

ՀՀ ԿՐԹՈՒԹՅԱՆ, ԳԻՏՈՒԹՅԱՆ, ՄՇԱԿՈՒՑԹԻ ԵՎ ՍՊՈՐՏԻ
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ՄԱՆՈՅԱՆ ՋԵՄՄԱ ԳՈՒՐԳԵՆԻ

ՖԻԶԻԿԱՔԻՄԻԱԿԱՆ ԳՈՐԾՈՆՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ *PARACHLORELLA*
KESSLERI և *CHLORELLA VULGARIS* ԿԱՆԱԶ ՋՐԻՄՈՒՌՆԵՐՈՒՄ ՋՐԱԾՆԻ
ԼՈՒՍԱԿԱԽՅԱԼ ԱՐՏԱԴՐՈՒԹՅԱՆ ՎՐԱ

Գ.00.04 - Կենսաքիմիա մասնագիտությամբ
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գիտական աստիճանի հայցման ատենախոսության

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YEREVAN STATE UNIVERSITY

MANOYAN JEMMA GURGEN

THE EFFECT OF PHYSICOCHEMICAL FACTORS ON THE LIGHT-
DEPENDENT HYDROGEN PRODUCTION BY GREEN ALGAE
PARACHLORELLA KESSLERI AND *CHLORELLA VULGARIS*

SYNOPSIS

of dissertation for conferring of science degree of
Candidate of Biological Sciences
In the specialty of 03.00.04 - Biochemistry

YEREVAN 2025

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝

կ.գ.թ., դոցենտ Լիլիթ Սերգեյի Գաբրիելյան

Պաշտոնական ընդդիմախոսներ՝

կ.գ.դ., պրոֆ. Աստղիկ Զավենի Փեփոյան

կ.գ.թ., դոցենտ Հռիփսիմե Լյուդվիկի
Հայրապետյան

Առաջատար կազմակերպություն՝

ՀՀ ԳԱԱ Հ. Բունիաթյանի անվան
կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2025թ. դեկտեմբերի 12-ին
ժամը 15:00-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲԿԳԿ-ի
Կենսաֆիզիկայի 051 մասնագիտական խորհուրդի նիստում (0025, Երևան, Ալեք
Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությունը կարելի է ծանոթանալ Երևանի պետական համալսարանի
գրադարանում:

Ատենախոսության սեղմագիրը առաքված է 2025թ. նոյեմբերի 12-ին:

051 մասնագիտական խորհրդի գիտական
քարտուղար, կ.գ.դ., դոցենտ՝



Մ.Ա. Փարսադանյան

The theme of dissertation has been approved at Yerevan State University

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Leading organization:

H.Buniatian Institute of Biochemistry

of NAS RA

The defence of the dissertation will be held on 12th December, 2025, at 15:00, at the session of
051 Scientific Specialized Council on Biophysics of HESC of RA at Yerevan State University
(0025, Yerevan, Alex Manoogian str. 1, YSU, faculty of Biology).

The dissertation is available at the library of Yerevan State University

The synopsis has been sent on 12th November, 2025.

Scientific Secretary of 051 Specialized Council,
D.Sc., Assoc. Prof.



M.A. Parsadanyan

INTRODUCTION

Topic's significance. The increasing demand for energy around the globe has prompted a critical analysis of our dependence on conventional fossil fuels. Finding and utilizing cleaner, more sustainable energy sources is crucial as worries about climate change and environmental sustainability grow. Hydrogen (H_2) presents a viable option due to its high energy (142 MJ kg^{-1}) and low environmental impact when utilized as a fuel. In recent years, biological H_2 production has gained significant interest as a promising and eco-friendly approach with low production costs.

Green algae are photosynthetic microorganisms that exhibit high metabolic flexibility and have been widely used as producers of valuable compounds, such as carbohydrates, lipids, proteins, pigments, vitamins, etc. Recently, they have gained attention for their potential in sustainable energy production. The ability of microalgae to produce H_2 as a result of light-dependent reactions was first discovered in 1942 by Gaffron and Rubin in the green alga *Scenedesmus obliquus*, marking a foundational moment in the study of H_2 production by algae. In green algae, H_2 production is linked to the electron transfer during photosynthesis and is facilitated by [FeFe]-hydrogenase. The main electron donor for this enzyme is ferredoxin, and a key condition for H_2 production is the absence of oxygen. Green algae use photosystems (PS) II and I to carry out oxygenic photosynthesis. Green algae have two pathways for H_2 production: PS II-dependent and independent. PS II-dependent pathway involves water oxidation and is commonly referred to as “direct photolysis”, while the PS II-independent pathway includes an endogenous substrate uptake. Most of the studies use *Chlamydomonas reinhardtii* as a model eukaryotic organism for H_2 generation research and the best H_2 producer; however, *Chlorella* species can be suggested as alternative organisms for sustainable H_2 generation due to their potentially high H_2 production capacity and synthesis of other by-products of high interest and commercial value, making these species more suitable for industrial scale. However, the H_2 -producing potential of another species, *Parachlorella kessleri*, has not been studied yet. Although the potential of green algae for H_2 production is well recognized, a comprehensive understanding of the mechanisms governing this process is still lacking. Current research is focused on identifying novel, high H_2 -producing algal strains, as well as optimizing H_2 production.

Green algae isolated in Armenia offer an unexploited resource for sustainable energy production. This research focuses on understanding the mechanisms of light-dependent H_2 production within green algae isolated and identified in Armenia to unlock their full potential for applications in biofuel generation. Shedding light on the biochemical processes and mechanisms involved in H_2 generation in these algae will help optimize conditions that favor increased H_2 yield for possible future large-scale applications. Moreover, this exploration aligns with the broader goals of reducing greenhouse gas emissions and transitioning towards a sustainable energy landscape, thus supporting the objectives of the European Green Deal.

Research goals and tasks. The purpose of this work is to study the properties and mechanisms of light-dependent H₂ production by green algae (*Parachlorella kessleri* and *Chlorella vulgaris*) from the *Chlorellaceae* family, isolated in Armenia, under various physicochemical conditions.

Constituted tasks of the research were:

- Determination of the growth characteristics and light-dependent H₂ production by green algae in the presence of various carbon sources.
- Study the effect of extremely high frequency electromagnetic irradiation (EHF EMI) on the growth and H₂-producing ability of green algae.
- Investigation of the effects of protonophores, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and dinitrophenol (DNP), on the H₂ production.
- Examination of the effect of biogenic elements (nitrogen and sulfur) deprivation on the light-dependent H₂ production by green algae.
- Assessment of the potential applications of ethanol and brewery waste in H₂ production by green algae.
- Clarification of the role of photosystem II (PS II) in the H₂ production process by algae under different cultivation conditions.

Scientific novelty and practical value of the study. In the current work, the effects of various physicochemical factors on the anaerobic metabolism and light-dependent H₂ production by green algae *P. kessleri* and *C. vulgaris* isolated in Armenia are investigated for the first time. It was found that acetate as a carbon source significantly stimulates H₂ production by *P. kessleri* during photoheterotrophic anaerobic growth, compared to photoautotrophic conditions. The study also revealed a stimulating effect of protonophores on H₂ yield in *P. kessleri*. This effect is attributed to the dissipation of the proton motive force in the thylakoid membrane, which increases the availability of electrons for hydrogenase via PS I. It was confirmed that protonophores suppress oxygen evolution by inhibiting PS II, thereby creating anaerobic conditions and enhancing H₂ production. Additionally, the effect of EMI at frequencies of 51.8 and 53.0 GHz on *P. kessleri* was investigated for the first time. It was shown that the response of algae to these frequencies depends on the oxygen availability and algae cultivation conditions. For the first time, the effect of sulfur and nitrogen deprivation on growth characteristics, PS activity and H₂ production by green algae isolated in Armenia was determined. H₂ production is stimulated under all tested conditions, with the highest yield of H₂ observed under combined sulfur and nitrogen deprivation. The study also examined the effect of the specific inhibitor of PS II, diuron, on *P. kessleri*, confirming that H₂ production in this species primarily follows the PS II-dependent pathway. Additionally, the potential use of ethanol and brewery wastes as cultivation media for green algae was explored. It was found that these wastes stimulated light-dependent H₂ production. Moreover, algae cultivated in waste-containing media used both PS II-dependent and independent H₂ production pathways.

The obtained results are crucial for understanding hydrogen metabolism in green algae isolated in Armenia. Furthermore, the investigations of H₂ production pathways and mechanisms, as well as the improvement of cultivation conditions for higher yields, are important steps toward unlocking the potential of algae as an energy source.

Main points to present at the defence:

1. Green alga *P. kessleri* isolated in Armenia performs light-dependent H₂ production under photoheterotrophic anaerobic conditions. A PS II-dependent H₂ production pathway operates in *P. kessleri*.
2. Protonophores stimulate H₂ production by *P. kessleri*. The dissipation of the proton gradient in the thylakoid membrane promotes the activation of the PS II-independent H₂ production pathway.
3. The effect of electromagnetic irradiation at frequencies of 51.8 and 53.0 GHz on *P. kessleri* depends on the algae's cultivation conditions and the presence of oxygen in the environment.
4. Nitrogen and sulfur deprivation stimulates light-dependent H₂ production by *P. kessleri* and *C. vulgaris*. In the H₂ production process, PS II plays a significant role as the primary electron donor.
5. The use of ethanol and brewery waste as cultivation media for *P. kessleri* and *C. vulgaris* significantly stimulates light-dependent H₂ production. Under these conditions, the PS II-independent H₂ production pathway operates.

Work approbation. Main results of the dissertation were discussed at seminars at the Department of Biochemistry, Microbiology, and Biotechnology and the Research Institute of Biology of Yerevan State University, and at scientific conferences: “V Int. Conference on Biotechnology and Health” (Yerevan, Armenia, 2020); “Lomonosov-2021” (Moscow, Russia, 2021); Scientific-Practical Conference “Biotechnology: Science and Practice, Innovation and Business” (Yerevan, Armenia, 2021); The Biochemistry Global Summit (Lisbon, Portugal, 2022); FEMS Conference on Microbiology (Belgrade, Serbia, 2022); Int. Conference “The Present and Future of Plant Biotechnology” (Minsk, Belarus, 2023); Pan-Armenian Scientific Conference-2023 (Yerevan, Armenia, 2023); 48th FEBS Congress “Mining Biochemistry for Human Health and Well-being” (Milan, Italy, 2024); Annual Summary Scientific Conference-2024 (Yerevan, Armenia, 2024); FEMS MICRO 2025 (Milan, Italy, 2025).

Publications. On the basis of the experimental data observed in the dissertation, 23 papers, including 5 articles in international peer-reviewed journals and 4 articles included in the list of HESC of the RA, as well as 14 abstracts were published.

Volume and structure of the dissertation. The dissertation contains the following chapters: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and

discussion (Chapter 3), summary, conclusions, and cited literature (a total of 151 papers and books). The dissertation consists of 136 pages, 2 tables and 33 figures.

MATERIALS AND METHODS

Research objects. Green algae *P. kessleri* RA-002, *P. kessleri* MDC6524, and *C. vulgaris* Pa-023 (Microbial Depository Center, NAS, Yerevan, Armenia) and *C. vulgaris* IBCE C-19 (Algae Collection, Institute of Biophysics and Cellular Engineering, NAS, Minsk, Belarus) were used.

Green algae cultivation conditions. Algae were grown in Tamiya medium under aerobic conditions upon illumination and shaking at 100 rpm (WideShake SHO-1D, DAIHAN Scientific, Korea) (Manoyan et al., 2022). Acetate or glucose was used as the carbon source for photoheterotrophic cultivation of algae. Algal growth was monitored by measuring the optical density at 680 nm using a Spectro UV-Vis Auto spectrophotometer (Genesys 10S UV-VIS-Thermo Fisher Scientific and UV 2700 Shimadzu). The specific growth rate (μ) was calculated as: $\mu = (\ln OD_t - \ln OD_0)/\Delta t$, where OD_0 is the initial OD_{680} , and OD_t is OD_{680} after t days. The initial pH of the algae cultivation media was adjusted to 7.5, and was measured by a pH/ORP benchtop meter (HANNA Instruments, Portugal). Redox potential was measured using a platinum electrode (EPB-1, Measuring Instruments Enterprise, Gomel, Belarus) (Manoyan et al., 2019, 2020). The cells' morphology was determined using a light microscope (Microscope XSP-136C, $\times 1000$, China) and an inverted microscope (OPTIKA IM-5, LED 8W with C-P8 digital camera, Italy) (Manoyan et al., 2025).

Determination of photosynthetic pigment content. Concentrations of pigments, including chlorophylls (Chl) *a* and *b*, and carotenoids, extracted from algae cells using 96% ethanol, were determined. Absorption spectra of extracts were recorded by a Spectro UV-Vis Auto spectrophotometer (Manoyan et al., 2022, 2025).

Determination of H₂ production. For the H₂ production assay, algae cultivated under aerobic conditions were harvested by centrifugation and transferred to Tamiya or waste-containing media as described (Manoyan et al., 2025). H₂ production assays were conducted under anaerobic conditions and illumination. H₂ yield was assessed by potentiometric and gas chromatography methods (Manoyan et al., 2024, 2025). H₂ concentration in the gas-phase was determined by standard gas chromatography techniques (GC 7820A, Agilent Technologies, USA). To identify the H₂ production pathways, the effects of the specific PS II inhibitor, diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), and protonophores, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP), were studied.

Determination of electromagnetic irradiation effects. Algal suspensions were exposed to EMI with a frequency of 51.8 and 53.0 GHz using a high-frequency EMI generator (model G4-141) with a conical antenna (Scientific-Production Enterprise "Istok", Fryazino, Moscow Region, Russia) (Manoyan et al., 2020).

Determination of photochemical activity of photosystems. Photochemical activity of PSs was recorded using a PAM-fluorometer (DUAL-PAM 100, Heinz Walz, Germany) (Manoyan et al., 2022, 2024).

Physicochemical analysis of waste. The FTIR spectra of algae biomass, untreated wastes, and algae cultivated in waste-containing media were acquired using a Nicolet™ iS50 FTIR spectrometer (Manoyan et al., 2025). High-performance liquid chromatography (HPLC, Agilent 1260 Infinity, Germany) was used to analyze the composition of waste, specifically sugars and organic acids (Manoyan et al., 2025).

Determination of chemical oxygen demand (COD) and total nitrogen content in waste. Total nitrogen concentration was determined by the Kjeldahl method, and COD was determined as described (Manoyan et al, 2025).

Chemicals and data processing. Various reagents of analytical grade were used to perform this research. The data were collected from at least three to four independent experiments. Standard deviations were calculated using Microsoft Excel. The statistical significance of the obtained results was confirmed using single-factor ANOVA analysis or Student's t-test (p); the difference is valid if $p \leq 0.05$.

RESULTS AND DISCUSSION

Growth characteristics and H₂ production by green algae *Parachlorella kessleri* in the presence of different carbon sources

The choice of carbon source is important for the cultivation of photosynthetic microorganisms, as it affects both their growth and H₂ yield. Selecting carbon sources that are efficiently utilized by algae can enhance biomass accumulation and stimulate H₂ production. In this study, the growth and H₂ photoproduction by *P. kessleri* were investigated in the presence of various organic carbon sources (acetate, glucose, and fructose). Algae were cultivated under aerobic conditions in Tamiya medium with shaking at 100 rpm and upon illumination of 2000 lux. The optimal concentration of the organic carbon source was found to be 1 g L⁻¹. The addition of organic carbon sources promoted a high biomass yield of algae. The composition of algae's photosynthetic pigments is sensitive to environmental conditions. *P. kessleri* grown in acetate-supplemented medium exhibited the highest levels of Chl *a* and Chl *b*, ~1.2 times higher than in cultures grown with glucose (Fig. 1a). In contrast, carotenoid content was the highest in cultures grown with fructose, being 1.2–1.4 times greater than in those grown with acetate or glucose (Fig. 1a).

The effect of organic carbon sources on the H₂ production by *P. kessleri* during anaerobic growth was examined. H₂ production studies were conducted under anaerobic conditions, because green algae do not produce H₂ under aerobic conditions (Manoyan, Gabrielyan, 2018). *P. kessleri* produced H₂ in the presence of all three organic carbon sources tested. H₂ production under photoautotrophic anaerobic conditions was negligible compared to that under

photoheterotrophic conditions (Fig. 1b). Maximum H₂ yield (~1.4 mmol L⁻¹) was observed in cultures grown with acetate (Fig. 1b). A similar effect of acetate on H₂ production by *Ch. reinhardtii* was reported by Kosourov and co-authors (2007). This enhancement in H₂ yield is likely due to oxygen uptake and the induction of [FeFe]-hydrogenase, which is active only under anaerobic conditions. Green algae in order to generate ATP perform “anaerobic oxygenic photosynthesis”, where the oxygen produced during photosynthesis is consumed by respiration, creating anaerobic conditions that activate [FeFe]-hydrogenase and initiate H₂ generation.

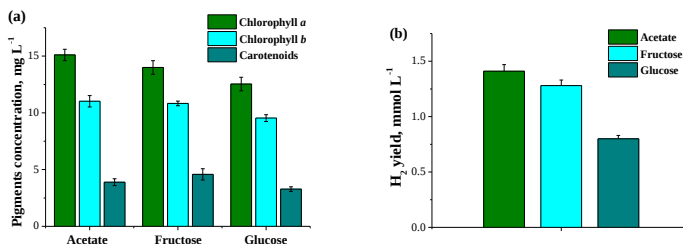


Fig. 1. Effect of carbon sources on the concentration of photosynthetic pigments (a) and light-dependent H₂ production (b) by *P. kessleri* RA-002 ($n=3$; $p<0.05$).

The pathways of H₂ production by *P. kessleri* have not been explored yet. To explore these routes, the effects of protonophores: CCCP and DNP, as well as a PS II-specific inhibitor, diuron, have been investigated. Both protonophores used show a positive effect on H₂ production by *P. kessleri* during growth under anaerobic conditions (Fig. 2a). The maximal H₂ yield was obtained in the presence of 15 μ M CCCP and 50 μ M DNP, which was three-fold higher in comparison with the control without protonophores addition (Fig. 2a). Other studies have also noted the enhancing effect of CCCP on H₂ production by *Ch. reinhardtii*, *Platymonas subcordiformis*, and *Aphanoteche halophyta* (Yang et al., 2014; Pansook et al., 2019). During dark conditions, H₂ production by this alga was not observed even in the presence of protonophores, indicating that H₂ generation in *P. kessleri* was mediated by light.

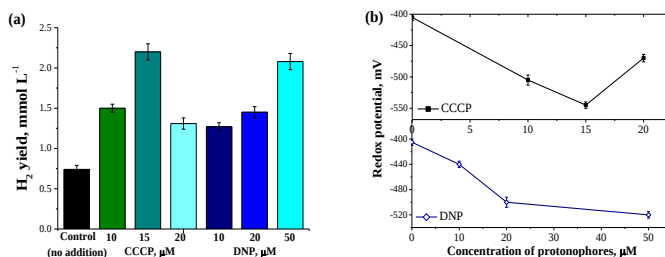


Fig. 2. Effect of protonophores on H₂ production (a) and redox potential (b) in *P. kessleri* ($n=4$, $p<0.05$).

The effects of CCCP and DNP on the redox potential of the *P. kessleri* culture medium were also examined (Fig. 2b). Samuilov with co-workers (1995) suggested that some protonophores can act as redox carriers, interacting with components of the photosynthetic electron transport chain. This interaction led to a decrease in O₂ production and the creation of anaerobic conditions. In the presence of 15 μ M CCCP and 50 μ M DNP, the maximal negative values of redox potential were obtained. The change of redox potential kinetics was coupled with the maintenance of anaerobic conditions and H₂ formation in *P. kessleri*, as well as with the synthesis of various end-products (Manoyan et al., 2019).

The enhancing effect of protonophores on the H₂ production can be coupled with dissipation of proton motive force (Δp) through thylakoid membrane in *P. kessleri*, increasing the availability of protons and electrons to [FeFe]-hydrogenase, because hydrogenase is located in chloroplast stroma in green algae (Fig. 3). This hydrogenase can work as an additional sink under anaerobic conditions. Protonophores can also decrease O₂ evolution via suppressing PS II activity and the water-splitting complex. In this case, respiration is retained via inactivation of water-splitting complex, resulting in the creation of anaerobic conditions, expression of hydrogenase activity, and H₂ formation via PS I (Fig. 3). Diuron is a specific inhibitor of PS II activity that blocks electron transfer from PS II to plastoquinone (Manoyan et al., 2019, 2024). H₂ production was not observed in the presence of diuron. The results showed that in this alga operates a PS II-dependent H₂ production pathway (Fig. 3).

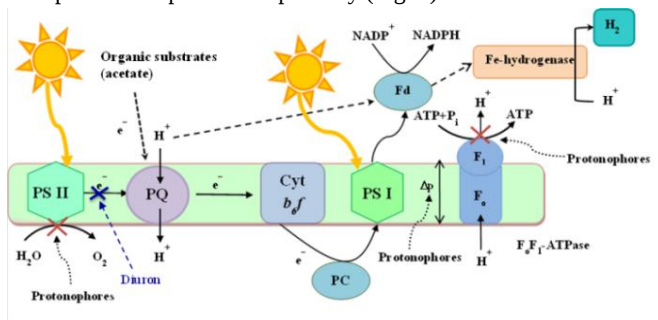


Fig. 3. Schematic representation of the proposed mechanisms of protonophores and diuron action on H₂ production by *P. kessleri* RA-002 (Manoyan et al., 2019).

The effect of high-frequency electromagnetic irradiation on the growth properties and biohydrogen production by *Parachlorella kessleri*

In this work, the effects of low-intensity EMI of extremely high frequency (51.8 and 53.0 GHz) on the growth rate and content of photosynthetic pigments of green algae *P. kessleri* during growth under aerobic and anaerobic conditions have been investigated. The choice of these frequencies is coupled with the resonance of water molecules, since the effects of EMI can be mediated by water (Manoyan et al., 2020).

After irradiation of *P. kessleri* by EMI with 51.8 and 53.0 GHz for 1 h, an increase in the specific growth rate (under aerobic conditions) was observed compared to non-irradiated control cells (Fig. 4a). The effect of EMI depended on the EMI frequency: 51.8 GHz led to a more pronounced (43%) increase in the growth rate compared to 53.0 GHz. Anaerobic growth conditions caused a decrease in the growth rate by 26% and 35% for 51.8 and 53.0 GHz, respectively (Fig. 4a). To reveal the mechanisms of EMI action, the absorption spectra of cell extracts in 96% ethanol were obtained for algae, cultivated under aerobic conditions (Fig. 4b). After irradiation an increase in the levels of photosynthetic pigments was observed for both frequencies (Fig. 4b). The frequency of 51.8 GHz gives a more pronounced effect than EMI with 53.0 GHz.

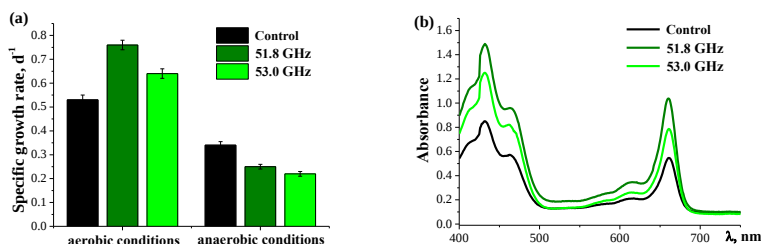


Fig. 4. Effect of EMF at frequencies of 51.8 and 53.0 GHz on the specific growth rate (a) and the absorbance spectra (b) of *P. kessleri* RA-002. Control was a non-irradiated culture of *P. kessleri* ($n=3$, $p<0.05$).

The effects of EMI on the content of photosynthetic pigments in *P. kessleri* grown under aerobic and anaerobic conditions are shown in Fig. 5. Under aerobic conditions, after irradiation of algae by EMI with a frequency of 51.8 GHz the content of Chl *a* and Chl *b* was increased 1.5- and 1.7-fold, respectively, while at 53.0 GHz the concentration of Chls was not significantly increased (Fig. 5a). However, the total carotenoid content enhanced 2 and 1.76 fold for 51.8 and 53.0 GHz frequencies, respectively (Fig. 5a). The enhancing effect of EMI on other photosynthetic organisms has been reported. In cyanobacteria *Spirulina platensis* at a wavelength of 7.10 mm, the intensification of photosynthesis was observed, accompanied by an increase in the production of biomass and the amount of Chl *a* (Pakhomov et al., 1998; Tambiev et al., 2012). Under anaerobic conditions, EMI caused a considerable decrease in the content of Chls and total carotenoids (Fig. 5b). The frequency of 53.0 GHz demonstrates the maximal inhibitory effect: the pigment concentration decreases ~3 fold in comparison with the control. Thus, a study of the EMI effects on the growth properties of *P. kessleri* has shown that the effects depend on assay conditions (Manoyan et al., 2020). The data indicate a significant role of O₂, since the enhancing effect of EMI on green algae was observed only under aerobic conditions. Moreover, under anaerobic conditions, an inhibition of the algal growth rate and a decrease in the pigments amount were obtained.

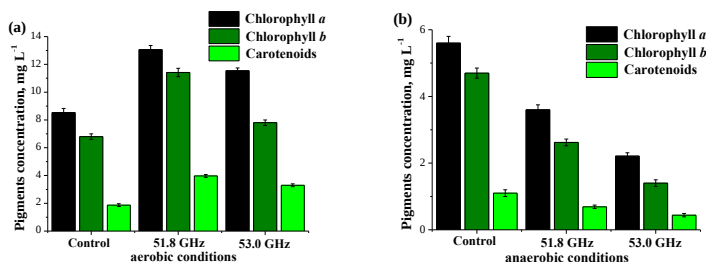


Fig. 5. Effect of EMF at frequencies of 51.8 and 53.0 GHz on the photosynthetic pigments content in *P. kessleri* RA-002 grown under aerobic (a) and anaerobic (b) conditions. Control was a non-irradiated culture of *P. kessleri* ($n=3$, $p<0.05$).

H₂ production by a non-irradiated *P. kessleri* was determined within 3-5 days of anaerobic growth (Fig. 6). Irradiation of algae inhibited H₂ production by *P. kessleri* during 3-4 days of anaerobic growth. However, the yield of H₂ partially recovered after 5 days of growth, indicating the existence of protective mechanisms in the studied algae (Fig. 6). But the yield of H₂ was 3-fold lower compared with the non-irradiated control, which suggests a suppression of the activity of the responsible enzyme, [FeFe]-hydrogenase. Similar data were shown for various microorganisms, such as *Escherichia coli* and *Rhodobacter sphaeroides* (Soghomonyan et al., 2016).

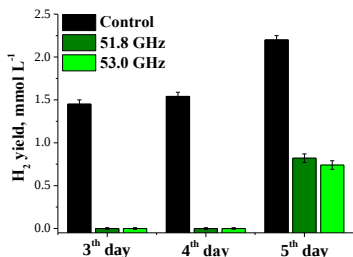


Fig. 6. The effects of EMI at frequencies of 51.8 and 53.0 GHz on the H₂ yield in *P. kessleri* RA-002, cultivated under anaerobic conditions. Control was a non-irradiated culture of *P. kessleri* ($n=3$, $p<0.05$).

Thus, the response of *P. kessleri* to high frequency EMI is a complex process, which depends on the algae cultivation conditions and the presence of O₂ (Manoyan et al., 2020). The results indicate that the EMI affects the growth rate of algae, the level of photosynthetic pigments, and H₂ yield, which may be associated with the changes in membrane properties, as well as with the energy conversion processes. The changes in water structure during the irradiation at resonant frequencies can also affect the structure and properties of the membrane. The results obtained can be used to regulate the production of biomass and pigment formation in green algae.

Growth characteristics and biohydrogen production by green algae under nitrogen- and sulfur-deprived conditions

In the present study, for the first time, for green algae *P. kessleri* MDC6524 and *C. vulgaris* IBCE C-19, isolated in Armenia and Belarus, respectively, the combined effect of sulfur (S) and nitrogen (N) deprivation was investigated, and it was compared with the individual effects of deprivation of only one of these elements. For the creation of a N-deprived medium, KNO₃ was omitted from Tamiya medium, and in S-deprived media, all sulfates were replaced by chlorides (Manoyan et al., 2024). The growth rates and photosynthetic pigment contents of *C. vulgaris* and *P. kessleri* were analyzed under growth conditions with N or S deprivation and combined N and S deprivation. The specific growth rates of *C. vulgaris* and *P. kessleri* were $0.49 \pm 0.02 \text{ d}^{-1}$ and $0.45 \pm 0.02 \text{ d}^{-1}$, respectively, after 48 h of cultivation in standard Tamiya media upon illumination. N and S deprivation inhibited the growth rate of *C. vulgaris* by ~1.3 and ~1.7 times, respectively; similar results were obtained for *P. kessleri* (Fig. 7). Combined deprivation of S and N inhibited *C. vulgaris* and *P. kessleri* growth rates by ~4 and ~3.5 times, respectively (Fig. 7).

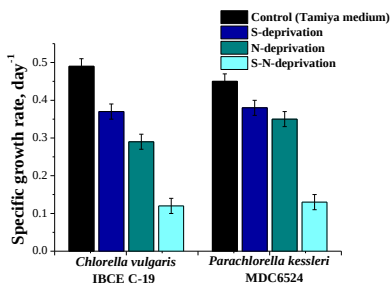


Fig. 7. Specific growth rates of *C. vulgaris* IBCE C-19 and *P. kessleri* MDC6524 cultivated under S- and N-deprived conditions ($n=3$, $p \leq 0.05$). Culture of algae grown on a standard Tamiya medium served as controls.

Deprivation of N and S significantly decreased the content of photosynthetic pigments in both algae. *C. vulgaris* showed a greater loss of chlorophyll pigments under N or both N and S deprivation (~35% loss) as compared to S deprivation treatment (21% loss) (Fig. 8a). The total carotenoid content decreased by ~20 % under combined N and S deprivation. In *P. kessleri* cells, deprivation of N decreased the content of Chl *a* and Chl *b* by ~20% relative to the control and did not change the levels of carotenoids (Fig. 8b). The absence of S in the nutrient medium had a less significant effect. The largest decrease (35%) in Chl *a* and Chl *b* in *P. kessleri* was registered under combined N and S deprivation (Fig. 8b). In both algae, the Chl *a/b* ratio did not change significantly. A decrease in the level of pigments indicates potential significant modifications in the functioning of the photosynthetic apparatus of the tested algae species. PAM-fluorescence analyses were used to evaluate the photochemical activity of two photosystems in nutrient-deprived conditions. Under anaerobic conditions, nutrient deprivation decreased the photochemical activity of PS II in both algae, whereas no significant effect of deprivation on PS I activity was found.

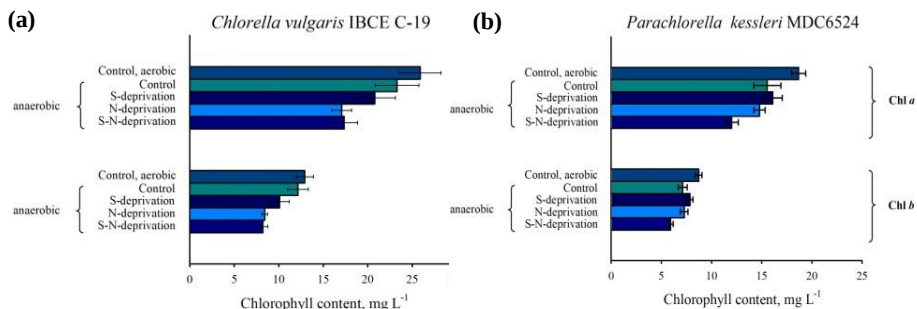


Fig. 8. Changes in the amounts of chlorophyll a and b in *C. vulgaris* IBCE C-19 (a) and *P. kessleri* MDC6524 (b) grown in S- and N-deprived media ($n=3$, $p \leq 0.05$).

H₂ production as a result of PS II dysfunction is widely discussed. The H₂ yield in *Ch. reinhardtii* significantly increased as a result of the partial suppression of PS II activity (Antal et al., 2020). Our data indicate that in *C. vulgaris* and *P. kessleri*, the nutrient deprivation leads to the suppression of PS II photochemical reactions, which, in the absence of the effect on PS I activity, stimulates the H₂ production by algal cells (Manoyan et al., 2022, 2024). It was shown that both algae demonstrated the ability to generate H₂ under S and N deprivation in anaerobic conditions. H₂ production by both algae started after 24 h and was detected at least during 96 h with a maximum level at 48-72 h. H₂ production was stimulated in all cases of nutrient deprivation, with the most significant H₂ yield detected in combined S- and N-deprived media (Fig. 9). Under these conditions, H₂ yield reached the maximal levels (*C. vulgaris*: 60 mL L⁻¹; *P. kessleri*: 68 mL L⁻¹), which were 3-4 times greater than control. Overall, the H₂ yield in *P. kessleri* was higher compared to that of *C. vulgaris* under all tested conditions (Fig. 9).

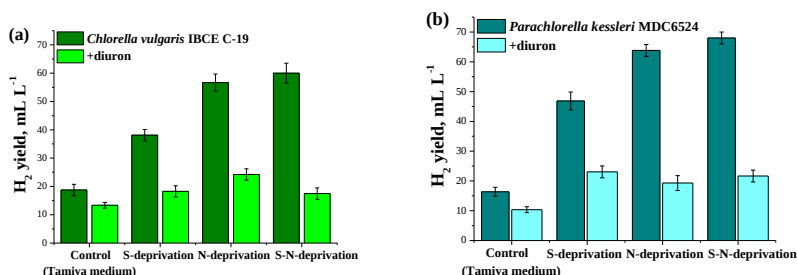


Fig. 9. H₂ production by *C. vulgaris* IBCE C-19 (a) and *P. kessleri* MDC6524 (b) cultivated under S- and N-deprived conditions ($n=3$, $p \leq 0.05$). Cultures grown in standard Tamiya medium served as controls.

Thus, S and N deprivation in algae can lead to an increase in H₂ production due to [FeFe]-hydrogenase induction. Nutrient deprivation triggers metabolic changes that include upregulation of hydrogenase enzymes. Algae undergo a shift in their metabolic pathways, favoring the fermentation of organic compounds. As a result, excess reducing equivalents, such as reduced NADH, are generated within the cells. The induced hydrogenase enzymes then utilize these excess reducing equivalents to catalyze the production of H₂. In other words, the H₂ generation provides an alternative route for algae to dispose of excess reducing equivalents and maintain redox balance under nutrient stress.

Two pathways, such as PS II-dependent and independent, provide light-dependent H₂ production by green algae. Diuron has been used to determine the pathway of H₂ generation in algae tested under nutrient deprivation. Diuron inhibited the H₂ production by *C. vulgaris* IBCE C-19 and *P. kessleri* MDC6524 showing the involvement of PS II in this process (Fig. 9). Addition of diuron to *C. vulgaris* and *P. kessleri* cultivated under N and S deprivation conditions decreased H₂ yield by up to 70% (Fig. 9). Overall, this indicates that H₂O is the main electron donor for hydrogenase in these algae and that up to 70% of electrons for H₂ generation are provided by PS II, whereas the PS II-independent pathway possibly donates only 30% of electrons (Manoyan et al., 2024). The same results were reported by Zhang and co-workers for *C. protothecoides* grown under N-limited and S-deprived conditions in the presence of diuron (Zhang et al., 2014). By blocking electron transfer from the primary electron acceptor in PS II to the plastoquinone pool, diuron disrupts the normal flow of electrons in the photosynthetic electron transport chain. This may result in disruption of redox balance and the generation of reducing equivalents, such as NADPH and NADH, which serve as electron donors for hydrogenase. The disruption of their balance can impair the enzymatic reactions involved in H₂ production. It is important to note that while S and N deprivation can enhance H₂ production in algae, the overall efficiency and yield of H₂ are influenced by various factors, including the specific strain of algae, cultivation conditions, and availability of other cofactors and electron donors. Therefore, optimizing the nutrient-deprivation strategy and understanding the underlying metabolic mechanisms are crucial for maximizing H₂ production by algae.

Growth properties and H₂ production by another alga *C. vulgaris* Pa-023 under S- and N-deprived condition have been investigated. S and N deprivation led to a decrease in the specific growth rate in *C. vulgaris* by ~1.4 and ~1.2-fold, respectively, compared with the control cultivated in complete Tamiya medium (Fig. 10a). H₂ production was recorded in *C. vulgaris* during anaerobic growth under N- and S-deprived conditions (Fig. 10b). An increase of H₂ yield in *C. vulgaris* under N- (2.0 mmol L⁻¹) and S-deprived (2.2 mmol L⁻¹) conditions was observed, which was ~3 times higher than the H₂ yield of algae grown in complete Tamiya medium. For comparison, Kosourov with co-workers (2007) reported H₂ yields of 1.50 and 0.9 mmol L⁻¹ in *Ch. reinhardtii* CC-124 cultivated under N- and S-deprived photoheterotrophic conditions, respectively. To identify the H₂ generation route in this alga, diuron was used, which was added after 24 h of algae growth, when H₂ production was recorded. In the presence of diuron, H₂ production significantly (~5 times) decreases in S- and N-deprived cells,

indicating that a PS II-dependent pathway is operating here (Fig. 10b). In *C. vulgaris* Pa-023 under nutrient deficiency, PS II supplies ~85% of electrons for hydrogenase.

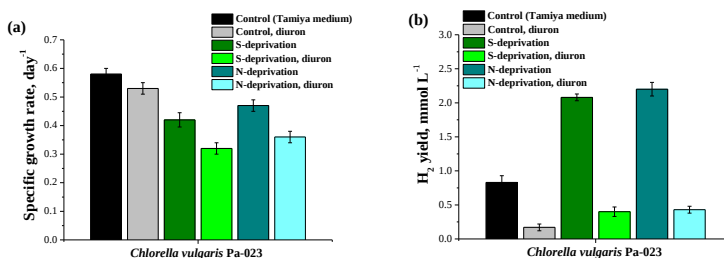


Fig. 10. Specific growth rate (a) and H₂ yield (b) in *C. vulgaris* Pa-023 grown under N- and S-deprived conditions ($n=3$, $p<0.05$).

Growth properties and biohydrogen production by green algae in ethanol and brewery waste-containing media

Alcohol production is frequently regarded as one of the most environmentally taxing industries due to the variety and large volume of waste generated during the process. However, using microorganisms to harness alcohol production wastes, such as ethanol and brewery wastes, offers a promising approach to reduce waste and enhance sustainability in this pollution-prone sector. By converting these wastes into useful products like biofuels and valuable secondary metabolites, photosynthetic microorganisms provide a way to reduce the environmental impact of alcohol production while simultaneously producing valuable products for various industries.

It is well-established that algae grow better under photoheterotrophic conditions than under photoautotrophic conditions. Ethanol (EtW) and brewery (BW) production wastes were selected as cultivation media for *P. kessleri* MDC6524 due to their availability, nutrient content, suitability for microbial use, and low cost. These industrial wastes are a rich source of carbon and other nutrients that are beneficial for microorganisms' growth and H₂ production.

To explore the potential of brewery and ethanol production waste as cultivation media for *P. kessleri*, experiments were conducted using both undiluted and diluted waste samples. The pH of the wastes was adjusted to 7.5, as this pH value is optimal for both algal growth and hydrogen production. *P. kessleri* was cultivated in media containing undiluted and 2-fold diluted BW, as well as undiluted and 2-, 5-, and 10-fold diluted EtW, where anaerobic conditions were maintained. Algae culture grown in standard Tamiya medium with glucose as a carbon source served as the control. Morphology of *P. kessleri* cultivated in Tamiya and waste-containing media was determined under an inverted microscope. Control cells of *P. kessleri* are large and ellipsoidal, whereas mature cells have a spherical shape and a clearly defined cell wall (Fig. 11a). In EtW-containing media, the cells were predominantly spherical in shape, with a

clearly defined cell wall and a cup-shaped chloroplast; some young cells exhibited an ellipsoidal shape (Fig. 11b, c). In BW-containing media, the cells appeared larger, with a cup-shaped chloroplast with a greenish-yellow hue. A higher frequency of dividing cells was observed with the formation of up to eight autospores (Fig. 11d, e).

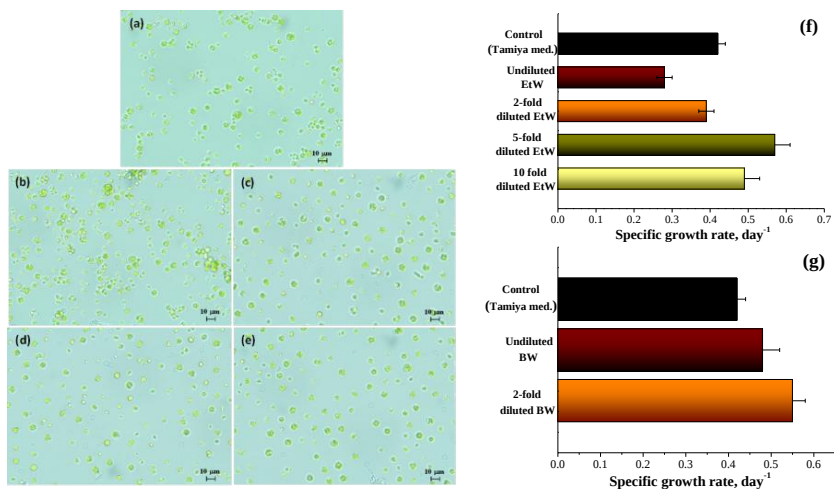


Fig. 11. Microscope images of *P. kessleri* MDC6524 cultivated in: (a) Tamiya medium (control); (b, c) 5- and 10-fold diluted EtW; (d and e) undiluted and 2-fold diluted BW. Specific growth rate of *P. kessleri* grown in EtW- (f) and BW-containing media (g) ($n=3$, $p<0.05$).

The specific growth rate of *P. kessleri* increased during cultivation in waste-containing media (Fig. 11f, g). The highest specific growth rate of algae was observed in 5-fold diluted EtW and 2-fold diluted BW-containing media, showing a 1.4 times increase compared to the control culture (Fig. 11f, g). Similar results were obtained for *C. vulgaris* grown in brewery wastewaters (Lois-Milevicich et al., 2020). In contrast, in undiluted EtW medium, the specific growth rate of algae decreased by 1.5 times compared to the control culture (Fig. 11f), due to the high concentration of organic compounds in the waste. Therefore, diluting the waste is essential to optimize the concentration of organic compounds and ensure efficient algae growth.

To investigate the levels of photosynthetic pigment in *P. kessleri* cultivated in waste-containing media, absorption spectra were recorded (Fig. 12). The highest pigment content of *P. kessleri* was observed during cultivation in 5-fold diluted EtW- and 2 fold diluted BW-containing media (Fig. 12). When *P. kessleri* cultivated in 5 fold diluted EtW- and 2 fold diluted BW-containing media, the total carotenoids and Chl *a* contents increased 2 times compared to the control, while the amount of Chl *b* remained unchanged (Fig. 12c). The high amounts of Chl *a* and carotenoids suggests an increase in the light-harvesting capacity of algae.

Diluted ethanol and brewery waste can promote the growth of green algae because they contain various nutrients that serve as essential sources for cell growth, potentially enhancing biomass production.

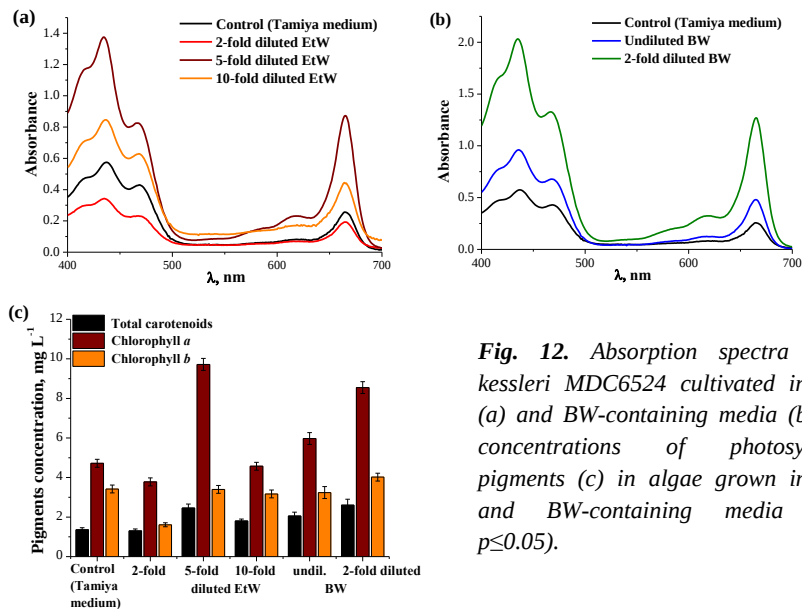


Fig. 12. Absorption spectra of *P. kessleri* MDC6524 cultivated in EtW- (a) and BW-containing media (b); and concentrations of photosynthetic pigments (c) in algae grown in EtW- and BW-containing media ($n=3$, $p \leq 0.05$).

To determine the composition of the waste used, Fourier transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC) were carried out. This analysis made it possible to identify which compounds are effectively utilized by green algae. The chemical composition of brewery and ethanol waste is very rich, mainly in organic compounds such as proteins, carbohydrates, organic acids, etc. Consequently, their FTIR spectra have a complex pattern, indicating the presence of vibrational bands that characterize different biomolecules (Fig. 13). Interpretation of FTIR spectra involves identifying the presence, location and intensity of bands corresponding to specific functional groups belonging to various biomolecules contained in the waste (Manoyan et al., 2025). During the cultivation of algae in waste-containing media, slight changes in the peak positions and their intensities are observed, which indicate the utilization of waste components by algae and the formation of new compounds of metabolism (Fig. 13).

HPLC analysis of industrial wastes demonstrated that the content and composition are typical for these wastes. Brewery production waste contains sugars such as glucose, arabinose, xylose, and fructose, and organic acids such as malic, lactic and oxalic acids. During cultivation in BW-containing media, glucose, fructose and malic acid are completely consumed by the algae. In ethanol production, waste sugars such as glucose, fructose, arabinose, galactose,

xylose, and sucrose, and organic acids such as lactic, oxalic, and tartaric acids are present (Manoyan et al., 2025). *P. kessleri* completely utilized glucose, oxalic, and tartaric acids in EtW-containing media, while the concentrations of fructose and lactic acid decreased by ~1.4 and 1.2 fold, respectively. *P. kessleri* was unable to utilize xylose, which is present in both wastes. Important characteristics of waste, such as chemical oxygen demand (COD) and total nitrogen concentration were also determined. The COD of effluents is crucial for the cultivation of microalgae, as elevated carbon levels in waste can suppress their growth. During our experiments the COD values of EtW and BW were 6670 mg O₂ L⁻¹ and 4380 mg O₂ L⁻¹, respectively, which indicate that these industrial wastes can be used as promising media for photoheterotrophic cultivation of *P. kessleri*. The total nitrogen concentrations in EtW and BW are 38 and 42 mg L⁻¹, respectively. During *P. kessleri* cultivation, the nitrogen concentration in EtW and BW-containing media decreased by 68% and 43%, respectively. The algae utilized total nitrogen and used it for growth.

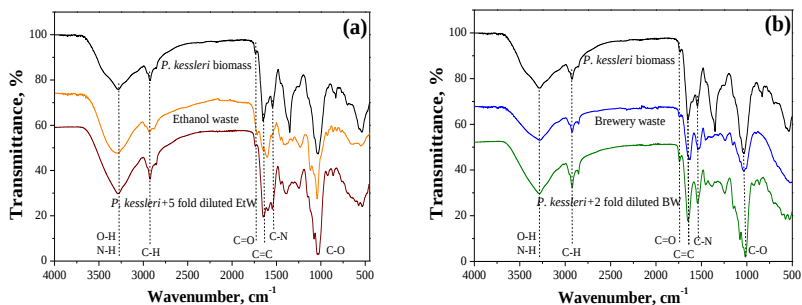


Fig. 13. FTIR spectra of *P. kessleri* biomass, wastes, and algae cultivated in (a) 5-fold diluted EtW- and (b) 2-fold diluted BW-containing media.

The H₂ production by *P. kessleri* MDC6524 cultivated in waste-containing media under anaerobic conditions and continuous illumination was determined. Waste dilution increased the H₂ production by *P. kessleri*: the highest H₂ yield was observed in 5- and 10-fold diluted EtW, and 2-fold diluted BW media (Fig. 14). Moreover, H₂ production by *P. kessleri*, cultivated in waste-containing media, began at 24 h and continued for at least 120-144 h, with a stable yield observed between 48 and 96 h. In control cells cultivated in standard Tamiya medium, H₂ production continued for 96 h with a maximum yield at 48-72 h (Manoyan et al., 2025). The highest H₂ yields in *P. kessleri* were detected in 5- and 10-fold diluted EtW-containing media after 48 h cultivation, and achieved to 72 and 66 mL L⁻¹, respectively, which were ~5 times higher compared to the control cultivated in Tamiya medium (Fig. 14a). Moreover, H₂ production rate also reached its maximum level in 5- and 10-fold diluted EtW. In 2-fold diluted BW-containing media the maximum yield of H₂ production was 55 mL L⁻¹, which was 4 times higher compared to the control grown in Tamiya medium (Fig. 14b). No H₂ generation was

observed when algae were grown on undiluted EtW-containing media, whereas in 2-fold diluted EtW and undiluted BW the H₂ yield was not significant (Fig. 14). This may be due to the high content of organic compounds in the wastes. Therefore, waste dilution is essential to optimize the concentration of organic compounds for biomass and H₂ generation by green algae.

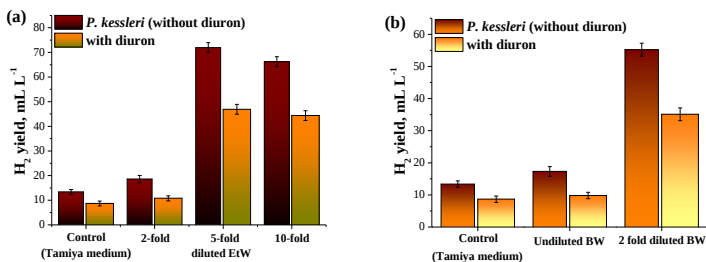


Fig. 14. H₂ yield in *P. kessleri* MDC6524 cultivated in EtW- (a) and BW-containing media (b) ($n=3$, $p \leq 0.05$).

To elucidate the mechanisms of H₂ generation and the functional activity of PS II, which provides reductants for H₂ production, the effects of diuron, a known PS II inhibitor, on H₂ yield in *P. kessleri* were determined. Diuron suppressed the H₂ production by *P. kessleri*, cultivated in diluted waste-containing media by approximately 40% (Fig. 14). The data obtained indicate that in algae cultivated in waste-containing media, only 40% of the electrons for H₂ generation are provided by PS II during water splitting, while the PS II-independent pathway contributes 60% of the electrons. Thus, diuron inhibits the transfer of electrons from the PS II reaction center to the plastoquinone pool. Organic compounds found in waste support the PS II-independent pathway by providing electrons to the plastoquinone pool.

In addition, the H₂ generation in another algae strain, *C. vulgaris* IBCE C-19, using ethanol production waste, was investigated. The results showed that H₂ generation in these algae, in EtW-containing media was also started at 24 h growth, whereas in control cells, cultivated in standard Tamiya medium, H₂ yield was detected at 48 h. No H₂ generation was observed when undiluted waste was used. During the cultivation in diluted EtW-containing media H₂ production by *C. vulgaris* was higher in comparison with the control (Fig. 15a). The highest H₂ yield was detected at 48 h growth in 5- and 10-fold diluted EtW, which was ~3-fold higher than the H₂ yield in the control, grown on Tamiya medium (Fig. 15a).

To determine the contribution of PS II in H₂ generation during *C. vulgaris* cultivation in waste-containing media, the effect of diuron was investigated. Diuron suppressed the H₂ generation in *C. vulgaris* by ~70% in 5- and 10-fold diluted EtW, which indicates the significant role of PS II as a supplier of electrons (~70%) for H₂ production in this algae (Fig. 15a). The effect of diuron on H₂ yield in *C. vulgaris*, cultivated in diluted EtW-containing media, indicates that PS II provides the main electrons for H₂ production during splitting of

water, this pathway of H_2 generation is known as PS II-dependent route (Manoyan, Gabrielyan, 2021). H_2 production by microorganisms is coupled with a decrease in the value of the redox potential. During cultivation of *C. vulgaris* in undiluted EtW-containing media, the decrease in the redox potential up to -306 ± 5 mV was observed, whereas in 5- and 10-fold diluted EtW-containing media, the redox potential decreased up to -565 ± 25 and -520 ± 20 mV, respectively (Fig. 15b).

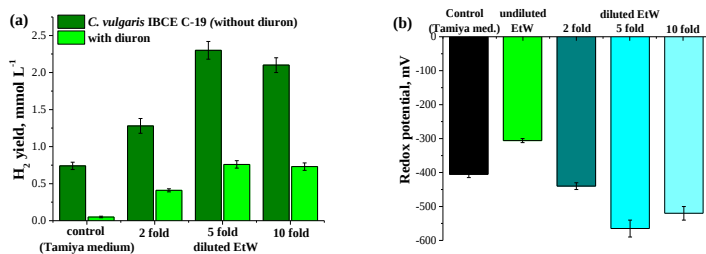


Fig. 15. The H_2 yield (a) and redox potential (B) in *C. vulgaris* IBCE C-19 cultivated in Tamiya (control) and 2-10 fold diluted EtW-containing media ($n=3$, $p<0.05$).

Thus, the data obtained indicate that the used alcohol production wastes represent valuable sources of natural compounds and can effectively serve as efficient substrates for obtaining biomass and H_2 production by both *P. kessleri* and *C. vulgaris*.

In the presented dissertation, using modern biochemical and microbiological methods, the characteristics of light-dependent hydrogen production by green algae isolated in Armenia were investigated, and integrated approaches for stimulating biomass and H_2 yields were developed.

CONCLUSIONS

The following conclusions were made based on experimentally obtained results:

1. Green alga *Parachlorella kessleri* carries out light-dependent H_2 production under photoheterotrophic anaerobic conditions. Moreover, in *P. kessleri*, the main pathway for H_2 production is the photosystem (PS) II-dependent pathway.
2. Protonophores stimulate H_2 production in *P. kessleri*. The dissipation of the proton gradient across the thylakoid membrane promotes the activation of the PS II-independent pathway for H_2 production.
3. The effect of electromagnetic irradiation (EMI) at frequencies of 51.8 and 53.0 GHz on *P. kessleri* depends on the algae cultivation conditions and the presence of oxygen in the environment. Under aerobic conditions, extremely high frequency EMI stimulates algal growth and the content of photosynthetic pigment, whereas under anaerobic conditions, an inhibitory effect of EMI is observed.

4. Nitrogen and sulfur deprivation stimulate light-dependent H₂ production by *P. kessleri* and *C. vulgaris*. The maximum H₂ yield is recorded under combined nitrogen and sulfur deprivation conditions. Under nitrogen and sulfur deficiency, PS II acts as the main electron donor.
5. The use of ethanol and brewery wastes as cultivation media for *P. kessleri* and *C. vulgaris* stimulates the light-dependent H₂ production. The maximum H₂ yield is observed with diluted wastes and pH 7.5. It was found that in *P. kessleri* grown in waste-containing media, two H₂ production pathways are active: PS II-dependent and independent. Moreover, the waste can be utilized by the algae to support H₂ production via the PS II-independent pathway.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. **Manoyan J.**, Hakobyan D., Gabrielyan L., Shahzizyan I., Sahakyan L., Minasyan E., Gabrielyan L. (2025) Improvement of biomass and biohydrogen generation in *Parachlorella kessleri* using ethanol and brewery production wastes as cultivation media. *International Journal of Hydrogen Energy*, 102: 400-410, <https://doi.org/10.1016/j.ijhydene.2025.01.075>.
2. **Manoyan J.**, Hakobyan L., Samovich T., Kozel N., Sahakyan N., Muravitskaya H., Demidchik V., Gabrielyan L. (2024) Comparison of sulfur and nitrogen deprivation effects on photosynthetic pigments, polyphenols, photosystems activity and H₂ generation in *Chlorella vulgaris* and *Parachlorella kessleri*. *International Journal of Hydrogen Energy*, 59: 408-418. <https://doi.org/10.1016/j.ijhydene.2024.02.035>.
3. Mysleiko MA, Vecherek MS, Manoyan JG, Gabrielyan LS, Samovich TV, Kozel NV, Muravitskaya AO, Demidchik VV. (2024) Synthesis of biohydrogen by green microalgae and methods of increasing its production. *Experimental Biology and Biotechnology*. 2: 36–45. In Russian. EDN: NCZWSI.
4. **Manoyan J.**, Samovich T., Kozel N., Demidchik V., Gabrielyan L. (2022) Growth characteristics, biohydrogen production and photochemical activity of photosystems in green microalgae *Parachlorella kessleri* exposed to nitrogen deprivation. *International Journal of Hydrogen Energy*, 47(38): 16815-16823, <https://doi.org/10.1016/j.ijhydene.2022.03.194>.
5. **Manoyan J.** (2022) Growth and hydrogen production by *Chlorella vulgaris* Pa-023 under sulfur and nitrogen deprivation. *Biological Journal of Armenia* 4(74):, 6-11 <https://www2.flib.sci.am/journal/Biology/>
6. **Manoyan JG.**, Gabrielyan LS. (2021) The ethanol industry waste as a valuable feedstock for hydrogen photoproduction by green algae *Chlorella vulgaris*. *Proceedings of Yerevan State University, B: Chemical and Biological Sciences* 55, 3 (256): 232-239. <https://doi.org/10.46991/PYSU:B/2021.55.3.232>.

7. **Manoyan J.**, Gabrielyan L., Kalantaryan V., Trchounian A. (2020) Growth properties and hydrogen yield in green microalga *Parachlorella kessleri*: Effects of low-intensity electromagnetic irradiation at the frequencies of 51.8 GHz and 53.0 GHz. *Journal of Photochemistry and Photobiology B: Biology*, 211: 112016, <https://doi.org/10.1016/j.jphotobiol.2020.112016>.
8. **Manoyan J.**, Gabrielyan L., Kozel N., Trchounian A. (2019) Regulation of biohydrogen production by protonophores in novel green microalgae *Parachlorella kessleri*. *Journal of Photochemistry and Photobiology B: Biology*, 199: 111597. <https://doi.org/10.1016/j.jphotobiol.2019.111597>.
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10. **Manoyan J.**, Petrosyan A., Gabrielyan L. (2025) Enhancement of hydrogen photoproduction by *Parachlorella kessleri* using a mixture of brewery and ethanol production wastes. *FEMS Micro* 2025, July 14-17, Milan, Italy, p. 394.
11. **Manoyan J.**, Hakobyan L., Gabrielyan L. (2024) Biomass and biohydrogen production in green algae using alcohol industry wastes. *Proc. Annual conference-2024*, December 7-15, Yerevan, Armenia, p. 152.
12. **Manoyan J.**, Hakobyan L., Gabrielyan L. (2024) Enhancement of H₂ production by *Chlorella vulgaris* using potato peel waste. *FEBS Open Bio*, 14 (2): SpT-09-1, p. 68, doi: 10.1002/2211-5463.13836.
13. **Manoyan J.**, Hakobyan L., Muravitskaya H., Demidchik V., Gabrielyan L. (2023) Growth characteristics and biohydrogen production by algae of *Chlorellaceae* family under nutrients deprivation conditions. *Proc. Pan-Armenian conference*, November 17-22, Yerevan, Armenia, p. 186.
14. Muravitskaya HO., Svetlakov VI., Bondarenko VYu., Samovich TV., Kozel NV., Sokolik AI., Gabrielyan LS., **Manoyan JG.**, Demidchik VV. (2023) Assessment of biohydrogen production and digital analysis of the phenotype of Belarusian and Armenian algae strains of *Chlorellaceae* family. *Int. scientific conference "The present and future of plant biotechnology"*, NAS, Belarus, Minsk, May 24-26, p. 135, ISBN 978-985-880-344-5.
15. Gabrielyan L., **Manoyan J.**, Gabrielyan L. (2022) Brewer's spent grain as a potential substrate for hydrogen production by *Parachlorella kessleri*. *FEBS Open Bio*, 12: P-02.4-022, p. 184. doi: 10.1002/2211-5463.13440.
16. Hakobyan L, **Manoyan J.**, Panosyan E, Gabrielyan L. (2022) Hydrogen generation in sulfur-deprived green microalgae *Chlorella vulgaris*. *FEBS Open Bio*, 12: P-02.4-021, pp. 183-184. doi: 10.1002/2211-5463.13440.
17. Gabrielyan L, **Manoyan J.**, Hakobyan L, Samovich T, Kozel N, Demidchik V. (2022) Hydrogen photoproduction by nitrogen-deprived microalgae *Parachlorella kessleri* and

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ՄԱՆՈՑԱՆ ԶԵՄՄԱ ԳՈՒՐԳԵՆԻ

ՖԻԶԻԿԱԶԻՄԻԱԿԱՆ ԳՈՐԾՈՆՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ *PARACHLORELLA KESSLERI* և *CHLORELLA VULGARIS* ԿԱՆԱԶ ԶՐԻՄՈՒՈՆԵՐՈՒՄ ԶՐԱՄԻ ԼՈՒՍԱԿԱՆՑԱԼ ԱՐՏԱԴՐՈՒԹՅԱՆ ՎՐԱ

ԱՍՓՈՓԱԳԻՐ

Բանալի բառեր՝ *Parachlorella kessleri*, *Chlorella vulgaris*, H₂-ի արտադրություն, ֆոտոհամակարգեր, էլեկտրամագնիսական ճառագայթներ, պրոտոնոֆորներ, կենսածին տարրերի սակավություն, արդյունաբերական թափոններ:

Կանաչ ջրիմուռները ֆոտոսինթեզող օրգանիզմներ են, որոնք տարբեր միջավայրերում աճելու կարողության, աճման բարձր արագության և արտադրական ցածր ծախսատարության շնորհիվ լայն կիրառություն են ստացել արդյունաբերության տարբեր ոլորտներում: Հատկապես մեծ հետաքրքրություն է ներկայացնում կանաչ ջրիմուռների կիրառությունը H₂-ի արտադրության մեջ, որը կարող է հաջողությամբ կիրառվել որպես վերականգնվող էներգիայի աղբյուր:

Ներկայացվող հետազոտության մեջ՝ օգտագործելով ժամանակակից կենսաքիմիական և մանրէաբանական մեթոդներ, ուսումնասիրվել է ՀՀ-ում մեկուսացված *Parachlorella kessleri* և *Chlorella vulgaris* կանաչ ջրիմուռներում H₂-ի լուսակախյալ արտադրությունը՝ ֆիզիկաքիմիական տարբեր գործոնների ազդեցությամբ:

Հետազոտությունների արդյունքում պարզվել է, որ *P. kessleri*-ն արտադրում է H₂ ֆոտոհետերոտրոֆ անաերոբ պայմաններում: Ընդ որում, *P. kessleri*-ում հիմնականում գործում է ֆոտոհամակարգ (ՖՀ) 2-կախյալ H₂-ի արտադրության ուղին: Ցույց է տրվել նաև պրոտոնոֆորների դրական ազդեցությունը *P. kessleri*-ում H₂-ի արտադրության վրա, ինչը կապված է թիլակոիդային թաղանթում պրոտոնային գրադիենտի ցրման հետ՝ հասանելի դարձնելով ՖՀ 1-ի միջոցով էլեկտրոնները հիդրոգենազի համար: Պարզվել է, որ *P. kessleri*-ի վրա 51.8 և 53.0 ԳՀց հաճախությամբ էլեկտրամագնիսական ճառագայթների ազդեցությունը կախված է ջրիմուռների աճի պայմաններից: Որոշվել է *P. kessleri*-ում և *C. vulgaris*-ում H₂-ի արտադրությունը ծծմբի և ազոտի անբավարարության պայմաններում: H₂-ի ելքը խթանվել է կենսածին տարրերի անբավարարության փորձարկված բոլոր դեպքերում, իսկ H₂-ի առավելագույն ելք գրանցվել է համակցված S-ի և N-ի սակավության պայմաններում: Պարզվել է, որ S-ի և N-ի անբավարարության

պայմաններում ՖՀ 2 գործում է որպես էլեկտրոնների հիմնական մատակարար: Գարեջրի և էթանոլի արտադրության թափոնների, որպես ջրիմուռների աճման միջավայրերի, կիրառումը խթանել է H_2 -ի լուսակախյալ արտադրությունը: H_2 -ի առավելագույն ելք գրանցվել է նոսրացված թափոնների կիրառմամբ: Հետազոտությունները ցույց են տվել, որ թափոն պարունակող միջավայրում աճեցված *P. kessleri*-ում գործում են H_2 -ի արտադրության 2 ուղի՝ ՖՀ 2-կախյալ և անկախ, ընդ որում թափոնների օրգանական միացությունները կարող են օգտագործվել՝ ՖՀ 2-անկախ ուղիով H_2 արտադրությունը ապահովելու համար:

Այսպիսով, աշխատանքում ստացված արդյունքները վկայում են, որ ՀՀ-ում մեկուսացված կանաչ ջրիմուռները H_2 -ի խոստումնալից արտադրիչ են: Կենսաջրածնի արտադրության ուղիների ու մեխանիզմների ուսումնասիրությունը, և H_2 -ի բարձր ելքին նպաստող պայմանների օպտիմալացումը կարևոր են տվյալ ջրիմուռների՝ որպես կայուն էներգիայի աղբյուրների ներուժի պարզաբանման համար:

МАНОЯН ДЖЕММА ГУРГЕНОВНА

**ВЛИЯНИЕ ФИЗИКО-ХИМИЧЕСКИХ ФАКТОРОВ НА СВЕТОЗАВИСИМОЕ
ВЫДЕЛЕНИЕ ВОДОРОДА ЗЕЛЕНЫМИ ВОДОРОСЛЯМИ *PARACHLORELLA*
KESSLERI И *CHLORELLA VULGARIS***

РЕЗЮМЕ

Ключевые слова: *Parachlorella kessleri*, *Chlorella vulgaris*, выделение H_2 , фотосистемы, электромагнитное излучение, протонофоры, дефицит биогенных элементов, промышленные отходы.

Зеленые водоросли – фотосинтезирующие организмы, которые, благодаря способности расти на различных средах, высокой скорости роста и низким производственным затратам, нашли широкое применение в различных областях промышленности. Особый интерес представляет использование зеленых водорослей для получения биоводорода (H_2), который может успешно применяться в качестве возобновляемого источника энергии.

В данной работе было исследовано влияние различных физико-химических факторов на светозависимое выделение H_2 зелеными водорослями *Parachlorella kessleri* и *Chlorella vulgaris*, изолированными в Армении, с использованием современных биохимических и микробиологических методов.

Исследования показали, что *P. kessleri* производит водород в фотогетеротрофных анаэробных условиях. При этом, в *P. kessleri* в основном действует фотосистема (ФС) 2-зависимый путь продукции H_2 . Также показано положительное влияние протонофоров на выделение H_2 в *P. kessleri*, что связано с рассеиванием протонного градиента в тилакоидной мембране, что увеличивает доступность электронов для гидрогеназы через ФС 1. Установлено, что действие электромагнитного излучения с частотами 51.8 и 53.0 ГГц на *P. kessleri* зависит от условий культивирования водорослей. Также было исследовано производство H_2 в *P. kessleri* и *C. vulgaris* в условиях дефицита серы и азота. Во всех случаях дефицита биоэлементов наблюдалось увеличение продукции H_2 , при этом максимальный выход H_2 был зарегистрирован при комбинированном дефиците серы и азота. Было установлено, что при дефиците серы и азота ФС 2 действует как основной донор электронов. Использование отходов производства пива и этанола в качестве среды для культивирования водорослей стимулировало светозависимое выделение H_2 . Максимальный выход H_2 был получен при использовании разбавленных отходов. Исследования показали, что в *P. kessleri*, выращенной на средах, содержащих отходы, активны оба пути продукции H_2 – ФС 2-зависимый и ФС 2-независимый, причем органические соединения отходов могут быть использованы для обеспечения продукции H_2 через ФС 2-независимый путь.

Таким образом, результаты, полученные в данной работе, свидетельствуют о том, что зеленые водоросли, изолированные в Армении, являются перспективными продуцентами H_2 . Изучение путей и механизмов продукции биоводорода, а также оптимизация условий, способствующих высокому выходу H_2 , имеет важное значение для раскрытия потенциала данных водорослей, как устойчивых источников энергии.

