. "Open pond cultures of indigenous algae grown on non-arable land in an arid desert using wastewater." *International Aquatic Research* 7 (2): 221–233. [doi:10.1007/s40071-015-0107-9](https://doi.org/10.1007/s40071-015-0107-9).
- [8] Zhou, G.J, F.Q.Peng,L.J.Zhang, and GG Ying GG. 2012." Biosorption of zinc and copper from aqueous solutions by two freshwater green microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*". *Environmental Science and Pollution Research* 7(19):2918–2929. [https:// doi. org/ 10. 1007/s11356- 012- 0800-9](https://doi.org/10.1007/s11356-012-0800-9)
- [9] Qin, L., B.Wang ,P. Feng , Y.Cao, Z.Wang, and S. Zhu.2022. "Treatment and resource utilization of dairy liquid digestate by nitrification of biological aerated filter coupled with assimilation of *Chlorella pyrenoidosa* ".*Environmental Science and Pollution Research* 3 (3): :3406–3416. [https:// doi. org/10. 1007/ s11356- 021- 15903-1](https://doi.org/10.1007/s11356-021-15903-1)

- [10] Ma, X., W.Zhou, Z.Fu , Y.Cheng,M. Min, Y.Liu,Y. Zhang, P.Chen , and R.Ruan R .2014. Effect of wastewater-borne bacteria on algal growth and nutrients removal in wastewater-based algae cultivation system. *Bioresour Technol* 167(2):8–13.
[https:// doi. org/ 10. 1016/j. biort ech.2014. 05. 087](https://doi.org/10.1016/j.biortech.2014.05.087)
- [11] Rahmani, A., D. Zerrouki, A.Tabchouche, and L.Djafer L. 2022. "Oilfield-produced water as a medium for the growth of *Chlorella pyrenoidosa* outdoor in an arid region ": *Environmental Science and Pollution Research*. 29(3): 87509–87518 <https://doi.org/10.1007/s11356-022-21916-1>
- [12] Ruiz, J., G.Olivieri, J.D.Vree , and R.Bosma. 2016. "Towards industrial products from microalgae". *Energy & Environmental Science* 9(4): 3036-3043 DOI: 10.1039/C6EE01493C
- [13] Rawat, I., R. Kumar, T. Mutanda, and F. Bux. 2011. "Dual Role of Microalgae : Phycoremediation of Domestic Wastewater and Biomass Production for Sustainable Biofuels Production." *Applied Energy* 88 (10): 3411–3424.
[doi:10.1016/j.apenergy.2010.11.025](https://doi.org/10.1016/j.apenergy.2010.11.025).
- [14] Ding, J., F. Zhao, Y. Cao, L.Xing, W.Liu, S.Mei, and S. Li. 2015."Cultivation of Microalgae in Dairy Farm Wastewater without Sterilization." *International Journal of Phytoremediation* 17 (3): 222-227.[doi:10.1080/15226514.2013.876970](https://doi.org/10.1080/15226514.2013.876970).
- [15] Ramanna, L., A. Guldhe, I. Rawat, and F. Bux. 2014. "The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources." *Bioresour Technol* 168(2): 127–135. [doi:10.1016/j.biortech.2014.03.064](https://doi.org/10.1016/j.biortech.2014.03.064)
- [16] Lizzul, A. M., P. Hellier, S.Purton, F. Baganz, N. Ladommatos, and L. Campos. 2014."Combined remediation and lipid production using *Chlorella sorokiniana* grown on wastewater and exhaust gases." *Bioresour Technol* 151(3): 12–18.
- [17] Ebrahimian, A., H.R.Kariminia, and M.Vosoughi. 2014. "Lipid production in mixotrophic cultivation of *Chlorella vulgaris* in a mixture of primary and secondary municipal wastewater." *Renewable Energy* 71 (4): 502–508. [doi:10.1016/j.renene.2014.05.031](https://doi.org/10.1016/j.renene.2014.05.031).
- [18] Pittman, J.K., A.P.Dean, and O.Osundeko. 2011. "The potential of sustainable algal biofuel production using wastewater resources." *Bioresour Technol* 102 (1): 17–25. [doi:10.1016/j.biortech.2010.06.035](https://doi.org/10.1016/j.biortech.2010.06.035)
- [19] Koreivienė, J., R.Valčiukas, J.Karosienė, and P.Baltrėnas. 2014. "Testing of *Chlorella*/*Scenedesmus* microalgae consortia for remediation of wastewater, CO₂ mitigation and algae biomass feasibility for lipid production." *Journal of Environmental Engineering and Landscape Management* 22 (2): 105–114.
[doi:10.3846/16486897.2013.911182](https://doi.org/10.3846/16486897.2013.911182)
- [20] Bhatnagar, A., S.Chinnasamy, M.Singh, and K. C. Das. 2011. "Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters." *Applied Energy* 88 (10): 3425–3431.
[doi:10.1016/j.apenergy.2010.12.064](https://doi.org/10.1016/j.apenergy.2010.12.064)

- [21] Prajapati,S.K., P.Kaushik, A.Malik, and V.K.Vijay.2013. “Phycoremediation and biogas potential of native algal isolates from soil and wastewater”. *Bioresource Technology* 135 (2): 232–238.doi:10.1016/j.biortech.2012.08.069.
- [22] Dahmani S, Zerrouki D, Ramanna L, Rawat I, Bux F (2016) Cultivation of *Chlorella pyrenoidosa* in outdoor open raceway pond using domestic wastewater as medium in arid desert. *Bioresour Technol* 219:749–752. [https:// doi. org/ 10. 1016/j. biort ech. 2016. 08. 019](https://doi.org/10.1016/j.biortech.2016.08.019).
- [23] Ding, Jinfeng, Fengmin Zhao, Youfu Cao, Li Xing, Wei Liu, Shuai Mei, and Shujun Li. 2014. “Cultivation of Microalgae in Dairy Farm Wastewater without Sterilization.”*International Journal of Phytoremediation*, no. March 2014: 140221132517006. doi:10.1080/15226514.2013.876970.
- [24] Pittman, Jon K, Andrew P Dean, and Olumayowa Osundeko. 2011. “The Potential of Sustainable Algal Biofuel Production Using Wastewater Resources.” *Bioresource Technology* 102 (1): 17–25. doi:10.1016/j.biortech.2010.06.035.
- [25] Koreivienė, Judita, Robertas Valčiukas, Jūratė Karosienė, and Pranas Baltrėnas. 2014. “Testing of *Chlorella*/*Scenedesmus* Microalgae Consortia for Remediation of Wastewater, CO₂ Mitigation and Algae Biomass Feasibility for Lipid Production.” *Journal of Environmental Engineering and Landscape Management* 22 (2): 105–114. doi:10.3846/16486897.2013.911182