Eco-Efficient biohydrogen production from Algae: Effect of Media Composition and Environmental Factors

Doaa G. Abed[1], Asawer A. Alwasiti[1], Riyadh S Almukhtar[1], Zainab Y. Shnain[1]

[1] Chemical Engineering College, University of Technology, Baghdad, Iraq

Corresponding Author: Asawer. A. Alwasiti, asawer.a.alwasiti@uotechnology.edu.iq

Abstract

The increasing demand for sustainable energy sources worldwide has led to an upsurge in the consideration of biologically produced hydrogen as an alternative clean energy resource. Hence, this research investigates the production of biohydrogen from the algal organism Chlorella vulgaris. The originality of this work is the quantifying and enhancing the production of hydrogen using Chlorella vulgaris through the integration of nutrient-stress induction, purple-wavelength illumination, and a low-cost real-time hydrogen monitoring system, using an optimized two-stage hybrid process. The effects of culture medium formula, light type, intensity, phosphate level, temperature, and pH levels on algae growth and hydrogen production was carried out. The results show that algal growth was maximum in NPK (20:20:20), representing a well-balanced nutrient composition compared to BG11 and industrial wastewater growth medium, which is essential for the enhancing the growth of Chlorella vulgaris for sustainable biohydrogen production. Indeed, the results revealed that algae growth and hydrogen production were increased under optimum conditions characterized by controlled limitations in phosphate levels at 0.4 ppm. The results revealed also that the use of violet lights at an intensity of 2000 lux had a practical enhancement for both algae growth and hydrogen production, with maximum production of 1000ppm, establishing the energy synergistic requirement based on photobiology aspects for effective algae growth stimulation and enhanced biohydrogen production based on the algal photosynthesis process. The Response Surface Methodology approach based on the Box-Behnken design results show that an optimal algae growth rate of 0.0225 hr^{-1} at a pH requirement of 6.58 and a temperature requirement of 27.57 °C.

Keywords: Algae, growth rate, hydrogen production, optimization

1. Introduction

Since 2020, an increase in the releasing of gases like methane and nitrous oxide. These factors put together create an escalating problem that needs immediate attention to maintain a balance within the environment, owing to the constant increase in population and the rate at which natural resources like fossil fuels are being consumed [1]. One of the effective solutions to such challenges would be the shift towards the use of clean and renewable sources of energy like solar power, wind power, biofuels, and hydrogen gas [2, 3].

Biofuels come in three generations. The first-generation biofuels include bioethanol, biodiesel, and biogas. These biofuels were criticized in 2010 for several environmental concerns and carbon emissions. Currently, the emission reduction capabilities of biodiesel technology are deemed inefficient. For that reason, there has been a growing emphasis on second-generation biofuels that can be obtained from lignocellulosic sources. Despite that, there remain technical difficulties in the use of biofuels in internal combustion engines [4]. The third generation of biofuels uses microorganisms such as green algae and cyanobacteria. The capacity of algal growth in simpler habitats and high photosynthesis efficiency makes them an ideal and clean energy alternative that emits very few pollutants[5-7].

In recent years, there has been increased attention towards the production of bioenergy from microalgae using microalgal biomass. Microalgae can be distinguished by low cost and the possibility of recycling materials from the biomass [8].

The current mechanisms used include direct photobiological analysis, indirect photobiological analysis, photo fermentation, and dark fermentation. These mechanisms make use of the hydrogenase and nitrogenase that act as active enzymes in the biological structure of the plants [9-11].

Chlamydomonas reinhardtii remains the model organism that has been studied the most for hydrogen production in photobiological processes due to the well-known genetic nature and efficient methods of genetic transformation [11]. Modern biological approaches of genetic engineering include improvements in hydrogenase expression levels, reduction of hydrogenase activity as an electron sink, and stress robustness [12]. Besides, certain green algal strains of Chlorella, Scenedesmus, and Dunaliella genus have been found to be

promising for hydrogen production at various scales of culturing [13]. The choice of such strains depends on various factors like hydrogen yield rates, stress robustness, and culturing needs [14].

The bio-hydrogen process from algal cultures involves several factors, such as operational factors like medium used in the algal cultures, pH, temperature, and nutrient availability, such as nitrogen and phosphate. Recently, it has been revealed that the role of physico-chemical factors, such as hydrogenase activity, in enhancing H2 synthesis from algal cultures, oxygen scavengers, and nanoparticles [14].

The general reaction scheme for microalgae hydrogen production includes two phases: firstly, in aerobic conditions to absorb energy from sources, followed by anaerobic conditions to obtain H2 gas. The hybrid process for hydrogen production by algal cultures has attracted widespread research due to the system's capacity to overcome challenges posed by unidirectional processes by combining algal and engineering principles [14-15]. In such processes, algal cultures can be grown in nutrient-rich wastewater medium to optimize algal growth [16]. The Cultures undergo fermentation or darkness fermentation processes towards activating hydrogen evolution. The dual-step process provides higher output than direct photobiologic methods of hydrogen evolution as well as avenues for contemporaneous waste treatment and CO2 capture [17]. The hybrid processes combine algal photobioreactor designs integrated with microbial electrolysis cell equipment or photofermenters. This will increase hydrogen production through removing the limitations results from the toxicity of algal caused by oxygen and enhancing the electron accessibility [18-19]. Advanced research shows that the commercial output of hydrogen gas can be increased by the integration of modified genetic algal and new process designs [15, 18]. This study aims to improve as well as optimizing an eco-efficient, two-stage hybrid process for biohydrogen production using Chlorella vulgaris through evaluating the effects of key cultivation parameters—including nutrient composition, light spectrum and intensity, phosphate concentration, pH, and temperature—on both algal growth and hydrogen yield. The novelty of this research involves the integrated strategy combines both of controlled nutrient-induced stress and spectral light optimization to enhance hydrogenase activity and

maximize biohydrogen output. In contrast to previous studies that have typically focused on either biomass accumulation or hydrogen production in isolation, this research employs a statistically driven Box–Behnken design to uncover synergistic conditions that effectively bridge the gap between these two objectives.

2. Experimental Work

2.1 Materials

Chlorella vulgaris green algae was purchased from the USA. The algae cells were pre-adapted under cooling conditions of about 6-8˚C. The algae were cultured using demineralized water and two types of culture media, N:P: K[20:20:20], which consists of (ammonia 20: phosphate20: potasium20) as a culture medium from India, and BG11, which consists of main material as Sodium nitrate 1.500 gm/l with a few other chemical materials from India.

The demineralized water characterization is pH:6.8-7.5, SC:≤0.4µs/cm. Phosphate (Na_3PO_4 H_2O ,with a concentration of 98% from Chania) was also used.

2.2 Experimental Set-up

The experiments were conducted in a glass basin measuring 30 cm x 19 cm x 21 cm with a tight plastic cover, connected to an air blower for aerations, as shown in the Figure. Fluorescent light sources (white, purple, blue) are positioned 5 cm away from the sides. The cover is equipped with a hydrogen sensor (QM-2) linked to an Arduino to measure hydrogen concentration. It also includes a temperature meter to monitor the fluctuation in temperature and pH sensors to regulate acidity using hydrochloric acid and sodium hydroxide. A nitrogen gas tube is used to displace oxygen during the anaerobic phase, and a separate device measures dissolved oxygen in the water.

The experiments were carried out in two stages. The first stage focused on the growth of the algae. In this stage, 2 ml of microalgae Chlorella vulgaris cells were added to 1 L of demineralized water with 0.25 g of NPK culture media, sterilized via autoclaving at 200-

220°C for 15-20 minutes. An aerobic The growth process was achieved by aerobic conditions, with air bubbles supplied to the cells for 5 hours each day. The algae continued to grow for 6 days under controlled conditions. Three types of light (white, blue, and purple) under two intensities (2,500 and 5,000 lux), as measured with a lux meter were used to irradiate the cultures were irradiated from. The experiments were done at different values of temperatures (27°C, 30°C, and 32°C) and pH (6.5, 7.5, and 8.5). The pH was adjusted using 1.0 mol of HCl or 1.1 mol of NaOH. The growth of the microalgae was monitored and measured every 24 hours using a UV device at a wavelength of 620 nm.

The algae growth rate for each 48 hr is calculated depending on the following equation [20]

$$C_{48} = C_o e^{\mu(48-0)} \quad 1$$

In which C is the biomass concentration and μ is the growth rate after 48 hours.

The second stage involved hydrogen production. The hydrogen was measured using the MQ-2 sensor, which was calibrated using the two-point method. This process was conducted under anaerobic conditions. The anaerobic phase includes two conditions: photolytic and dark, to compare their effects. Additionally, various phosphate concentrations are prepared to determine optimal levels for growth and biohydrogen production.

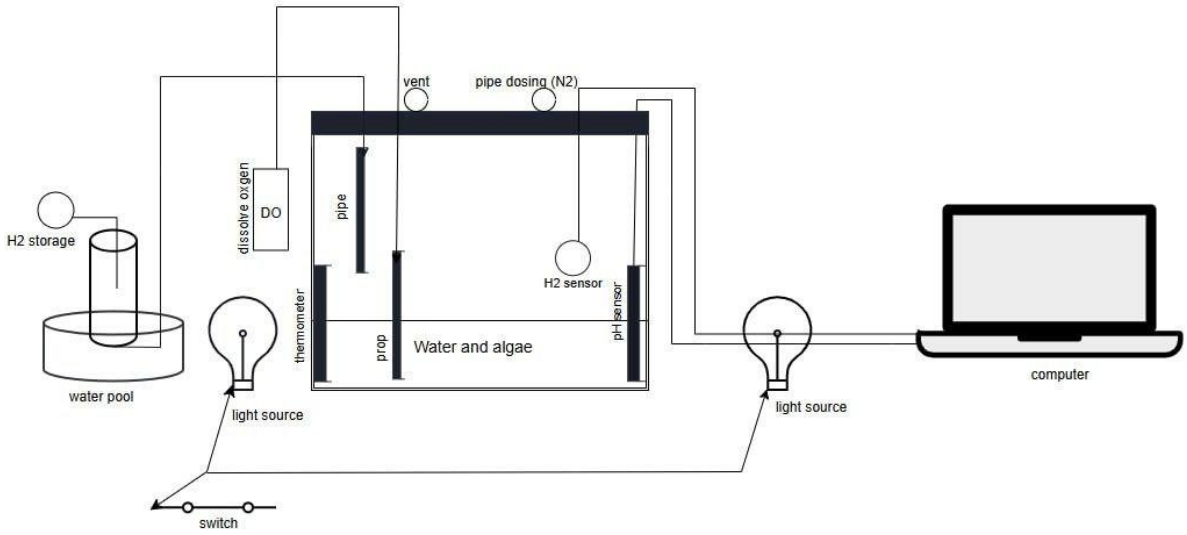

Fig.1: The schematic diagram

3. Results and Discussion

3.1 Growth Rate and Cultivation of Microalgae

The relation between algae biomass and optical density measured at 620 is linear, as shown in Figure 2. This is expected since, as the density increases, more light is absorbed by the cells, leading to higher biomass production. This behavior is aligned with numerous researchers [20-22]. The best-fit line obtained is

Biomass (g/l) = 0.489 OD_{620} + 0.0391, which is used to get the biomass of algae for the later experiments.

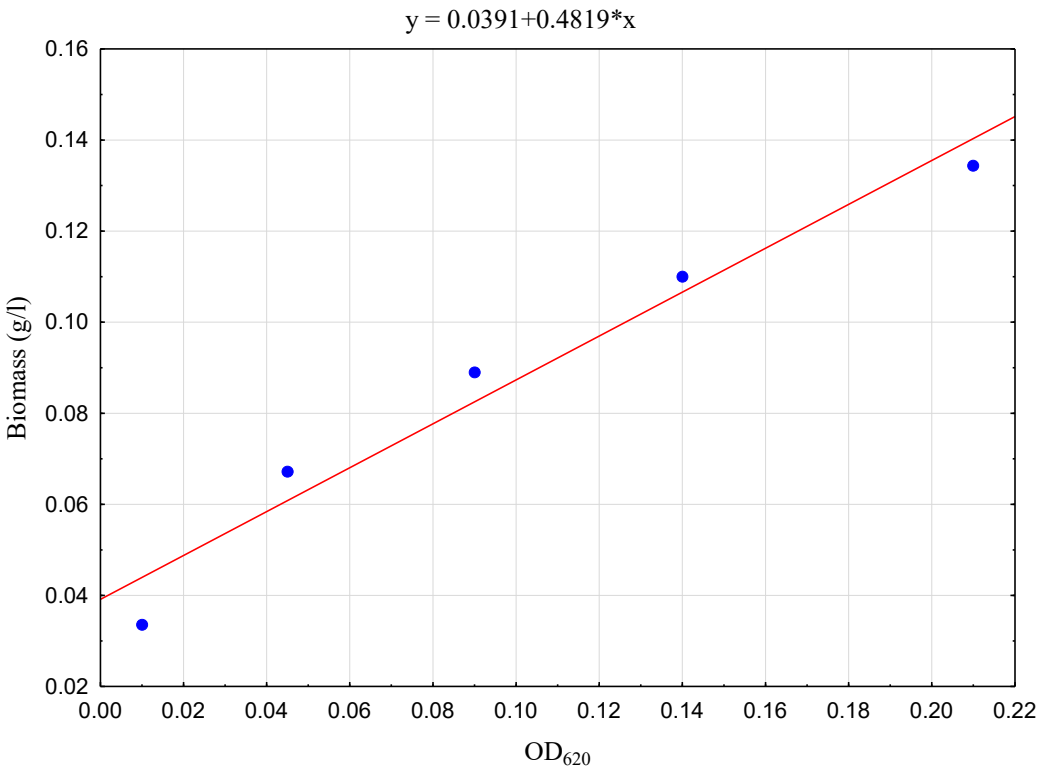

Fig. 2: The standard curve of the Biomass of CV algae with OD_{620}

3.2 Effect of the culture medium

Synthetic water media

The effect of different cultivation media on the microalgae growth dynamics and consequent hydrogen production was systematically investigated under controlled environmental conditions. Throughout all experiments, the temperature was kept at 27.2 °C and the pH at 6.7, and white fluorescent lighting at an intensity of 1200 lux to ensure uniform exposure across cultures.

The use of BG11 cultivation medium results in limited algal proliferation, as shown in Figure 3. During the first three days, the growth was detectable from 0.024 to 0.8 but rapidly declined thereafter, culminating in complete cessation of development. This stagnation specifies the limitations of BG11 media in supporting the accumulation of biomass and hydrogen production under the test parameters. Similar results of BG11 on growth and hydrogen yield have been reported in prior studies [23-24].

However, the use of NPK fertilizer (20:20:20) as the culture medium results in improving the growth of algae to 0.133 after six days. Biomass accumulation was not only rapid but also sustained. This medium showed to be highly supportive of metabolic activity, corroborating findings from earlier reports where nutrient-balanced fertilizers enhanced algal performance [25].

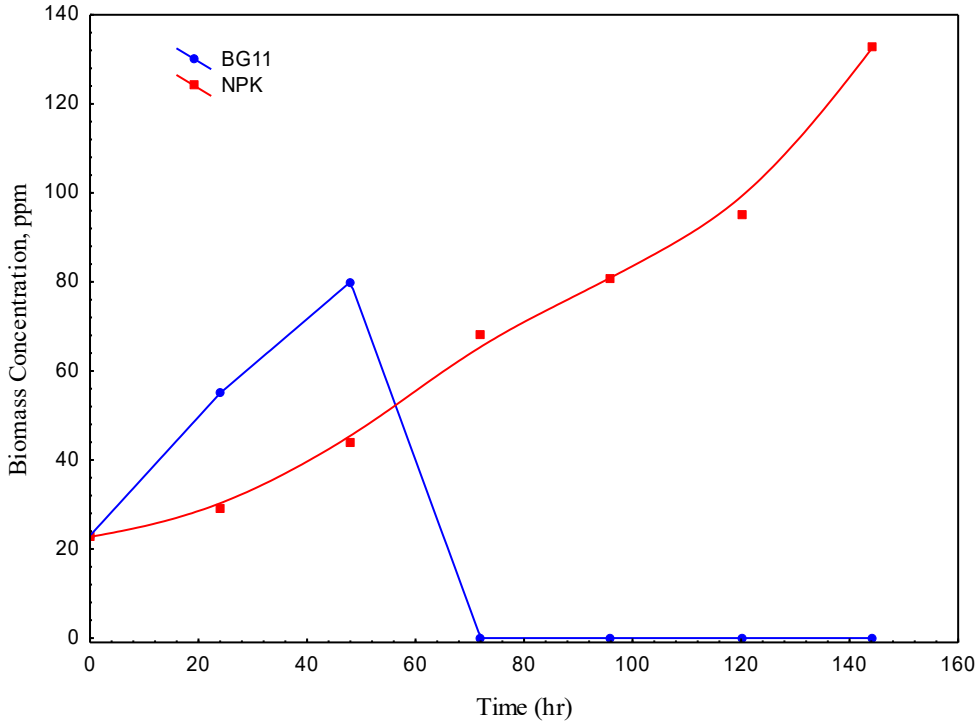

Fig.3: The relation between biomass CV algae and time under different culture media

The Industrial wastewater media

Water samples supplied from the cooling towers of the Wassit Thermal Power Plant (Iraq) was used as cost-effective media. The water was analyzed and tested for cultivation. The analysis showed that pH (8.31), phosphate concentration (1.56 ppm), and sulfate concentration (600 ppm), signifying a challenging chemical environment. The results reveal that the cultures failed to grow under these conditions, indicating that the elevated sulfate and phosphate levels exerted inhibitory effects on algal physiology and hydrogen generation (Fig. 3). This finding is reproducible with documented toxicity of excess phosphate and sulfate on algal metabolic processes [26].

In other side, the collected industrial wastewater from downstream of the cooling tower discharges promises more favorable characteristics with a pH of 7.55, a sulfate concentration of 430 ppm, and a phosphate concentration of 0.3 ppm. The results show a rapid and robustly growth, from 0.023 to 0.0285 under these conditions leading to significant hydrogen production within 6–10 days (Fig. 4). This performance can be due to the lower phosphate levels that create a more balanced nutrient environment, supporting biomass productivity as well as hydrogen yield.

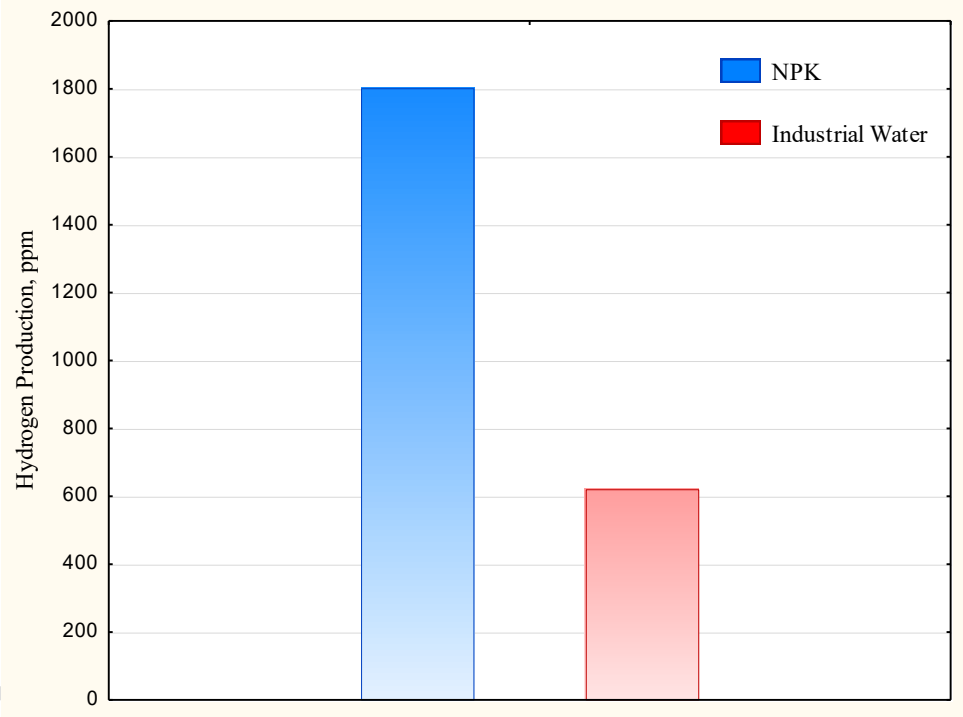

Figure (4): The production of hydrogen under different culture media

The Role of phosphate concentration

The microalgae biomass concentration during cultivation period (144 hours) under different initial nutrient concentrations (0.1, 0.4, 0.7, and 1 ppm) are presented in figure 5. The figure shows an increase in all cases, reflecting the exponential growth pattern typical

of algal cultures during the logarithmic phase. Though, the growth extent differs depending on the phosphate concentration.

At lower concentrations (0.1–0.4 ppm), The biomass growth was more obvious reaching values exceeding 110 ppm at the end of cultivation. This observation indicates that the cellular metabolism and photosynthetic activity are stimulated using moderate nutrient, providing an optimal balance for algal growth. In spite of that, the increasing of nutrient supply (e.g., 1 ppm) will impose stress on the system and reduce the growth. This reduction is due to osmotic stress, nutrient toxicity, or metabolic imbalances caused by oversaturation of the growth medium [27-28].

During the first 24 hours the growth curves show a rapid increase in biomass for all concentrations, corresponding to the initial adaptation and exponential growth phase. Afterward, the difference in growth rate is observed, with the 0.4 ppm condition consistently outperforming others. This shows that the balance of nutrient uptake and assimilation efficiency plays a significant role in biomass accumulation [29]. The lower biomass concentration at 1 ppm supports the principle of "nutrient inhibition" which is documented phenomenon presenting that excess nutrients interfere with photosynthetic efficiency and chlorophyll stability [30].

The figure shows a close alignment between 0.7 ppm curve and the 0.1 ppm condition proposes that as the cells surpass their optimal metabolic capacity then higher nutrient concentrations do not necessarily translate into higher productivity. Instead, they can slow down growth by altering cellular homeostasis. This observation is similar to other studies on Chlorella vulgaris and Scenedesmus obliquus, in which the optimum nutrient ranges were found to be narrow and strongly species-dependent [31].

In general, the results indicate that the most favorable condition was at 0.4 ppm, supporting both rapid biomass accumulation and sustained growth throughout the culture period. This highlights the importance of fine-tuning nutrient concentrations to avoid both deficiency and excess, thereby maximizing algal productivity.

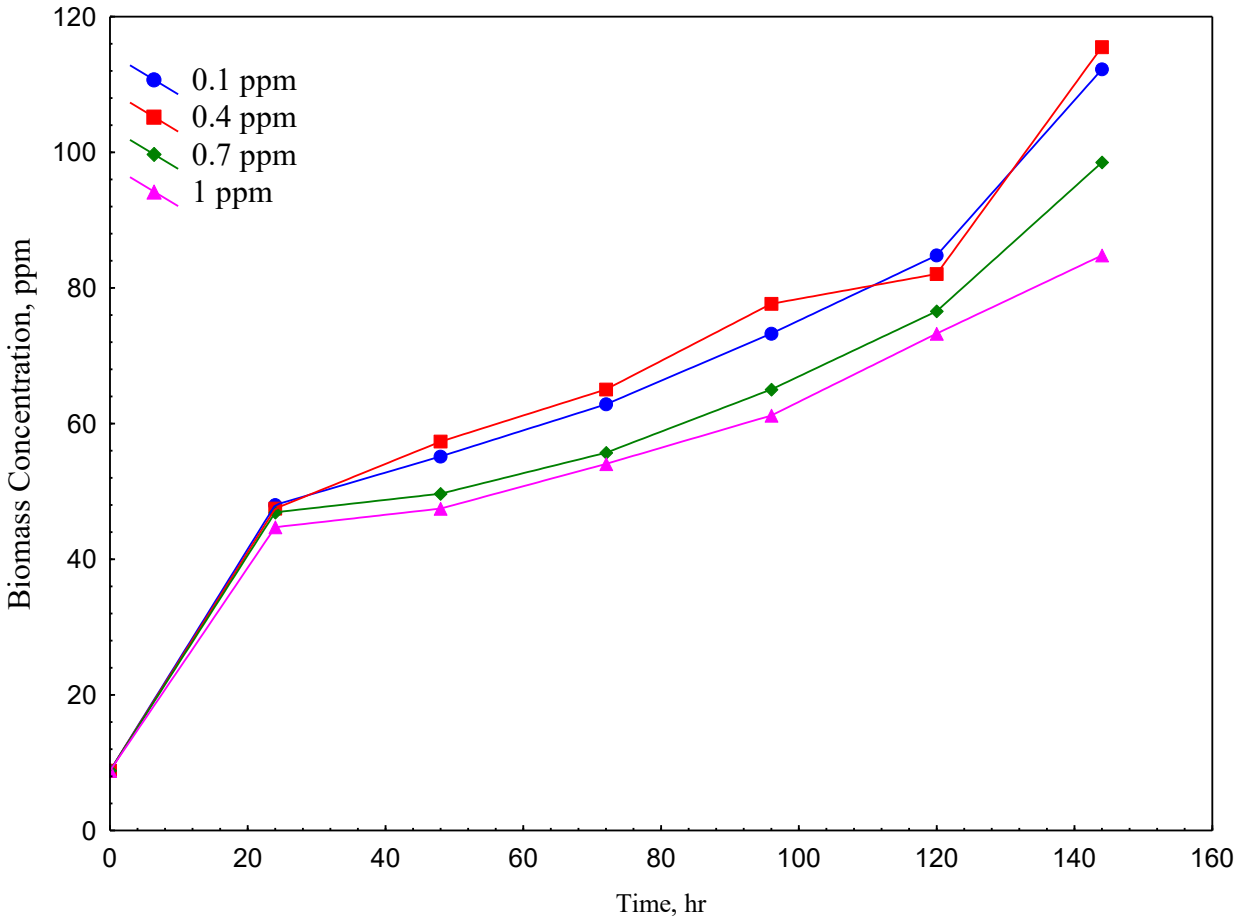

Fig.5: The relation between biomass concentration and time under different phosphate concentrations

Figure 6 shows that Chlorella vulgaris achieved its highest hydrogen production (~10,000 ppm) at 0.4 ppm phosphate, while both lower (0.1 ppm) and higher concentrations (0.7–1 ppm) yielded significantly reduced outputs. This indicates that moderate phosphate stress stimulates hydrogenase activity and redirects metabolism toward hydrogen evolution, whereas excessive phosphate favors biomass growth at the expense of hydrogen production.

Similar observations have been reported in Chlamydomonas reinhardtii and other microalgae, where nutrient limitation suppresses oxygen evolution and enhances hydrogenase activity [32-34]. Recent studies on Chlorella vulgaris also confirmed that controlled phosphate stress enhances biohydrogen yields, provided the stress is not too

severe [35-36]. Thus, 0.4 ppm emerges as the optimal condition, balancing cellular activity and stress to maximize hydrogen output.

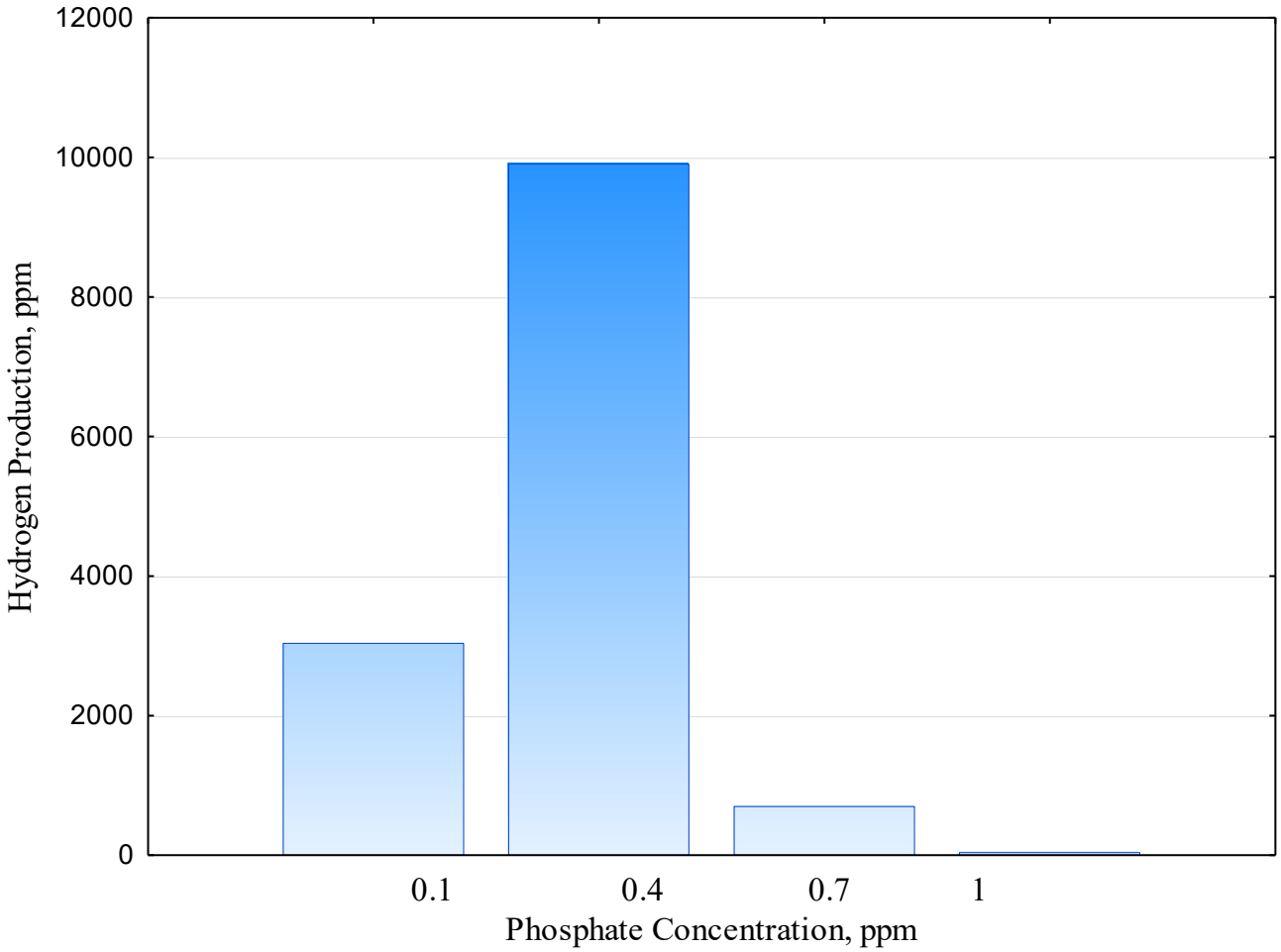

Fig.6: The hydrogen production under different phosphate concentrations

3.3 Influence of Light Type and Intensity

The effect of the type of lighting on the growth rate of algae and on the rates of biohydrogen production was studied, where different types of white, blue, and violet light were used with different lighting intensities at 1200 and 2000 lux under similar conditions in terms of temperature and pH at 27.5 °C and 6.7, respectively. The experimental results are shown

in figures (7-10), which demonstrate that light quality and intensity exert a decisive influence on both algal growth and hydrogen evolution in Chlorella vulgaris. The figures show that in all types of light, increasing the light intensity from 1200 to 2000Lux caused an increase in algae growth with about 10%, 66%, and 40% for white, purple, and blue types, respectively. Indeed, the highest growth rates occurred within a remarkably short period of 6–7 days, with values reaching 0.149 under white light, 0.200 under blue light, and 0.420 under violet light. Correspondingly, the hydrogen production was increased by 44% for both white and blue types and 88% for purple ones.

Contrasting between the used types of light, the purple light showes promising results of both algae growth and hydrogen production, demonstrating the spectral composition of illumination modulates algal physiology. The use of white light facilitated moderate growth rate and hydrogen production, contemplating its role in supporting overall photosynthetic activity. The blue light is essential for regulating photosystem II activity and chlorophyll absorption, however, it causes lower growth and yield production. This behavior can be due to its partial inhibition and limited penetration of the metabolic pathways [37]. On the other hand, the violet light at an optimized intensity of 2000 lux enhanced the biomass growth and hydrogen yield. This can be attributed to its shorter wavelength, results in providing higher photon energy that effectively stimulates both of chlorophyll and accessory pigments, enhancing metabolic pathways associated with photobiological hydrogen production [38–40].

The higher performance of violet light proposes that the biohydrogen productivity of Chlorella vulgaris cultivation can be maximized under selective light regimes. This result agrees with previous research highlighting that nutrient stress and spectral optimization act synergistically in redirecting photosynthetic electron flow toward hydrogenase-mediated hydrogen evolution [41]. This results is critical for designing cost-effective photobioreactors, in which tailored lighting conditions can promotes both growth kinetics and clean energy generation.

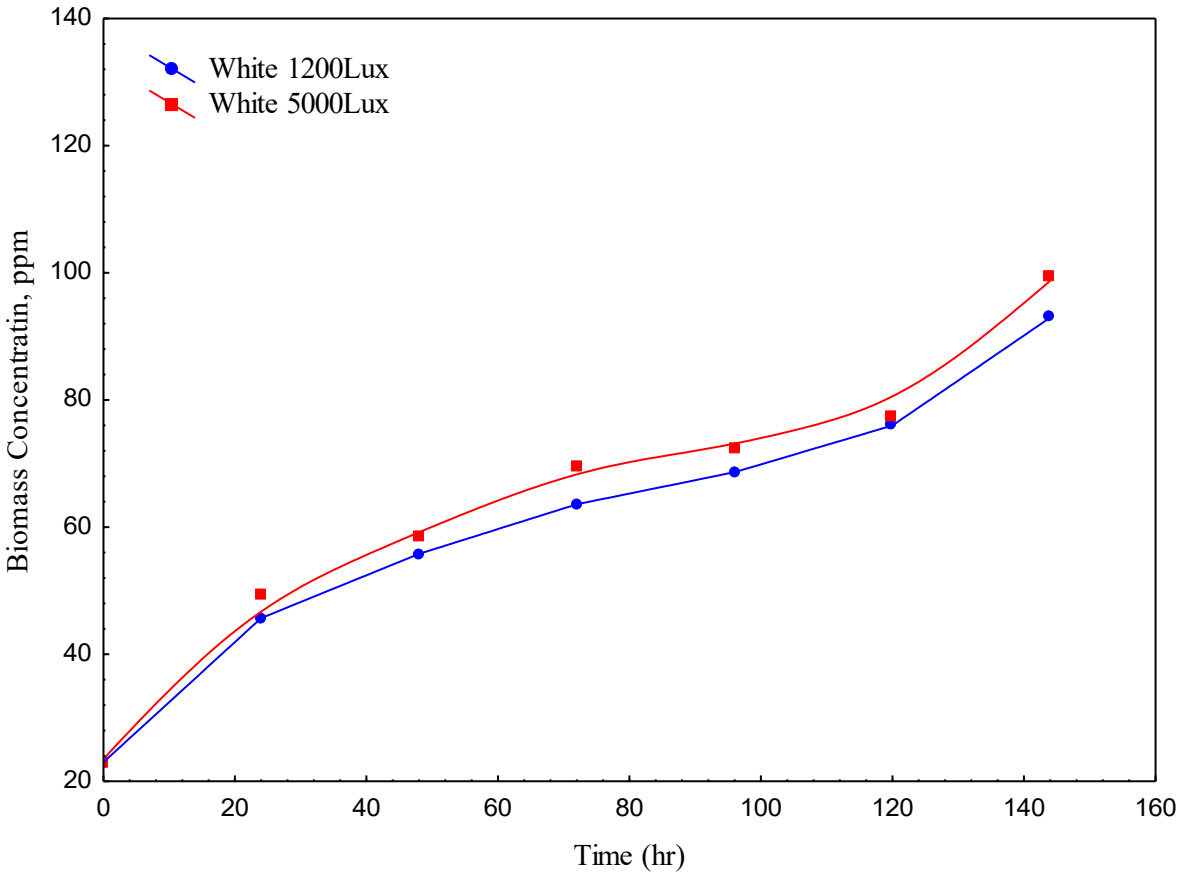

Fig.7: The relation between biomass growth and time under different white light intensities

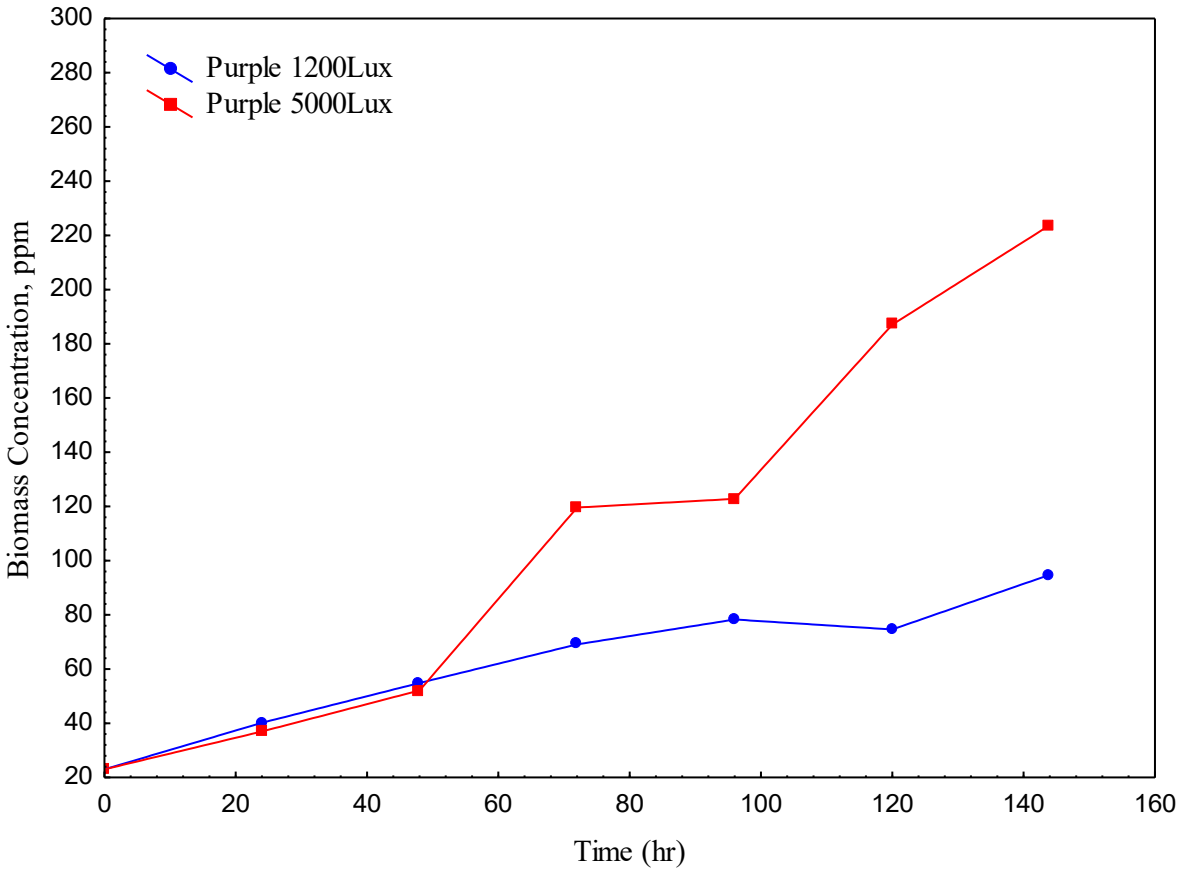

Fig.8: The relation between biomass growth and time under different purple light intensities

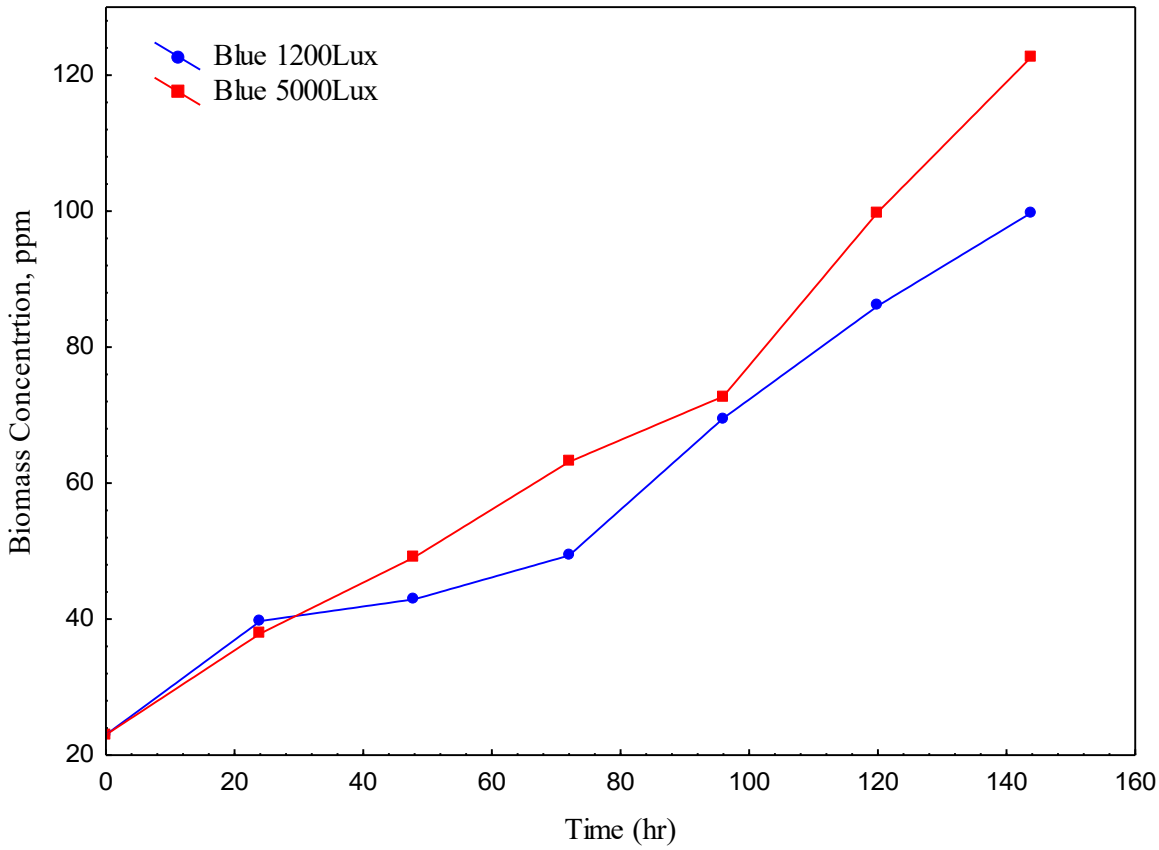

Fig.9: The relation between biomass growth and time under different blue light intensities

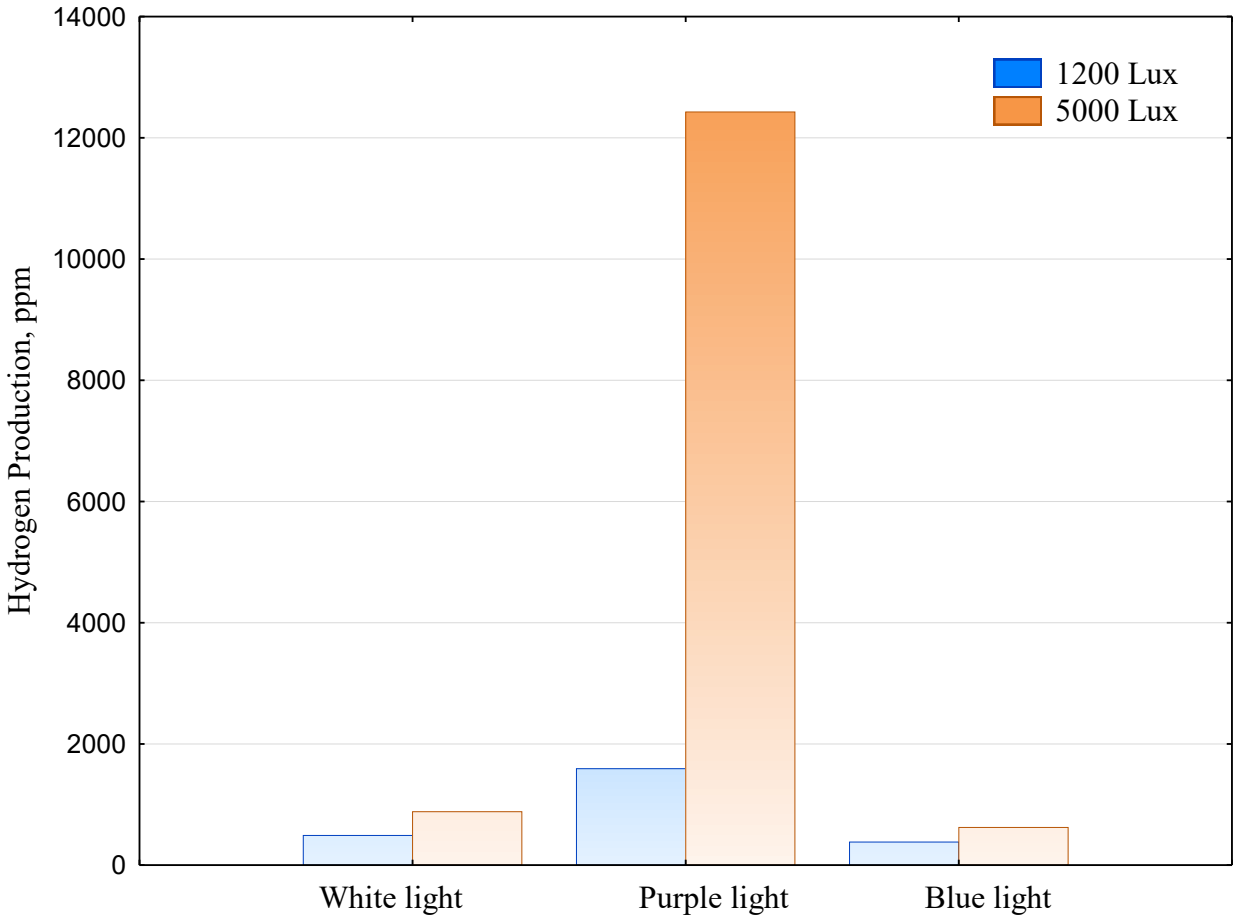

Fig.10: The production of hydrogen at different light types and intensities

3.4 Effect of operating variables

The knowledge of the impacts of environmental factors like temperature and pH on algal growth is an important consideration in the maximization of the growth of the alga Chlorella vulgaris for bioenergy production. In the research work presented here, the impacts of temperature ranging from 27 to 32°C and pH levels of 6.5 to 8.5 on algal growth, as depicted by cell concentration, were determined (Figures 11-13). The outcomes show that C. vulgaris behaves differently based on both factors.

In contrast to several earlier research reports that showed increased algal growth due to moderate increases in temperatures, it has been found that increased temperatures had a negative effect on the growth of C. vulgaris at temperatures within the range. At pH levels

of both 6.5 and 8.5, it was observed that the biomass concentration increased maximally at 27°C and decreased steadily as it increased to 30°C and 32°C. The above observation indicates that C. vulgaris in the system studied responds to temperature.

The decreased growth at high temperatures can be explained by the heat-stress-mediated physiological stress, which may affect the integrity of membranes and proteins as well as various key enzyme reactions that occur during photosynthesis and carbon assimilation processes [27].

High temperatures may decrease the availability of dissolved oxygen as well as increase the occurrence of photorespiration. These factors can affect algal growth.

These findings differ from those of Converti et al. [42], who found that 30°C represented an optimum temperature for growth of C. vulgaris. Such discrepancies may reflect variation due to strains or system specificity. Additional factors such as light intensity or substrate composition may influence temperature sensitivity.

The effect of pH on algal growth was studied at a constant temperature of 30°C and pH levels of 6.5 to 8.5, fig. 8. The findings showed that there was a specific growth optimum at pH 7, where C. vulgaris had the maximum cell growth. The growth regime at pH 6.5 showed a mild inhibition, whereas a moderate inhibition of growth occurred at pH 8.0 as well as at 8.5.

The increased performance at neutral pH supports the biological preference of most freshwater microalgae, including C. vulgaris, for a mild environment around neutral pH, as stabilizing internal pH and nutrient transport mechanisms work better at mildly neutral pH values [43]. At lower pH values, excessive influx of protons may negatively affect cell functions, and at higher pH values, vital nutrient components like phosphate may form precipitates, thereby preventing growth.

These findings are aligned with previous research by [44-45], which highlight the maintaining importance of optimal pH levels for microalgal productivity. Similar results also obtained by the work of Converti et al. [42], who showed an optimum growth value obtained at pH 8, again highlighting the influence of system-specific

The results reveal that the limiting factor on C. Vulgaris growth is temperature, in which the stress caused by high temperatures begins above 27°C. On the other hand, the most optimal condition is at pH 7, probably due to the balance it maintains around enzymatic reactions.

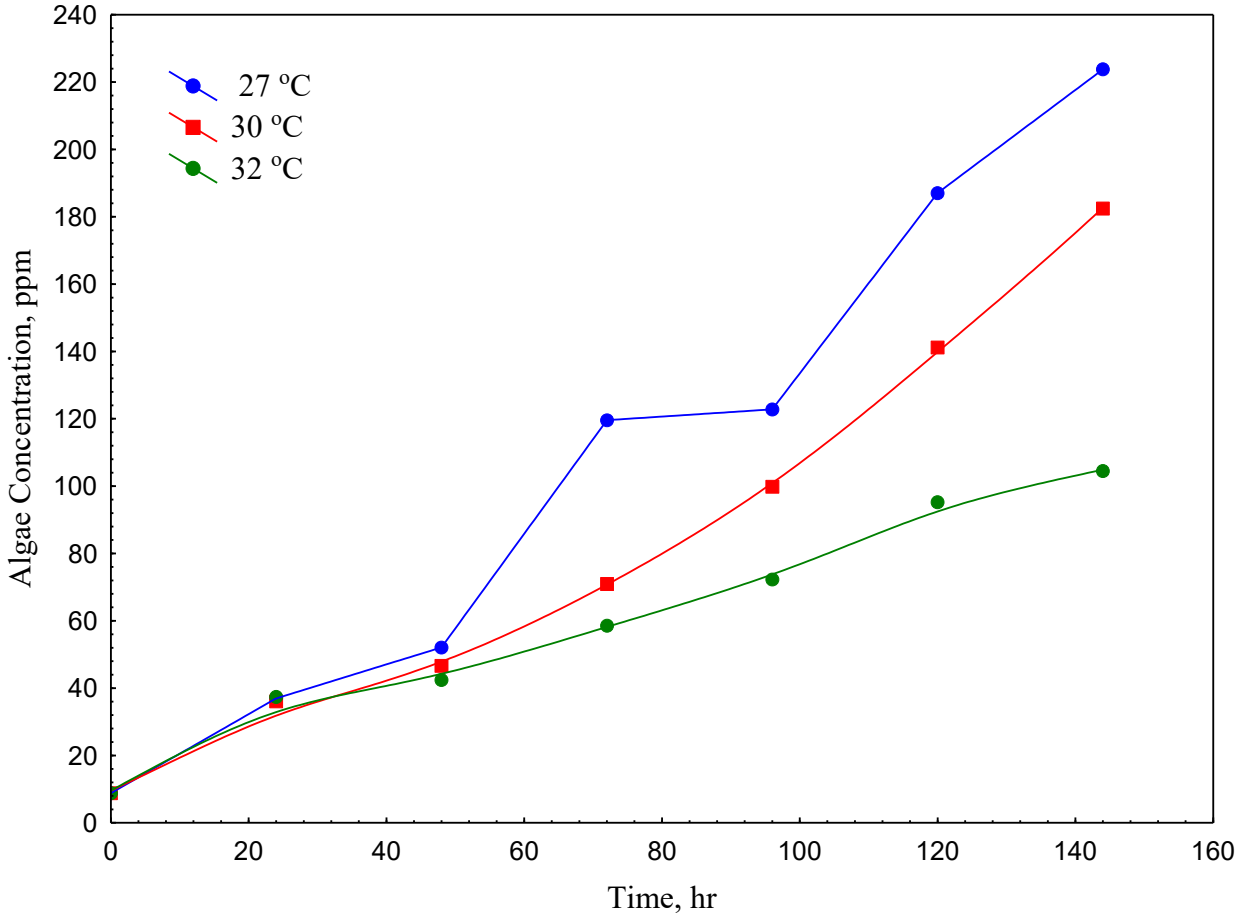

Fig.11 The relation between algae concentration and time under different temperatures at pH=6.5

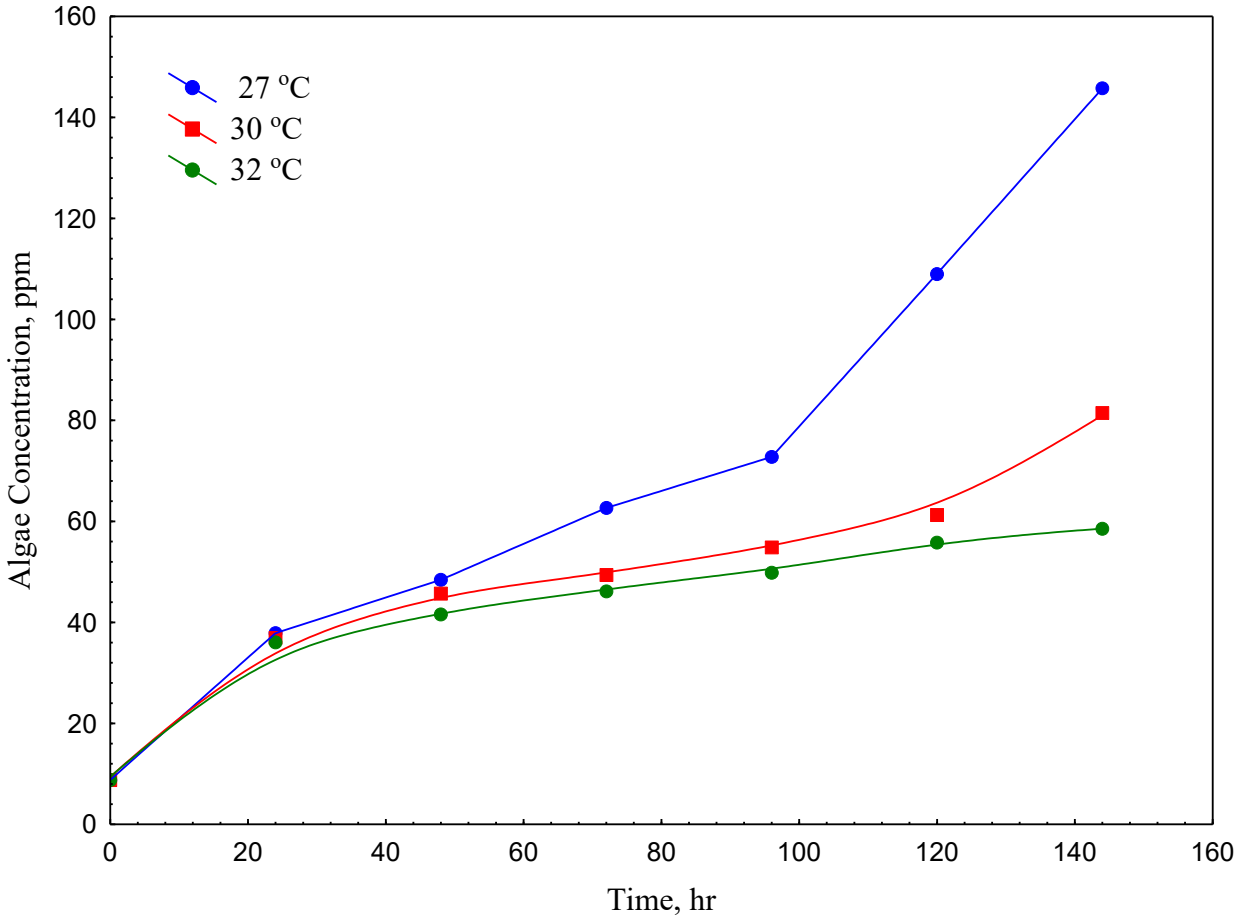

Fig.12 The relation of algae concentration and time under different temperatures at pH=8.5

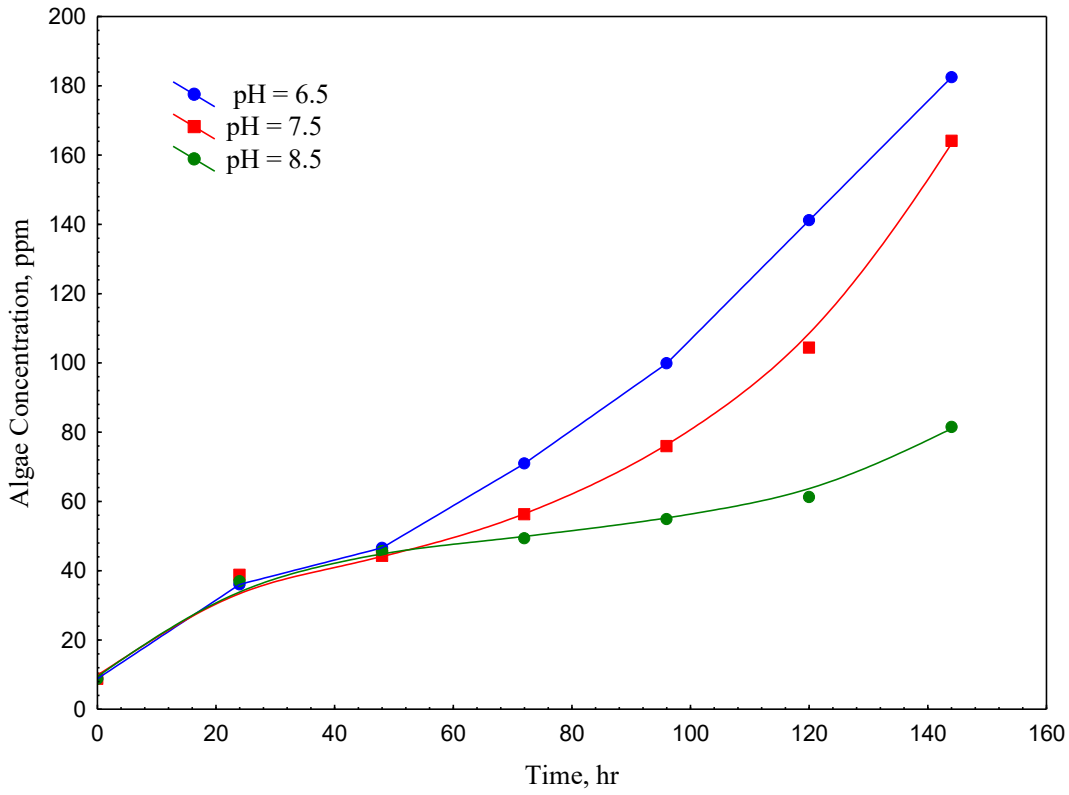

Fig.13 The relation of algae concentration with time under different pH levels at a temperature of 30 °C

3.5 Statistics and Optimization Results

In order to evaluate the individual and interactive effect of the operating conditions on the growth rate of the culture, The Box–Behnken method was applied. This approach was achieved through three stages: (i) the design of the experimental trials by a statistical model, (ii) the prediction of the response with different conditions, and (iii) the development of a mathematical model, calculating regression coefficients, and testing the model's adequacy.

The pH and temperature were designated as the independent variables, their range were varied within the ranges between 6.5–8.5 and 27–32 °C, respectively. The selected Box–Behnken design is the two factors at three levels model. The construct experimental matrix comprises of 13 randomized runs with nine factorial points and four center points. The response variable is the growth rate measured after six days.

A second-order polynomial model was generated (equation 2) by multiple regression techniques within Design Expert software, to describe the response surface. The dependent variable (growth rate) was mathematically expressed as a quadratic polynomial:

$$r\% = 0.0204 - 0.002T - 0.002pH - 0.0024T^2 \quad (2)$$

in which (r%) represents the growth rate. The selection of the second-order polynomial regression model was justified by its superior coefficient of determination (R^2) and consistency between predicted and adjusted (R^2) values, which was 0.904.

Table 1 summarized the obtained experimental results from the Box–Behnken runs, while Figure 14 illustrated the corresponding response surface plots. The graph demonstrated that the growth rate enhanced by lowering both pH and temperature till reaching an optimum at specific operating points. Indeed, the comparison between experimental and predicted growth rate values (Figure 15) revealed that most data points aligned closely with the 45° diagonal line, indicating minimal deviation between observed and model-predicted responses. The close alignment confirms the robustness of the model indicating a strong correlation between experimental results and quadratic predictions.

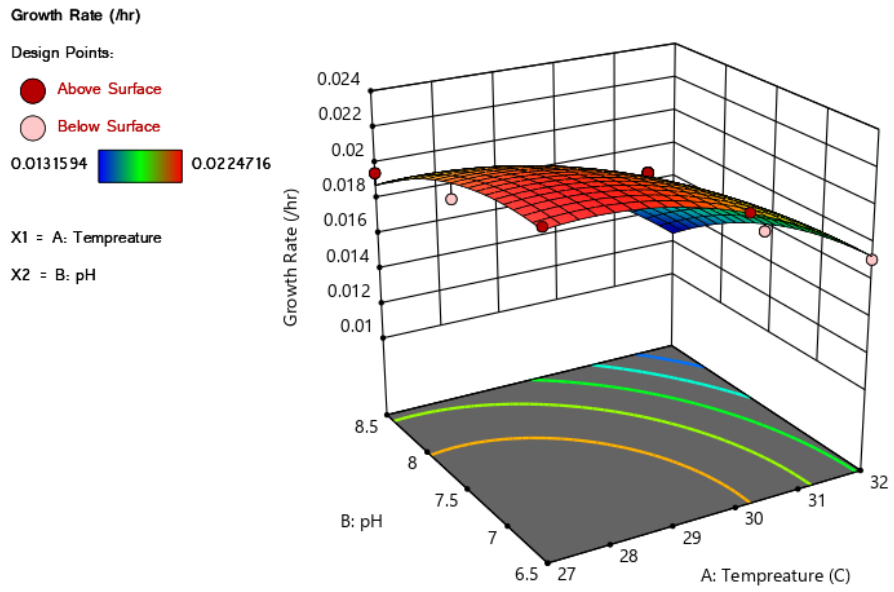

Fig.14: The 3D response surface plots

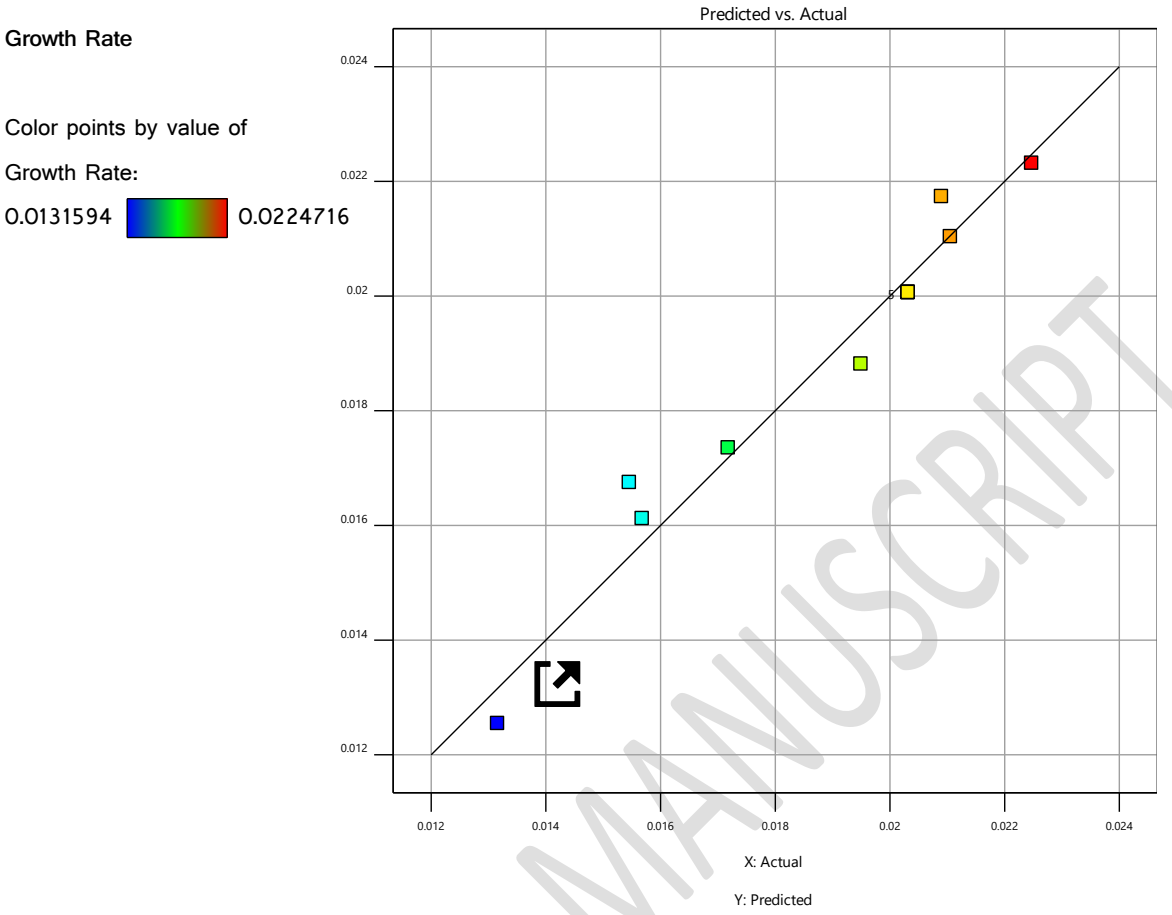

Fig.15: The experimental values vs. the predicted values

Table(1): The experimental and predicted values of growth rate

Run	Temperature, °C	pH	Experimental Growth Rate /hr	Predicted Growth Rate /hr
1	30	7.5	0.02032	0.0201
2	27	8.5	0.01949	0.0188
3	32	7.5	0.01568	0.0161
4	30	8.5	0.01546	0.0168

5	30	7.5	0.02032	0.0201
6	27	6.5	0.02247	0.0223
7	30	7.5	0.02032	0.0201
8	30	6.5	0.02105	0.021
9	30	7.5	0.02032	0.0201
10	32	8.5	0.01316	0.0125
11	27	7.5	0.0209	0.0217
12	32	6.5	0.01718	0.0174
13	30	7.5	0.02032	0.0201

The statistical model was further confirmed through ANOVA, the results are summarized in Table 2. A p-value below 0.05 showed that the regression model was statistically significant. The F-values indicate that temperature exhibited a stronger influence than pH on growth rate, among the two factors.

The central goal of this response surface methodology (RSM) analysis was to determine the optimal operating conditions that maximize algal growth rate. Numerical optimization of the model explored the design space and identified the most favorable configuration. The optimum conditions were determined to be pH 6.58 and temperature 27.57 °C, achieving a maximum predicted growth rate of 0.0225 hr^{-1}, as illustrated in Figure 16.

Table 2. The ANOVA analysis of the model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.0001	5	0	31.84	0.0001	significant

A-Temperature	0	1	0	87.52	< 0.0001	
B-pH	0	1	0	47.6	0.0002	
AB	4.32E-07	1	4.32E-07	0.7983	0.4013	
A^2	7.81E-06	1	7.81E-06	14.45	0.0067	
B^2	3.78E-06	1	3.78E-06	7	0.0332	
Residual	3.78E-06	7	5.41E-07			
Lack of Fit	3.78E-06	3	1.26E-06			
Pure Error	0	4	0			
Cor Total	0.0001	12				

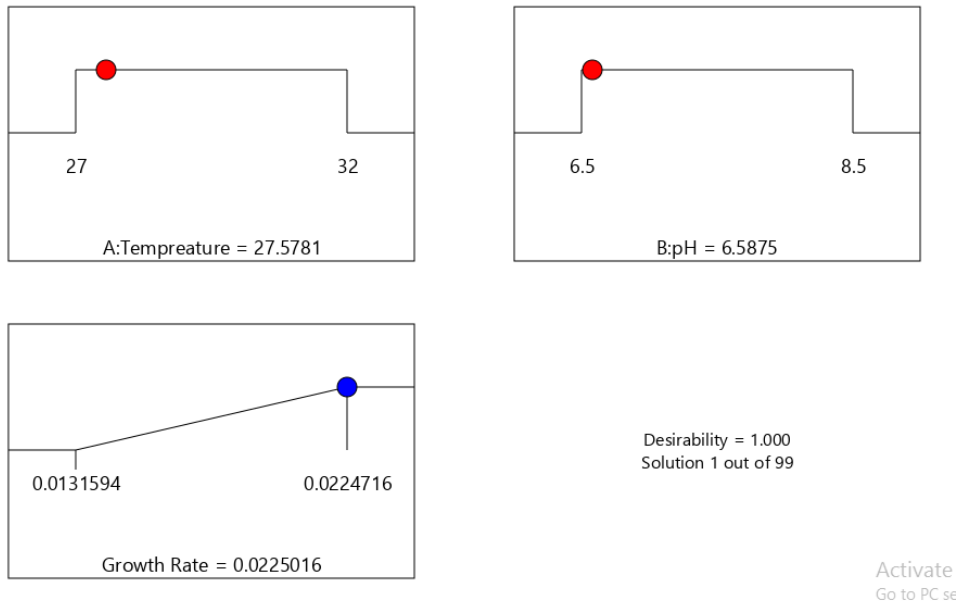

Fig. 16: The optimization results

Conclusion

This study reveals the promising use of Chlorella vulgaris bio-factory capability for sustainable hydrogen production via eco-efficient cultivation techniques. The significance of the study appears in the effective combination of optimized growth conditions and controlled phosphate limitation to effectively alleviate the trade-off between algal growth and hydrogen production. The NPK medium was very appreciable for algae biochemistry, whereas violet light exposure at an intermediate intensity level contributed the most for the favorable photon surroundings within photosynthetic processes and hydrogenase enzyme-catalyzed hydrogen production. The Box-Behnken approach for statistical optimization confirmed the importance of sustaining the growth process around a neutral pH level of about 7 and an intermediate temperature level of about 27°C for optimal growth rate attainment. In light of the above discussions, the combined importance of biostress conditions, photoreceptors, and thermoregulation represents an effective synergistic role within the stimulation of enhanced hydrogenase activity at the cell level for successful bio-hydrogen production processes via hybrid photobiological processes for the sustainable production of hydrogen for the transition towards green energy conversion technologies. Future studies should therefore concentrate on the integration of the process at the photobioreactor level and the genetic engineering of algae for enhanced bio-hydrogen production.

Acknowledgments: The authors would like to thank the Chemical Engineering Department at the University of Technology for their support.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: Not applicable.

Ethical approval: Not applicable.

Informed consent: Not applicable.

References

1. Hou, Z.; Liu, J.; Pang, J. “Green Transition and Climate Adaptation: Empirical Evidence of Corporate Resilience in the Low-Carbon Economy,” *Global NEST Journal* 2025, 27(6), 1–22. https://doi.org/10.30955/gnj.07421.
2. Ntinyari, W., and Gweyi-Onyango, J.P. (2020). Greenhouse gas emissions in agricultural systems and climate change effects in Sub-Saharan Africa. In African Handbook of Climate Change Adaptation (pp. 1–25). Cham: Springer International Publishing.
3. Wang, J., and Azam, W. (2024). Natural resource scarcity, fossil fuel energy consumption, and total greenhouse gas emissions in top emitting countries. Geoscience Frontiers , 15(2), 101757. https://doi.org/10.1016/j.gsf.2023.101757
4. Naik, S.N., Goud, V.V., Rout, P.K., and Dalai, A.K. (2010). Production of biofuels from renewable sources: A review. Renewable and Sustainable Energy Reviews , 14, 578–597.
5. Alalwan, H.A., Alminshid, A.H., and Aljaafari, H.A. (2019). Promising evolution of biofuel generations: A subject review. Renewable Energy Focus , 28, 127–139.
6. Aslan, S., and Kapdan, I.K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecological Engineering , 28, 64–70. https://doi.org/10.1016/j.ecoleng.2006.04.003
7. Zhang, L., Wang, Y., and Liu, H. (2024). Recent advances in sustainable hydrogen production from microalgae: Mechanisms, challenges, and future perspectives. Ecotoxicology and Environmental Safety , 268, 115908. https://doi.org/10.1016/j.ecoenv.2023.115908
8. Kumar, S., Patel, A., and Singh, M. (2021). Biohydrogen production from microalgae—Major bottlenecks and future research perspectives. Biotechnology Journal , 16(3), 2000124. https://doi.org/10.1002/biot.202000124
9. Neihaya Z. Heikmat; Ghaidaa H. Abd Ali; Shurooq L. Al-Razaq; Nada Z. Mahdi. (2019). Biofilm Inhibitory Potential of Westiellopsis prolifica Extract Against Some Pathogenic Microorganisms.37,1C,Page 101-108. https://doi.org/10.30684/etj.37.1C.16.

10. Marwa M. Jasim; Thamer J. Mohammed; Laith S. Sabri (2022). Air-lift Reactor's Characterization via Computational Fluid Dynamic (CFD): Review. 40, 3 , Page 484-497. https://doi.org/10.30684/etj.v40i3.2261

11. Sharma, A., Gupta, R., and Verma, K. (2022). Current prospects and future developments in algal bio-hydrogen production: A review. Biomass Conversion and Biorefinery , 12, 1414–1432. https://doi.org/10.1007/s13399-021-01414-z
12. Johnson, M., Brown, S., and Davis, L. (2023). Comparative analysis of hydrogen production pathways in green microalgae. Renewable Energy , 187, 445–458.
13. Chandrasekhar, T., Obul Reddy, P.C., Swapna, B., Veeranjaneya Reddy, L., Anuprasanna, V., Dakshayani, L., Ramachandra Reddy, P., and Reddy, M.C. (2024). Algae: The game-changers in biohydrogen sector. Critical Reviews in Biotechnology , 1, 1–18. https://doi.org/10.1080/07388551.2024.2387176
14. Jiao, Y., Wang, X., and Zhang, C. (2024). Recent advances in microalgal hydrogen production: Mechanisms, hybrid systems, and process integration. Renewable and Sustainable Energy Reviews , 194, 113222. https://doi.org/10.1016/j.rser.2024.113222
15. Klähn, S., and Hagemann, M. (2024). Synthetic biology approaches to engineer cyanobacteria and algae for biohydrogen production. Biotechnology Advances , 67, 108183. https://doi.org/10.1016/j.biotechadv.2024.108183
16. Alaa Kareem Mohammed; Safaa Abdalrasool Ali; Ibtisam Fareed Ali (2016). Using locally isolated Chlorella vulgaris in Wastewater Treatment. Eng. And Techno. Journal. 34, 4A , Page 762-768. https://doi.org/10.30684/etj.2016.112937.

17. Singh, A., and Sharma, Y. (2025). Hybrid microalgal–fermentation systems for biohydrogen production and wastewater treatment: A review. Energy Reports , 11, 512–529. https://doi.org/10.1016/j.egyr.2025.01.045
18. Zhang, H., and Chen, Y. (2024). Coupling dark fermentation and microbial electrolysis with algal biomass: Current status and perspectives. Bioresource

Technology , 395, 130105. https://doi.org/10.1016/j.biortech.2024.130105

19. Nagy, T., Tóth, T.N., and Kovács, L. (2024). Engineering Chlamydomonas strains for sustained hydrogen evolution under nutrient stress and hybrid processes. Algal Research , 77, 103203. https://doi.org/10.1016/j.algal.2024.103203
20. Saifuddin, N., Ong, M.Y., and Priatharsini, P. (2016). Optimization of photosynthetic hydrogen gas production by green alga in sulfur-deprived conditions. Indian Journal of Science and Technology , 9(40), 1–13. https://doi.org/10.17485/ijst/2016/v9i40/93390
21. Sasi, S. (2009). Cultivation and biomass estimation of Chlorella vulgaris . MSc Thesis.
22. Wang, Y., Li, Z., Zhao, J., and Chen, D. (2016). Algal biomass quantification using OD calibration in airlift photobioreactors. Biotechnology and Bioengineering , 113(6), 1205–1213.
23. Melis, A. (2002). Green alga hydrogen production: Progress, challenges, and prospects. International Journal of Hydrogen Energy , 27(11–12), 1217–1228.
24. Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S., and de Lasa, H. (2013). Integrated CO_2 capture, wastewater treatment, and biofuel production by microalgae culturing—A review. Renewable and Sustainable Energy Reviews , 27, 622–653.
25. Singh, A., Nigam, P.S., and Murphy, J.D. (2011). Renewable fuels from algae: An answer to debatable land-based fuels. Bioresource Technology , 102(1), 10–16.
26. Wang, B., Li, Y., Wu, N., and Lan, C.Q. (2008). CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology , 79(5), 707–718.
27. Kumar, M.S., Miao, Z., and Wyatt, S.K. (2020). Nutrient stress-induced metabolic reprogramming in microalgae for enhanced biofuel production. Frontiers in Energy Research , 8, 60. https://doi.org/10.3389/fenrg.2020.00060
28. Zhang, Y., Kong, X., Wang, Z., and Ma, Y. (2018). Effect of nutrient concentrations on the growth and lipid accumulation of microalgae. Renewable Energy , 127, 246–253. https://doi.org/10.1016/j.renene.2018.04.064

29. Cheng, P., Ji, B., Gao, L., Zhang, W., Wang, J., and Liu, T. (2019). The growth, lipid accumulation, and antioxidant system of Chlorella vulgaris under different nitrogen levels. Biotechnology for Biofuels , 12, 33. https://doi.org/10.1186/s13068-019-1381-2
30. El-Sheekh, M.M., El-Kassas, H.Y., Eladel, H., and Matter, I. (2019). Algal responses to nutrient enrichment: Growth and biochemical composition. Environmental Technology & Innovation , 16, 100444. https://doi.org/10.1016/j.eti.2019.100444
31. Li, T., Zheng, Y., Yu, L., and Chen, S. (2019). Mixotrophic cultivation of Chlorella vulgaris for lipid production: Balancing nutrient limitation and stress. Bioresource Technology , 289, 121617. https://doi.org/10.1016/j.biortech.2019.121617
32. Ghirardi, M.L., Zhang, L., Lee, J.W., Flynn, T., Seibert, M., Greenbaum, E., and Melis, A. (2007). Microalgae: A green source of renewable H_2. Trends in Biotechnology , 18(12), 506–511. [https://doi.org/10.1016/S0167-7799(00)01840-4](https://doi.org/10.1016/S0167-7799%2800%2901840-4)
33. Kosourov, S., Seibert, M., and Ghirardi, M.L. (2012). Effects of extracellular pH on the metabolic pathways in green algae for hydrogen production. International Journal of Hydrogen Energy , 37(11), 8850–8860. https://doi.org/10.1016/j.ijhydene.2012.02.086
34. Melis, A., Zhang, L., Forestier, M., Ghirardi, M.L., and Seibert, M. (2000). Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga Chlamydomonas reinhardtii . Plant Physiology , 122(1), 127–136. https://doi.org/10.1104/pp.122.1.127
35. Arora, N., Patel, A., Mehtani, J., Pruthi, V., and Pruthi, P.A. (2021). Hydrogen production by microalgae: A review on enhancement approaches. International Journal of Hydrogen Energy , 46(1), 344–368. https://doi.org/10.1016/j.ijhydene.2020.08.180
36. Dasgupta, C.N., Singh, A., Mallick, N., and Kaushik, B.D. (2019). Role of nutrient stress in hydrogen production by algae. Renewable and Sustainable Energy Reviews , 104, 1–12.

https://doi.org/10.1016/j.rser.2019.01.008
37. Richmond, A. (2004). Handbook of Microalgal Culture: Biotechnology and Applied Phycology . Oxford: Blackwell Science.
38. Melis, A. (2002). Green alga hydrogen production: Progress, challenges, and prospects. International Journal of Hydrogen Energy , 27(11–12), 1217–1228.
39. Chen, M., and Blankenship, R.E. (2011). Expanding the solar spectrum used by photosynthesis. Trends in Plant Science , 16(8), 427–431.
40. Wang, C.Y., Fu, C.C., and Liu, Y.C. (2007). Effects of using light-emitting diodes on the cultivation of Spirulina platensis . Biochemical Engineering Journal , 37(1), 21–25.
41. Kosourov, S., Seibert, M., and Ghirardi, M.L. (2003). Effects of extracellular pH on the metabolic pathways of hydrogen production in green algae. Biotechnology and Bioengineering , 78(7), 731–740.
42. Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., and Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chemical Engineering and Processing: Process Intensification , 48(6), 1146–1151.
43. Mandal, S., and Mallick, N. (2011). Microalga Scenedesmus obliquus as a potential source for biodiesel production. Applied Microbiology and Biotechnology , 89(3), 583–591.
44. Lee, Y.K., and Shen, H.M. (2013). Basic principles of cultivation and nutrition of microalgae. In Richmond, A., and Hu, Q. (Eds.), Handbook of Microalgal Culture: Applied Phycology and Biotechnology (2nd ed.). Wiley-Blackwell.
45. Markou, G., and Georgakakis, D. (2011). Cultivation of filamentous cyanobacteria (Spirulina) in agro-industrial wastes and wastewaters: A review. Applied Energy , 88(10), 3389–3401.