

USE ALGA CHLORELLA SOROKINIANA BE REDUCED CARBON DIOXIDE AND PROTEIN PRODUCTION

Ahmed Aidan Al-Hussieny¹ , Mohammed Sadeq Salman² , Mohammed Mohsin Shallal³

Ministry of Science and Technology, Baghdad, IRAQ¹

Av-cenna E-Learning center University of Baghdad ²

AL-Nisour University College Department of Pharmacy, Baghdad-IRAQ³

Email: ahmed.edan85@gmail.com, Email:m.sadiq@uobaghdad.edu.iq

Abstract

A strain of the algae called *Chlorella sorokiniana* was used for the experiment. database of the human genome (accession No. MH923013.1), *Chlorella sorokiniana* was shown to be highly effective in reducing CO₂ gas through the treatment time of the gas, with reductions reaching 92.94% after 3 days from the start of the study for the highest level of gas, which is 25 L / min, which is equivalent to 6000 mg / l, when grown in Nitrogen Phosphorus Potassium (NPK) culture medium over the course of a 24-day laboratory study. In addition to the chemical contents shown by the protein proportions, *Chlorella sorokiniana* biomass also produced an exponential increase in the uniform optical density of the mass of *Chlorella sorokiniana* within the five gas levels, which peaked at 0.294, 0.311, 0.345, 0.431, and 0.511 nm, respectively, when compared to the adult control. As of culture day 24, we have a reading of 0.098 nm.

Keywords : protein , carbon dioxide , Nitrogen Phosphate Potassium (NPK) , and algae.

Introduction

Ozone depleting substance outflows by ventures and human exercises make the planet hotter. While, A report of the Intergovernmental Board on Environmental Change (IPCC) fourth Appraisal in 2007 demonstrates that an ascent in discharges could prompt a temperature expansion in the scope of 4-7°C, with significant effects on the climate and human movement. Likewise, It is generally concurred that portion of the energy-related CO₂ discharges are diminished by 2050, through the high temperature is declined to under 3 degrees. Hence, this accomplishes by an energy innovation transformation including expanded energy effectiveness, expanded sustainable power sources, atomic power, and the decarbonization of force age from petroleum products [1].

While CO₂ discharging ventures are adding to the primary job in the ascent of ozone harming substances in the environment for a long time. So the biggest wellspring of ozone depleting substance emanations from human activities on the planet, which is singed non-renewable energy sources for heat, power, transportation, and homegrown utilization. Thusly, carbon confinement, catching, and putting away carbon set free from the overall energy framework could be the principle apparatus for lessening environmental CO₂ focus [2].

Green growth can move bicarbonate into cells, empowering them to catch carbon dioxide. These capacities of green growth guarantee the progression of the development of the natural mass by holding onto carbon dioxide from the gases discharged through the applied organic frameworks

and working to persistently and for all time diminish the CO₂ gas outflows and nitrogen oxides by the photosynthetic reactors subsequent to utilizing the underlying period of sea-going plants and green growth enduring sharpness and restricting exercises. Also, the HR that increment gas discharges. Unicellular green growth are self-taking care of living life forms by photosynthesis and produce energy from carbon dioxide and water within the sight of green matter and daylight. These single green growth contain a lot of protein. Microalgae have extraordinary potential as sustainable fuel sources since they have a quick development rate and the capacity to product excellent of the protein [2].

The growth of microalgae and other marine organisms is being considered as a potential source of protein. Certain marine plants and microalgae have been found to rival the protein content of common food sources such meat, eggs, soy products, and milk. [3]; [4]. Using green growth for protein production provides certain efficiency and health benefits over using traditional high-protein crops. Comparatively, the protein output from seaweed and microalgae is higher per area (2.5-7.5 tons/Ha/year and 4-15 tons/Ha/year, respectively). [5].

Along these lines, the ongoing review expects to lessen carbon dioxide by utilizing a green growth and to deliver protein from algal biomass.

Materials and Methods

Diagnosis, isolation and development of algae:

The current research utilized the microalgae (*Chlorella sorokiniana*). The genetic diagnosis made by the researcher can be found in the GenBank database (accession number MH923013.1) [6]. They were cut off from contact with water and had to rely on doctors for diagnosis[7], [8]. Isolates of algae were cultivated using regional nitrogen, phosphorus, and potassium (NPK) under carefully managed circumstances. We're talking about the aquatic ecosystem in Iraq. [6](Fig 1 a , b).

Division: Chlorophyta

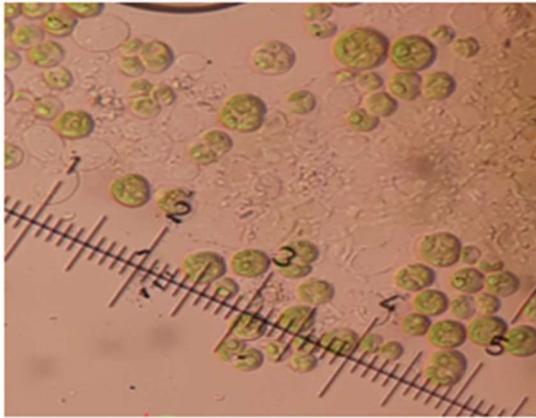
Class : Chlorophyceae

Order : Chlorococcales

Family : Chlorococcaceae

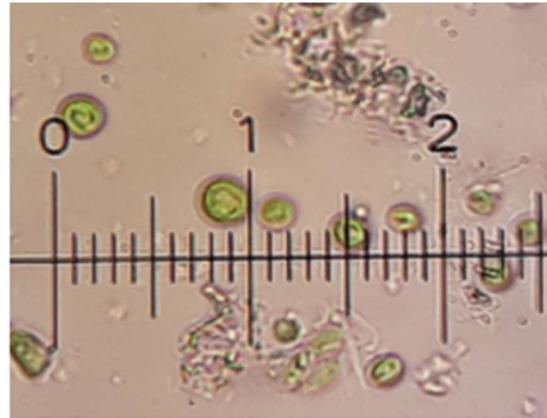
Genus : Chlorella

Species : Chlorella sorokiniana



Species : *Chlorella sorokiniana*

-b-



Genus : *Chlorella*

-a-

Fig(1a and b):Chlorella sorokiniana strain C3-ITS GenBank:MH923013.1

Laboratory experiments:

Microalgal isolates were grown in NPK and compared to a control culture grown with varying CO₂ flow rates (5, 10, 15, 20, and 25 L/min). The microalgae culture in each of the treatments is 10 liters. Every farm also had an air pump to ensure the gas was fully dissolved, and data on pH and lighting levels was kept. Chlorella sorokiniana, as demonstrated in the figures,.

Optical density:

Over the course of 24 days, the optical density of the microalgal culture was tracked using a spectrophotometer set to a wavelength of 680 nm in order to track the growth stages, achieve stability, and prepare for harvest.

There was a full set of duplicate measurements taken.

The growth rate (K) were calculated according to following equation [9].

$$K = \frac{(\text{Log } OD_t - \text{log } OD_0)}{t} \times 3.322$$

t: time (days)

OD_t: Growth after (t) days.

OD₀: algal growth at zero time.

Dissolved oxygen and pH

One of the most important positive data for microalgal farms is the primary productivity of algal cultures culture represented by the oxygen produced from bioprocess. This was achieved by

using oxygen dissolved device as well as following up the cultures by examining the pH as can be seen in the fig (2).

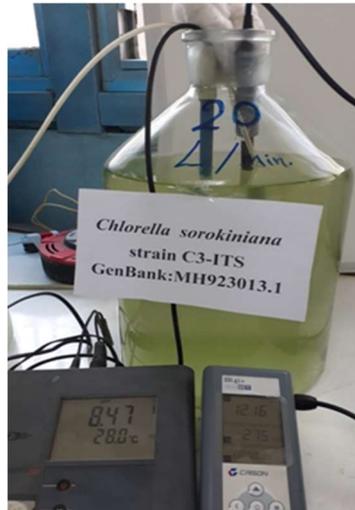


Fig (2): Examination of the productivity of microalgae farms through dissolved oxygen produced from algae while monitoring the pH of each culture .

Harvest experience

After 24 days of the trial starting, the farms were harvested, using 3000 rpm/min of time 15 min concentrated centrifugation, [6] The harvested biomass was dried in an oven at 40 ° C for 2 days [10], completely dried algae powder was stored in an opaque, well-closed container in the refrigerator, and processed to extract proteins Fig (3).

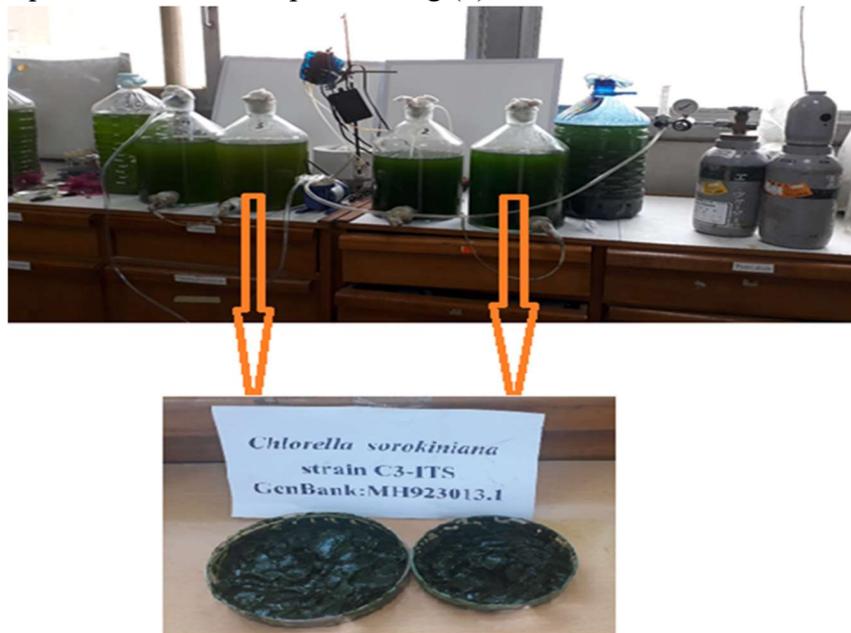


Fig.(3): Biomass of *Chlorella sorokiniana* after harvesting for culture .

Extraction of chemical contents

Proteins extraction

Protein content was estimated using the Keldahl method, as described in [11], which entailed placing a known weight of biomass (0.2 g) in a flask, adding 5 ml of sulfuric acid, and adding an appropriate amount of potassium sulfate and copper sulfate mixture. By raising the temperature, the contents were digested. The mixture was digested, and the result was a transparent, light blue liquid. The liquid was transported quantitatively to a Keldal distillation flask containing a 40% sodium hydroxide solution, and from there to a test tube immersed in a receiver flask containing a known volume of boric acid (20%), plus drops of red methyl index and the bromocresol blue. When the desired volume of distilled liquid has been collected in the distillation flask (often around 25 ml), the liquid is titrated with hydrochloric acid and the results are recorded (0.1 normality). It was decided to produce a blank solution (one that consists of all components except the sample). The following formula can be used to determine the protein proportion:

$$\text{Pr \%} = \left(\frac{V \times \text{ST} \times 0.014 \times 6.25}{W_s} \right) \times 100$$

Where, Pr%: Protein percentage

V: Volume of HCL consumer

ST: Standard

WS: Sample weight

Discussion and Result

Optical Density

All that verdant expansion Five different communities were set up with pneumatic machines to address different CO₂ gas rates, with the rates being 5, 10, 15, 20, and 25 L/min, and a sixth community served as a control without CO₂. At 680 nm, the optical thickness was calculated once per day for 24 days across all civilizations using the control culture. The lifestyle that utilized a siphoning rate of 25 L/min of gas had the highest growth rate compared to the other societies as measured by the striking increase in the microalgal living mass, followed by the other of the stream rates, which were addressed at 20, 15, 10, and 5 L/min. The control culture, as should be evident from Fig (4).

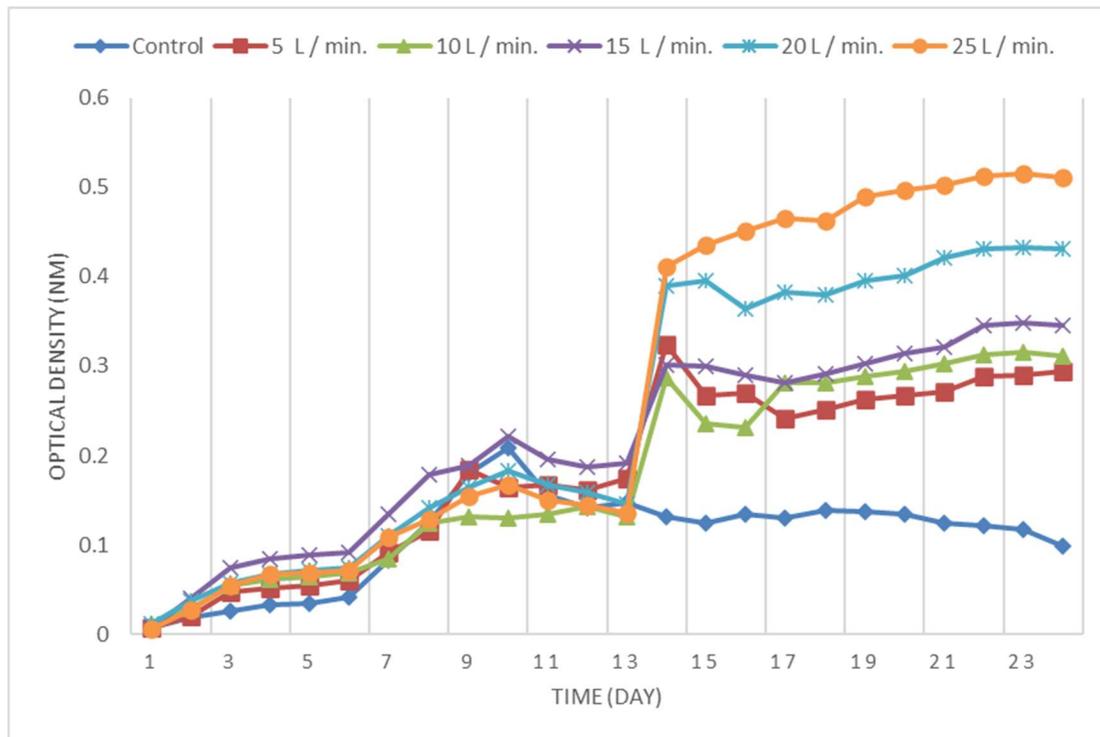


Fig (4): Chlorella sorokiniana optical density at varying CO2 concentrations

The transition of algal societies to lifestyle media equipped with gas was observed after the green development cycle inside the lifestyle outfitted with CO2 gas, and it was found that different cultures use gas at wildly different rates. A study [12] on cultivating Scenedesmus and Chlorella green growth in a culture medium with a high Centralization of CO2 confirmed this. The Analysis [13] additionally shows the duplication of algal societies and their transformation to the climate furnished with gas, Using a vacuum system to ensure the homogeneity of the farming medium, we created an environment with optimal temperature, light intensity, pH levels, and total surface area for the stable growth of our culture. A green growing cell, such as Chlorella sp., represents the realization that plants may absorb vast quantities of CO2 gas through their surface area. [14]

As a matter of fact, the catalyst frameworks spent significant time in the external divider play a part in directing the passage of groceries addressed by broke down salts of phosphates, nitrates and carbon sources, for their utilization in building layers, proteins and fats as well as building the external divider [15], with the suitable climate conditions for them with supplements that produce an outstanding expansion in biomass development [15]. Concerning the review [16], which demonstrated that the external mass of Chlorella vulgaris had a profoundly touchy Glutaminase compound to draw in carbon sources from the supplement media, notwithstanding the presence of cellulose supporting the external divider, as well as the presence of gatherings of genotypes that increment the living mass inside high paces of the sum or centralization of carbon dioxide [14]. For media-wealthy in essential materials, (for example, the media of the ongoing review NPK, which was outfitted with gas with a vacuum apparatus to guarantee dissolving gas) is a significant job in the speed of variation and development, which made the supplement medium a medium

immediately consumed by green growth through the dramatic increment that the review came to with a particular time.

pH

The pH values are one of the significant tests for crafted by green growth societies, as they affect expanding the development of green growth. Later, the consummation of the most common way of refined algal densities for each societies and setting them up with gas levels, the farmer's checking of the pH values factors started with the Control societies. As it came to in somewhere around 24 days for societies outfitted with CO₂ gas between (7.5-8.65), (7.32-8.64), (7.31-8.61), (7.32-8.79) and (7.43-8.65) L/min, individually, contrasted with the control adding up to (7.5-8.5). (Figure 5):

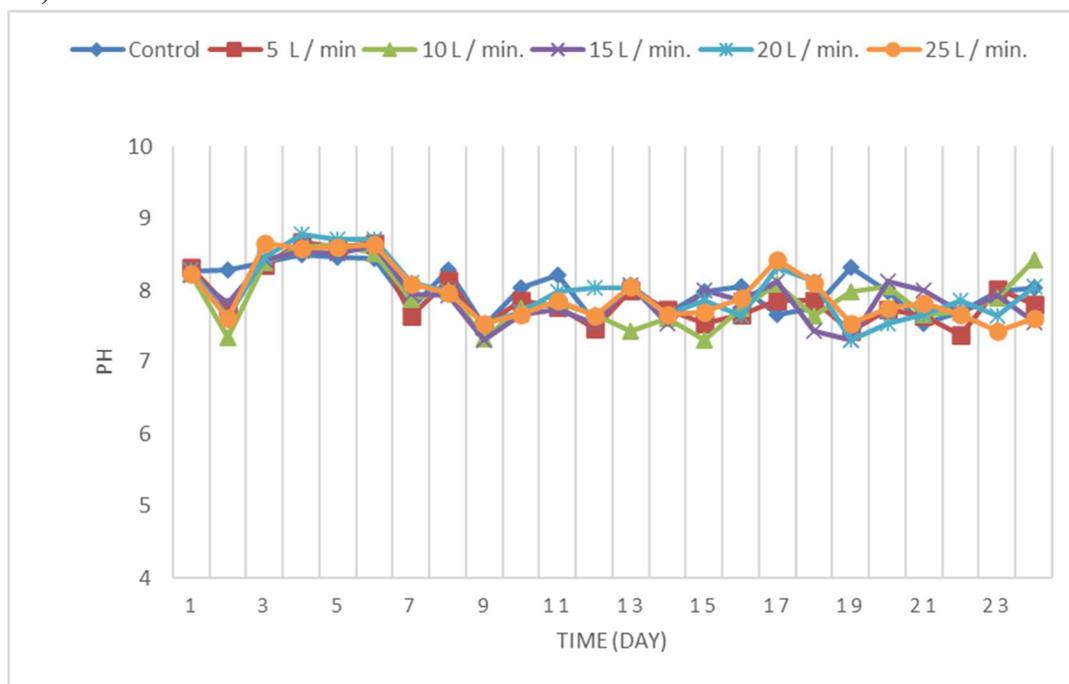


Fig (5) PH values of *Chlorella sorokiniana* cultivars with five different levels of CO₂.

The pH values are critical in the development and development of microalgae in light of the fact that they decide the dissolvability of minerals and carbon dioxide in the medium notwithstanding its impact on the biomass or the algal cell itself [17]. The pH influences a few things connected with algal societies, including the development and brief stockpiling of limit inside the cell, how much broke down carbon dioxide, the temperature and the metabolic movement of the cells [18]. *Scenedesmus* sp., and its relation to pH throughout development. The pH range studied was from 5 to 11, and the results showed that kelp development was not significantly different at pH levels of 7, 9, or 11. Notwithstanding, at pH 5, there was a huge limit in the development of microalgae because of the way that the pH variety was directed with the air circulation of the algal societies via air siphoning (0.03% carbon dioxide). Concerning the combination with carbon dioxide with the other different gases in the air, the pH upsides of the medium containing the algal cells are decreased [19].

Primary productivity of *Chlorella sorokiniana* for dissolved oxygen.

Disintegrated oxygen alignment means that the photosynthetic movement of green growth, being one of the significant essential items. The ongoing review kept oxygen fixations in *Chlorella sorokiniana* refined all through the 24-day concentrate on period. It was recorded between 10.02 - 12.07 mg/L for a stream pace of 5 L/min of CO₂ gas and 10.09-12.15 mg/L for a stream pace of 10 L/min and 10.74 - 12.33 mg/Liter for a stream pace of 15 liter/minute, 10.82 - 12.37 mg/liter for a stream pace of 20 liter/minute lastly 10.87 - 12.97 mg/liter for a pace of 25 liters/minute, contrasted with a control culture that had oxygen focuses from 9.41 - 11.98 mg/L (Fig. 6).

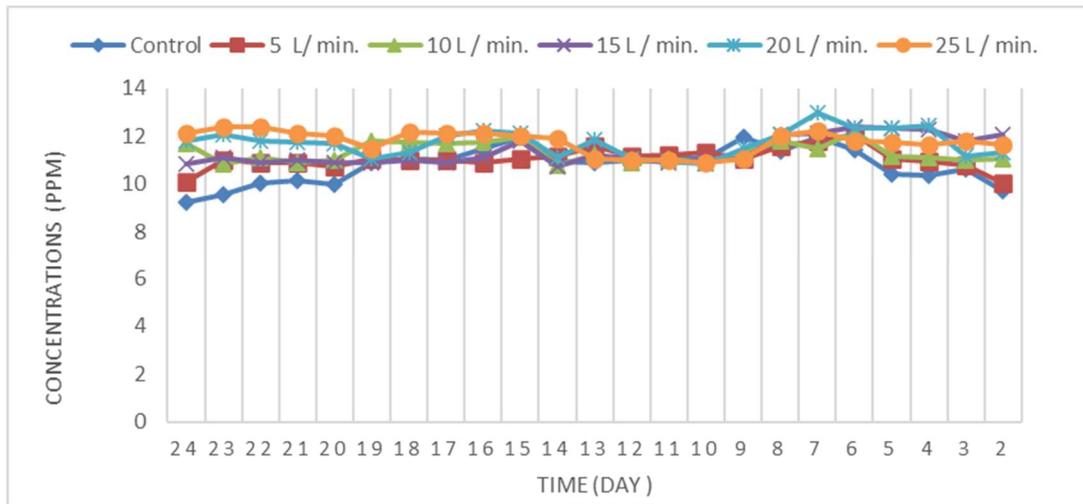


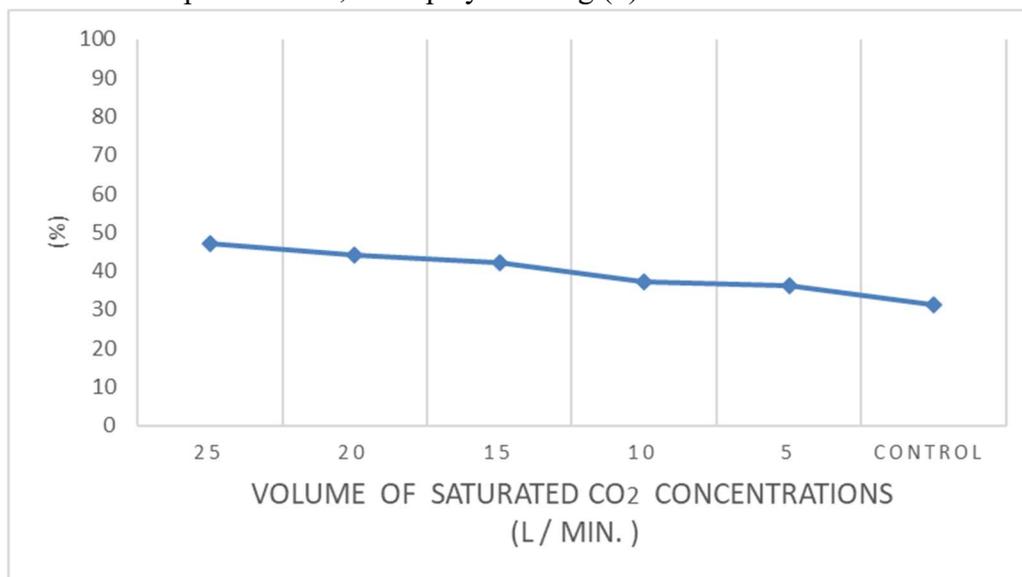
Fig. (6): Dissolved oxygen concentrations of *Chlorella sorokiniana* cultivars grown in the laboratory equipped with different rates of CO₂.

The Centralizations of broken up oxygen created by the microalgal societies, which develop and duplicate with various stream paces of CO₂ gas, increment with the feeding society media to expand the development pace of the algal living mass and increment the creation of oxygen and energy. The broke up oxygen creation might surpass 25% inside green growth societies during the day [20], [21]. Through the presence of huge amounts of CO₂ gas in green growth societies, the most common way of adjusting broke down CO₂ and delivering O₂ is a significant system. Counting the arrangement of the surface region of the way of life, through which the disintegration of CO₂ and its wide spread inside the algal societies. Notwithstanding the time of openness to daylight during the day and the wealth of fundamental supplements, this prompts a decrease of gas with a high efficiency of oxygen and energy [22].

Biochemical investigations of the Biomass of *Chlorella sorokiniana*

Sugars, lipids, proteins, glucose, cellulose, polyunsaturated unsaturated fatty acids, pigments, and food additives are only some of the many useful components found in the green growing biomass. Notwithstanding the presence of cancer prevention agents, drug materials and composts [23]. *Chlorella* sp. is one type of microalga that is very effective at producing these things. High quantities of green plant life have been sent to several countries, including China, Japan, Europe,

and the United States. As much as 2000 tons/year, through which numerous synthetic compounds utilized in the clinical, food and modern fields were extricated [24]. After 24 days, the green growing society harvesting system was complete, and biochemical combinations were detected. At a range of gas flow rates, we evaluated the protein content of *Chlorella sorokiniana*. Protein concentrations were 36.22, 37.26, 42.12, 44.25, and 47.08 percent, separately, contrasted with the control which added up to 31.32%, as displayed in Fig (7).



Fig(7): The proportion of protein produced from algae *Chlorella sorokiniana* within different rates of CO₂ gas.

Biochemical mixtures were recognized after the fulfillment of the collecting system for green growth societies following 24 days. The proteins of *Chlorella sorokiniana* were estimated at various gas stream rates. The extent of proteins came to 36.22, 37.26, 42.12, 44.25 and 47.08%, individually, contrasted with the control which added up to 31.32%, as displayed in Fig (7).

The suitable culture media assists with expanding the biomass through an expansion in the pace of cell development or an expansion in the substance of the inward cell, as indicated by a review [25]. During the improvement of *Scenedesmus* sp. green growth on a supplement medium to deliver and frame biomass in a wastewater culture. As the efficiency arrived at 0.578 g/liter of dry biomass and the creation of biochemical mixtures in high extents addressed by 19% proteins, 27.5% starches, and 17% fats, as in the review [26]. By adding CO₂ to the way of life medium took care of at various rates, which gave satisfactory extents to algal items like 28.5% protein, 64% starches, and 26.5% fats. Added to the focal point of the improvement carbon dioxide and due to that they had high efficiency and the extent of starches (64%).

A portion of the microalgae generally firmly connected with biotechnology are the green growth Chlorophyceae, *Chlorella vulgaris*, *Dunaliella salina*, and *Spirulina maxima* which are broadly utilized financially. It is predominantly utilized as human food supplement and creature feed added substance. *Spirulina platensis* blue green growth contains polyunsaturated unsaturated fats and

colors [27]. *Chlorella* sp. biomass likewise contained 25-30% protein, 6-10% starches and 30-40% lipid. On a culture furnished with second gas Organic CO₂ species *Chlorella* sp. also, *Spirulina platensis* showed CO₂ absorption effectiveness of 46% and 39%, respectively. CO₂ absorption combined with calcium precipitation is a biomass item that can be utilized financially [28].

References

- [1] Pulz O, Broneske J, Waldeck P (2013) IGV GmbH experience report, industrial production of microalgae under controlled conditions: innovative prospects. In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, 2nd edn. Wiley, Oxford, pp 445–460.
- [2] Elliott LG, Feehan C, Laurens LML, Pienkos PT, Darzins A, Posewitz MC. (2012). Establishment of a bioenergy-focused microalgal culture collection. *Algal Res*; 1: 102–113.
- [3] Stephen Bleakley and Maria Hayes. (2017). *Algal Proteins: Extraction, Application, and Challenges Concerning Production*, *J. Foods*, 6, 33.
- [4] Gouveia, L.; Batista, A.P.; Sousa, I.; Raymundo, A.; Bandarra, N. Microalgae in Novel Food Products. In *Food Chemistry Research Development*; Konstantinos, N., Papadopoulos, P.P., Eds.; Nova Science Publishers: New York, NY, USA, 2008; pp. 75–112.
- [5] Van Krimpen, M.; Bikker, P.; Van der Meer, I.; Van der Peet-Schwering, C.; Vereijken, J. (2013). Cultivation, Processing and Nutritional Aspects for Pigs and Poultry of European Protein Sources as Alternatives for Imported Soybean.
- [6] Abo Alheijaa, H. M, (2018). Optimization and modeling of some microalgae growth as potential source for biofuel production. M.Sc. Thesis, College of science/ University of Baghdad
- [7] Edward, G. Bellinger and David C. Sigeo (2010) *Freshwater Algae Identification and Use as Bioindicators*. Printed in Great Britain by Antony Rowe, Ltd. Chippenham, Wilts. pp 285.
- [8] Al-Hussieny, A.A. (2018). *Atlas of The Algae in The Iraqi Aquatic Environment*. Scholars press. Book. 195.
- [9] Huang, X.H.; Li, C.L.; Liu, C.W. and Zeng, D.Q. (2002). Studies on the Ecological Factors of *Oocystis borgei*. *Journal of Zhangjiang Ocean University*, Vol.22, No.3, 8–12.
- [10] Widjaja, A., C.-C. Chien and Y.-H. Ju, (2009). Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *Journal of the Taiwan Institute of Chemical Engineers*, 40, 13-20.
- [11] Van Dijk, D. ; Houba, V.J.G. (2000) Homogeneity and Stability of Material distributed within the Wageningen Evaluating Programmes for Analytical Laboratories Commun. *Soil.Sci.Plant.Anal*, 31 (11-14), 1745-1756.
- [12] Bhakta JN, Lahiri S, Pittman JK, Jana BB (2015) Carbon dioxide sequestration in wastewater by a consortium of elevated carbon dioxide tolerant microalgae. *Journal of CO₂ utilization* 10: 105-112.

- [13] Al-Hussieny, Ahmed Aidan, Hiba Thamer Hussein and Ameal H. Hmood .(2014). Increase algae culture by using various ways by different media culture. Journal of College of Basic Education 45(2): 157-164.
- [14] Al-Hussieny, A. A., Aljanabi, H. M., Imran, N. J., & Hussain, S. F. (2022). Efficiency testing of Algal *Chlorella Sorokin Ana* and *Coelastrella sp.* to reduce carbon dioxide. International Journal of Health Sciences, 6(S6), 6900–6914. <https://doi.org/10.53730/ijhs.v6nS6.11951>
- [15] Kefford, B.J., Paradise, T., Papas, P., J Fields, E. and Nugegoda, D.(2003). Assessment of a System to Predict the Loss of Aquatic Biodiversity from Changes in Salinity, Land and Water Australia. Products; Wageningen UR Livestock Research: Lelystad, The Netherlands; p. 48.
- [16] Tamure, H., Mine I., and Okuda, K.(1996). Cellulose- Synthesizing terminal complexes and microfibril structure in the brown algae *Sphacelaria rigidula* (Sphacelariales, Phaeophyceae) Phycological Research 44.pp:63-68.
- [17] Qiu, R.; Gao, S.; Lopez, P. A.; Ogden, K. L.(2017). Effects of pH on cell growth, lipid production and CO₂ addition of microalgae *Chlorella sorokiniana*. Algal Research, v.28, p.192-199.
- [18] Singh, N. K.; Dhar, D. W.(2011). Microalgae as second generation biofuel: A review. Agronomy for Sustainable Development, v.31, p.605-629.
- [19] Valdés, F. J.; Hernández, M. R.; Catalá, L.; Marcilla, A. (2012). Estimation of CO₂ stripping/CO₂ microalgae consumption ratios in a bubble column photobioreactor using the analysis of the pH profiles. Application to *nannochloropsis oculata* microalgae culture. Bioresource Technology, v.119, p.1-6.
- [20] Fernández I, Ací'en FG, Fernández JM (2012). Dynamic model of microalgal production in tubular photobioreactors. Bioresour Technol;126:172–81.
- [21] Bilanovic D, Holland M, Starosvetsky J (2016). Co-cultivation of microalgae and nitrifiers for higher biomass production and better carbon capture. Bioresour Technol;220:282–8.
- [22] Costache TA, Ací'en FG, Morales MM (2013).. Comprehensive model of microalgae photosynthesis rate as a function of culture conditions in photobioreactor. Appl Biotechnol;97:7627– 37.
- [23] Trivedi, J., Aila, M., Bangwal, D., Kaul, S., Garg, M.,(2015). Algae based biorefinery—how to make sense? Renew. Sustain. Energy Rev. 47, 295–307.
- [24] Thangave Mathimani and Arivalagan Pugazhendhi,.(2019). Utilization of algae for biofuel, bio-products and bio-remediation, Biocatalysis and Agricultural Biotechnology 17 : 326–330.
- [25] Shen, Q.-H.; Jiang, J.-W.; Chen, L.-P.; Cheng, L.-H.; Xu, X.-H.; Chen, H.-L.(2015). effect of carbon source on biomass growth and nutrients removal of *Scenedesmus obliquus* for wastewater advanced treatment and lipid production. Biores. Technol., 190, 257–263.
- [26] Ansari, F.A.; Ravindran, B.; Gupta, S.K.; Nasr, M.; Rawat, I.; Bux, F. (2019). Techno-economic estimation of wastewater phycoremediation and environmental benefits using *Scenedesmus obliquus* microalgae. J. Environ. Manag., 240, 293–302.
- [27] Sajilata, M., R. Singhal and M. Kamat, (2008). Fractionation of lipids and purification of γ -linolenic acid (GLA) from *Spirulina platensis*. Food Chemistry, 109, 580-586.

[28]Ramanan, R., K. Kannan, A. Deshkar, R. Yadav and T. Chakrabarti, (2010). Enhanced algal CO₂ sequestration through calcite deposition by *Chlorella* sp. and *Spirulina platensis* in a mini-raceway pond. *Bioresource technology*, 101, 2616-2622.