

Article

Production of Biohydrogen from Microalgae Biomass after Wastewater Treatment and Air Purification from CO₂

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Abstract: This article explores the feasibility of producing biohydrogen from microalgae following their use in wastewater purification from food industry facilities and the removal of high levels of carbon dioxide (CO₂) from the air. The authors investigated various methods for disrupting the cell membranes of microalgae and their impact on biohydrogen yield. The microalgae biomass obtained after wastewater and air purification underwent pre-treatment using physicochemical and chemical methods, including microwave radiation, acid treatment, and thermal treatment. The highest hydrogen production occurred during thermal and acid treatments of biomass with the addition of starch (44.24 mL/L of suspension). The use of microwave radiation for processing did not yield significant results. A comparison of the biohydrogen values obtained from untreated and treated biomass revealed that treatment enhances biohydrogen yield.

Keywords: biomass; biohydrogen; biogas; cell processing; water treatment; CO₂ absorption



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1. Introduction

The long-term depletion of fossil fuels and greenhouse gas emissions resulting from their combustion are global problems faced by humanity. In 2018, the primary energy sources were oil (33.6%), coal (27.2%), natural gas (23.9%), and nuclear (4.4%). Renewable energy constituted 10.8% of the total, with hydropower contributing 6.8% [1,2]. The development and use of alternative and renewable energy sources have been proposed as one of the key ways to solve these problems. Scientists are becoming more interested in biofuels because they are environmentally friendly and made from affordable, non-edible, and inexpensive feedstocks [3–5]. Hydrogen is expected to play an increasingly vital role in future developments over the next decade. It is assumed that it will be able to replace fossil fuels as an energy resource for regional funds or supplement it [6].

Hydrogen is one of the most common carbon-free fuels. Hydrogen is considered a cleaner and more promising fuel option due to its remarkable energy output (143 GJ tonne⁻¹) [7]. Hydrogen has a high energy density, which makes it attractive for use in a variety of energy-intensive applications such as automobiles, power generation, and some industries [8]. One of the main advantages of using hydrogen as a fuel is that when it is burned, carbon dioxide is not produced, which is the main gas that causes an increase in the greenhouse effect [7]. As a result of hydrogen combustion, only water is formed [9], which makes its use for energy generation environmentally friendly [10]. The use of hydrogen greatly contributes to the reduction in environmental problems such as

greenhouse gas emissions and acid rain. In addition, hydrogen can be produced from a variety of sources and technologies, making it promising for sustainable energy and helping to reduce dependence on fossil resources [11].

An important issue in terms of environmental impact is the method of hydrogen production. This is often conducted using natural gas steam reforming (SRM), in which natural gas is reacted with steam at a high temperature and pressure in the presence of a catalyst. The reaction leads to the formation of hydrogen and carbon dioxide. Another energy-intensive method of hydrogen production is water electrolysis, where an electric current is used to decompose water into hydrogen and oxygen. Gasification, thermochemical processes, and other photochemical processes are also applied. The main disadvantages of these methods of hydrogen production in terms of environmental impact are the formation of large volumes of carbon dioxide during steam reforming and the need for large amounts of electricity and water during electrolysis. The low concentration of hydrogen produced by thermochemical methods and the need for high-temperature complex operating conditions increase production costs and energy consumption, making them unsustainable [12–14].

In recent times, there has been a significant focus on biohydrogen production. Biohydrogen is generated by biological organisms, typically microorganisms, as part of their metabolic or biochemical processes [15,16]. The bulk of biologically produced hydrogen in the biosphere is released as a result of microbial fermentation processes, in which microorganisms decompose organic matter, releasing carbon dioxide and hydrogen [17]. The advantage of biological hydrogen production by microbes, when compared to chemical hydrogen production, is that it can occur at lower temperatures and pressures, which makes it a more energy-efficient process [18,19]. In the production of biohydrogen, the feedstock, its availability, and cost also play an important role.

Approximately half of the total biomass on the planet is represented by algae [20]. Such a rapid spread of algae can be explained not only by their natural life cycle but also by anthropogenic negative impacts on the environment, such as eutrophication. However, despite the negative effects of eutrophication, algae are a valuable natural resource for the production of biohydrogen. Microalgae are of particular interest among biomass resources [21]. Microalgae, containing a significant amount of carbohydrates, have great potential as a feedstock for the production of various types of biofuels, such as biodiesel, bioethanol, biohydrogen, and biogas [22,23].

Microalgae are a promising raw material for obtaining biohydrogen due to their properties. These microorganisms accumulate significant amounts of carbohydrates, such as starch, glycogen, and cellulose, which are considered efficient biohydrogen substrates [24]. They have a high capacity for photosynthesis and reproduction. Microalgae use available light, carbon dioxide, and some nutrients in the environment to grow. This reduces the need for external nutrient sources. Also, the use of carbon dioxide from the atmosphere as a source of inorganic carbon helps to reduce the carbon footprint. Another advantage of cultivating microalgae is their environmentally friendly production, which eliminates the need for various fertilizers, avoids competition with fertile agricultural soils, and requires less water than terrestrial crops, contributing to the efficient use of freshwater resources [25]. Microalgae can be cultivated in various types of water, including wastewater from food industry enterprises, which can be used by enterprises as an additional block for the biological treatment of polluted waters. Some species of microalgae can be used to extract nutrients from the water, which can help reduce eutrophication in polluted aquatic ecosystems [26]. There are technologies and methods for cultivating microalgae on an industrial scale, which makes their production and application for the production of biohydrogen more accessible, economically feasible, and environmentally safe [27].

Biohydrogen can be obtained from microalgae using several methods, namely photofermentation [28], biophotolysis [29], dark fermentation [22,30–32], and electrofermentation. The last method listed is less common due to its low biohydrogen output efficiency and the need for electrical energy. Biophotolysis and photofermentation are the most common methods for the biological generation of hydrogen. However, the implementation of these

methods of biohydrogen production requires light energy, which causes limitations in the application of these methods and reduces their economic efficiency [33].

Microalgae have the ability to produce biohydrogen as a result of their photosynthetic metabolism. Hydrogen gas is released by H₂-producing microorganisms (H₂ producers) as a metabolic byproduct of their fermentation [34–36]. They use light and water to form hydrogen gas, making it a renewable and environmentally friendly source of energy [37]. However, an important condition for successful photofermentation is the adaptation of microalgae to anaerobic conditions.

A more promising direction for obtaining biohydrogen from microalgae is the dark fermentation of microalgae biomass [22,38]. This method of biohydrogen production makes it possible to obtain hydrogen without the use of light, which expands the possibilities for its production under various conditions and at night [39]. This method differs in that the production of biogas is carried out in an anaerobic mode. Anaerobic microorganisms such as specific bacteria or archaea can be introduced to facilitate the fermentation process. The process of dark fermentation begins with the adaptation of microorganisms to anaerobic conditions. In the absence of light and oxygen, anaerobic microorganisms metabolize organic compounds in the biomass of microalgae [40]. During this metabolic process, complex organic molecules are broken down into simpler components. This process is facilitated by the activation of enzymes like hydrogenase. Hydrogenase, predominantly found in microalgae, plays a critical role in the initial step of hydrogen synthesis. It activates the process that initiates the formation of hydrogen molecules. This is followed by the action of nitrogenase, which performs the function of reducing chemical elements, turning them into hydrogen molecules [41]. The main product of interest is hydrogen gas, which is released as a by-product of these microbial reactions. In addition to hydrogen gas, dark fermentation can also produce other by-products, including volatile fatty acids such as acetic and butyric acids, carbon dioxide, and small amounts of methane.

Biomass pre-treatment improves carbohydrate conversion by preventing the formation of inhibitory chemicals and the breakdown of carbohydrates. This process remains the most critical and costly step in the process of biohydrogen production [42,43].

However, microalgae biomass contains minimal amounts of lignin and hemicellulose, which prevent biohydrogen formation. Therefore, the pretreatment of microalgae biomass becomes much more economically accessible and technically simpler [44]. For the pre-treatment of algae biomass, various methods are used, including heat treatment, the use of acids, and the use of microwave radiation [45].

Nguyen et al. [46] used the starch-rich *Chlamydomonas reinhardtii* biomass as a substrate for the production of biohydrogen by the amylolytic *Thermotoga neopolitana*. When intact cells were used as a substrate, no hydrogen was produced, and when the cell wall was destroyed by sonication or methanol treatment, hydrogen was produced in significant yield. In their studies, Liu et al. showed that biomass without pretreatment produces little hydrogen (474 mL/L) compared to biomass pretreated by various methods (1424 mL/L) [47].

Thermal pre-treatment involves the use of thermal energy to liquefy the biomass, which promotes hydrolysis of the system components. Xia et al. studied the effect of treatment temperature on biohydrogen yield. Combined heat treatment of *Nannochloropsis oceanica* biomass was used for the enzymatic production of hydrogen [48]. The biomass was microwave-heated, and it was observed that at temperatures exceeding 100 °C, the yield of reducing sugar reached 0.289 ± 0.002 g/g total volatile solids (TVSs), while at 100 °C and below only 0.047 ± 0.002 g/g TVS is achieved. Further, it was also shown that thermal pre-treatment alone is not sufficient to disrupt cell walls, decrease the degree of the polymerization of polysaccharides, and release fermentable sugars. The combination of thermal and chemical treatment has improved the hydrogen production potential of microalgae biomass [49,50].

Chemical treatments for biomass include the use of acids, alkalis, and other solvents to hydrolyze microalgae biomass. Although alkaline pretreatment is widely used for lignocellulosic biomass, it was shown in Xia et al.'s study [51] that alkaline pretreatment is

ineffective when it comes to the hydrolysis of microalgae biomass, and acid hydrolysis is the most preferred method. After acid pretreatment, the mixture is usually neutralized with alkali and used for fermentation. However, it was reported by Efremenko et al. [52] that when *Clostridium* was used in fermentation, the presence of acid during heat treatment at 121 °C reduced the hydrogen yield by a factor of three. According to Kumar et al. [53], heat treatment by simple autoclaving in combination with mild acid is the most commonly used pretreatment of microalgae biomass since the operation is simple and the yield of reducing sugars is quite high. Therefore, the yield of biohydrogen from algae is strongly affected by the initial treatment of microalgae biomass.

In this study, *Chlorella kessleri* VKPM AI-11 ARW microalgae were utilized for the first time after cultivation under high carbon dioxide conditions. Additionally, the biomass was cultivated for the first-time using wastewater from the food enterprise LLC «Baltika» Brewing Company in St. Petersburg, Russia. These aspects contribute to the novelty of this research.

The purpose of this work was to reduce the cost of obtaining biohydrogen from microalgae by using the residual biomass of microalgae *Chlorella kessleri* VKPM AI-11 ARW, the cultivation of which took place in conditions of high carbon dioxide content, after wastewater treatment. The aim was also to study the effect of various methods of processing the biomass of microalgae *Chlorella kessleri* VKPM AI-11 ARW, obtained during the purification of wastewater and air from CO₂, on the biohydrogen yield.

2. Materials and Methods

The main object of the study was a strain of unicellular green alga *Chlorella kessleri* VKPM AI-11 ARW. The strain is highly adaptable to various categories of water bodies and can develop in water bodies in different geographical zones, thereby preventing water blooms. It persists in water bodies at temperatures ranging from 4 to 16 °C and grows at temperatures ranging from 17 to 30 °C. Additionally, it exhibits higher productivity when cultivated in production facilities compared to other strains (*Chlorella vulgaris* KIEG, *Chlorella vulgaris* BIN) [54].

To reduce the cost of the obtained biomass and, subsequently, biohydrogen, the biomass was cultivated on the wastewater of the food enterprise LLC «Baltika» Brewing Company» (St. Petersburg, Russia). These wastewaters contain the macroelements necessary for the growth and development of microalgae (phosphate ions (PO₄³⁻), ammonium ions (NH₄⁺), nitrite ions (NO₂⁻), and sulfates (SO₄²⁻). Trace elements were added with a nutrient medium [55]. The composition of wastewater of LLC «Baltika» Brewing Company» (St. Petersburg, Russia) (mg/L) is presented in Table 1 [56].

Table 1. Wastewater composition of LLC «Baltika» Brewing Company».

Name of the Component	Concentration, mg/L
Chemical Oxygen Demand	5650
Total Nitrogen	43.77
NH ₄ –N	20.85
NO ₂ –N	0.022
NO ₃ –N	1.6
Total Phosphor	11.85
PO ₄ –P	25.86
SO ₄	207.2
Ca	22.3

Microalgae were preliminarily grown in a nutrient medium and then mixed in a ratio of 7:3 with wastewater. This ratio was chosen on the basis of previous studies [57,58]. It is optimal because, under these conditions, there is no loss of biomass; it grows well, using pollutants for its nourishment.

The removal efficiency is calculated by the Formula (1):

$$E = ((C_1 - C_2)/C_1) \cdot 100\%, \quad (1)$$

where C_1 is the initial concentration of the nutrient in wastewater, mg/L; C_2 is the final concentration of the nutrient, after purification by microalgae, mg/L.

CO₂ was used as a source of inorganic carbon. The content of CO₂ in the gas–air environment was 30%, which corresponds to the high content of CO₂ from thermal power plants emissions. Photobioreactor biofilter (PhBR-B) was supplied with 50 L of microalgae suspension, and the remaining 50 L was filled with a gas–air mixture with a high CO₂ content and aerated for 648 h (27 days). CO₂ was added daily through aerators in a volume of 7.5–17.5 L (15–39%) of the unoccupied volume of PhBR-B. Intensive bubbling with gases with a high content of CO₂ (15–39%) of the suspension of *Chlorella* microalgae makes it possible to intensify the processes of the absorption of CO₂ by the biomass of microalgae. CO₂ from gaseous emissions is not only a source of inorganic carbon for microalgae but also promotes the more rapid reproduction of microalgae cells and maintains the required pH of the solution in the range of 6.0 to 9.0 [59]. Thus, the microalgae cultivation process can be considered not only as a water purification process but also as an air purification process, which helps to reduce the carbon footprint. To supply carbon dioxide to the PhBR-B, a CO₂ cylinder was used, simulating emissions from gas power plants. The gas was supplied through an aeration pipe located at the bottom of the photobioreactor biofilter with holes. To measure the composition of the gas mixture in photobioreactors, a GEOTECH ga200plus gas analyzer (Geotechnical Instruments (UK) Ltd., Sovereign House, Queensway, Leamington Spa, Warwickshire, CV31 3JR, UK) was used (Figure 1).

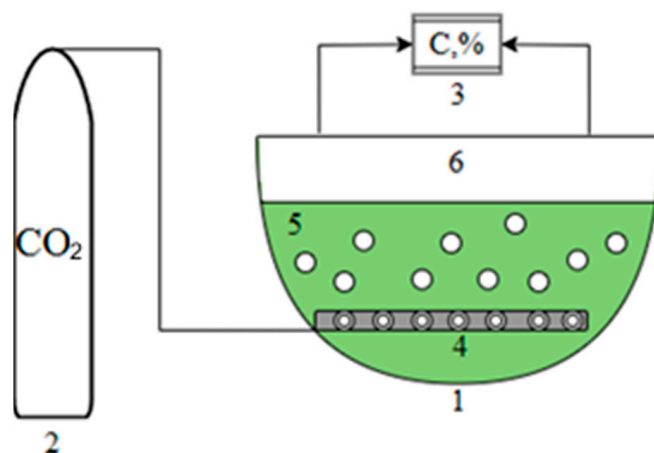


Figure 1. PhBR-B system with CO₂ source: 1—PhBR-B, 2—CO₂ supply system, 3—gas analyzer, 4—aerator, 5—microalgae biomass, 6—PhBR-B space for CO₂ supply.

The process of the cultivation and simultaneous purification of air and water was carried out in photobioreactors with a volume of 100 L (50 L of microalgae suspension and 50 L of free volume) with fluorescent lamps (2600 lux) turned on and a temperature of 26 ± 1 °C. Suspension aeration was carried out with a Xilong AP-003 aerator, (Xilong Scientific Co. Ltd., Shantou, China) with sequential aeration— 2×2.5 L/min. The optical density of the microalgae suspension was measured using a KFK-3 photoelectric colorimeter.

To increase the yield of biohydrogen, the obtained biomass of microalgae after the purification of wastewater and air was preliminarily treated with physicochemical and chemical methods. The following processing methods were used: microwave radiation and acid and heat treatment [48,60,61].

For acid treatment, 2% hydrochloric acid was used. To do this, 50 mL of 2% hydrochloric acid solution was added to 2 L of microalgae suspensions. After acid treatment, the pH dropped from 6.8 to 5.9. After acid treatment, the microalgae suspension was subjected to heat treatment at a temperature of $T = 100$ °C for 20 min. Microwave radiation was used

as a physical impact. Biomass after acid and heat treatment was subjected to microwave radiation with a wavelength of $\alpha = 0.12$ m, power = 800 W for 10 min. For the additional nutrition of hydrogen-producing bacteria, potato starch was used in the amount of 30 g (10% of the volume of microalgae suspension in the bioreactor).

The biomass of microalgae treated in various ways was loaded into hermetic bioreactors (8 pieces, bioreactor volume is 1 L); the suspension volume in the bioreactor was 300 mL.

For the process of obtaining biohydrogen, an inoculum was introduced in the amount of 30 mL (10% of the volume of microalgae suspension in the bioreactor). As an inoculum, a mixture of hydrogen-producing bacteria was used, which were cultivated for 6 months in a flow cultivator to obtain biohydrogen from various organic wastes [62,63]. This inoculum was provided to the research laboratory of bioenergy and supercritical technologies of the Federal State Budgetary Scientific Institution «Federal Scientific Agroengineering Center VIM» (FSAC VIM). The inoculum was subjected to sequencing analysis, which revealed the presence of hydrogen-producing bacteria such as *Aquifex pyrophilus*, *Calderobacterium hydrogenophilum*, and *Acidithiobacillus ferrooxidans*.

The composition of composite mixtures in bioreactors is presented in Table 2.

Table 2. Composition of composite mixtures for biohydrogen production from microalgae biomass.

Bioreactor No.	Volume of Suspension, mL	HCl (2%)	100 °C	Microwave Radiation	Additive (Potato Starch), g	Inoculum, mL
1, 2	300	–	–	–	–	30
3, 4	300	+	+	–	–	30
5, 6	300	+	+	–	30	30
7, 8	300	+	+	+	–	30

For the convergence of the experiment, 2 bioreactors of each composition were created.

After loading the composite mixtures to create anaerobic conditions, each bioreactor was purged with nitrogen. Next, the bioreactors were placed in a dark thermobox at a temperature of 38 °C to create mesophilic conditions. The fermentation process took place within 6 days.

The analysis of biogas obtained in bioreactors was determined on a Khromatek-Crystal 5000.2 gas chromatograph (ZAO SKB Khromatek, Yoshkar-Ola, Russia). Analysis mode: Analysis time, min: 10; Pressure, kPa: 220,000; Temperature, °C: 200; Number of detectors: 2; Type of detectors: thermal conductivity detector; Carrier gases: helium, argon.

Cell microscopy was performed using a Mikmed 5 microscope operating in transmitted light at a useful magnification of 400. Micrographs were obtained using a Toupcam video eyepiece. To detect dead cells, the biomass was treated with methylene blue.

All measurements were carried out in triplicate. The measurement error was 3–7%.

3. Results

Compounds of nitrogen, phosphorus, and sulfur, necessary for the cultivation of biomass, were present in the studied wastewater and replaced nutrients in the nutrient medium. In the process of cultivation, water was purified due to the absorption of nitrogen, phosphorus, and sulfur by microalgae as macroelements. After wastewater treatment and cultivation, according to the initial and final concentrations, the efficiency of wastewater treatment was calculated (according to Formula (1)), which for ammonium nitrogen was 75%, phosphates 52%, nitrites 94%, and sulfates 56% (Figure 2).

In order to assess the quality of water purified from pollutants, final concentrations were compared with the permissible concentrations of pollutants in wastewater, established in order to prevent a negative impact on the operation of centralized sewage systems [64]. The result of post-treatment with microalgae shows high efficiency in the removal of

biogenic elements and reaches the values of permissible concentrations for all substances except NH_4^+ .

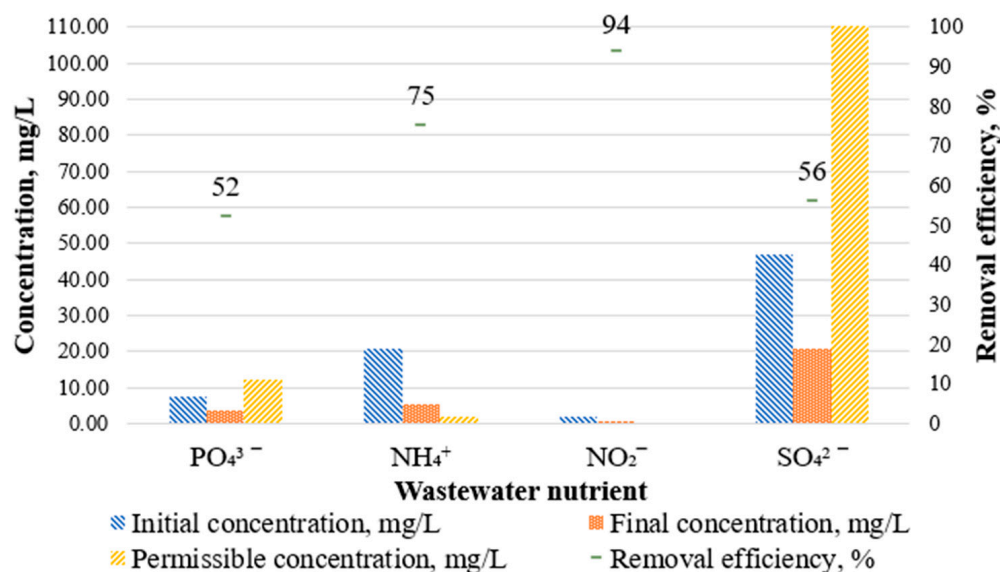


Figure 2. Initial (C_1) and final (C_2) concentration of substances in the nutrient medium and the efficiency of wastewater treatment in the process of microalgae cultivation.

Parallel to the process of water purification, the process of air purification from CO_2 by microalgae *Chlorella kessleri* was carried out. The uptake of CO_2 by microalgae is intense. The content of CO_2 in the photobioreactor biofilter decreased from 30% to almost 0 in 3 days. After the complete absorption of CO_2 in the gas–air environment, CO_2 was re-supplied with a content of 30%. The biomass optical density increased throughout the experiment according to the classical exponential biomass growth curve.

The main results of the study of carbon dioxide absorption are presented by the authors in [59]. In total, 50 L of *Chlorella kessleri* microalgae suspension absorbs 15.5 L of CO_2 in an average of 3 days. Thus, for 1 h, 1 L of *Chlorella kessleri* microalgae suspension (initial optical density—0.798) is able to absorb an average of 0.0046 L of CO_2 , and for 1 day, 1 L of *Chlorella kessleri* microalgae suspension is able to absorb on average of 0.11 l CO_2 .

To determine the mass of utilized CO_2 , the density of CO_2 (at a temperature of 27 °C) was used, which is equal to 1.773 kg/m³. A total of 1 L of suspension of microalgae *Chlorella kessleri* absorbs 0.195 ± 0.001 g of CO_2 per day, increasing its absorbing capacity as the number of biomass cells (optical density of the suspension) increases.

In the process of cultivation–purification of water and air, a biomass of microalgae was obtained with an optical density of 2.5 and pH = 6.8. After acid treatment, the color of the biomass changed from dark green to green-gray, which indicates the destruction of microalgae cells.

Microstructural studies before and after the application of physicochemical methods of microalgae suspension pretreatment recorded the destruction and disintegration of the cell wall of the microalgae biomass. Most of the cells after treatment with methylene blue are stained, which indicates the detection of dead cells with destroyed cell walls (Figure 3).

The treated cells coagulate into agglomerates (Figure 3b,c). Destroyed microalgae cells will enhance the process of biological conversion.

In order to obtain biohydrogen, microalgae biomass with various pretreatments was introduced into bioreactors, and the process of dark fermentation was carried out. On the 6th day, biogas was generated in all bioreactors, and its composition was analyzed using a chromatograph (Table 3).

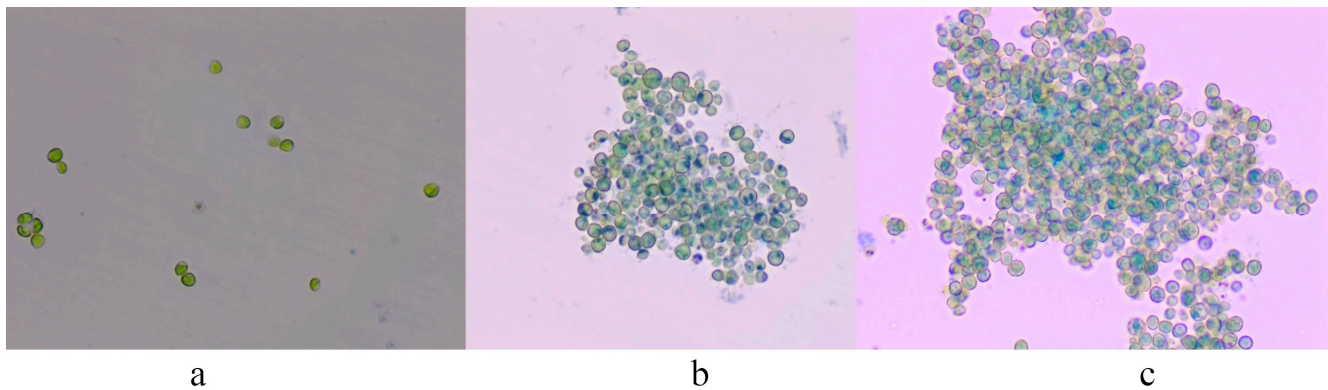


Figure 3. Microstructure of *Chlorella kessleri* microalgae cells: (a) without treatment; (b) after acid treatment; (c) after acid and heat treatment.

Table 3. Results of measurements of the composition of biogas obtained during dark fermentation of microalgae biomass without and with treatment.

Bioreactor No. *	H ₂ , mol.%	CO ₂ , mol.%	O ₂ , mol.%	N ₂ , mol.%
1, 2	0.00494 ± 0.00002	2.170	5.817	91.027
3, 4	0.03323 ± 0.00002	7.513	0.842	86.881
5, 6	2.17912 ± 0.00002	23.763	0.049	70.505
7, 8	0.02383 ± 0.00002	5.388	0.988	85.053

* The composition of bioreactors corresponds to the number in Table 1.

After loading all the components into the 1 L bioreactor, which resulted in biogas formation, the remaining free volume was 670 mL. Using this information and the data from Table 2, the volume of biohydrogen generated in milliliters was calculated. Subsequently, the volume of biohydrogen was determined per liter of suspension, with an initial volume of microalgae suspension of 300 mL. The calculated values for the volume of generated biohydrogen are presented in Table 4.

Table 4. The amount of generated biohydrogen.

Bioreactor No.	H ₂ , mL/L of Microalgae Suspension
1, 2	0.03
3, 4	0.67
5, 6	44.24
7, 8	0.52

The maximum volume of hydrogen was formed in bioreactors 5 and 6 (with the addition of starch) and amounted to 44.24 mL from 1 L of microalgae suspension.

The composition of biogas obtained in bioreactors 5 and 6 with the addition of starch was studied using a gas chromatograph (Table 5).

Table 5. The composition of biogas obtained in bioreactors 5 and 6 with the addition of starch.

Component	Time, min	Area, mV·s	Height, mV	Concentration	Unit of Measure	Detector
H ₂	0.930	1491.181	453.904	2.179	mol.%	2
CO ₂	0.983	40,157.867	9011.215	23.763	mol.%	1
O ₂	1.559	3.872	0.814	0.048729	mol.%	2
N ₂	2.333	4541.205	492.372	70.505	mol.%	2

Table 5. Cont.

Component	Time, min	Area, mV·s	Height, mV	Concentration	Unit of Measure	Detector
H ₂ S	0.000	0.000	0.000	0	mol.%	1
CH ₄	0.000	0.000	0.000	0	mol.%	2
COS	0.000	0.000	0.000	0	mol.%	1
CO	0.000	0.000	0.000	0	mol.%	2

Chromatograms of the composition of biogas obtained in bioreactor No. 6 are shown in Figure 4.

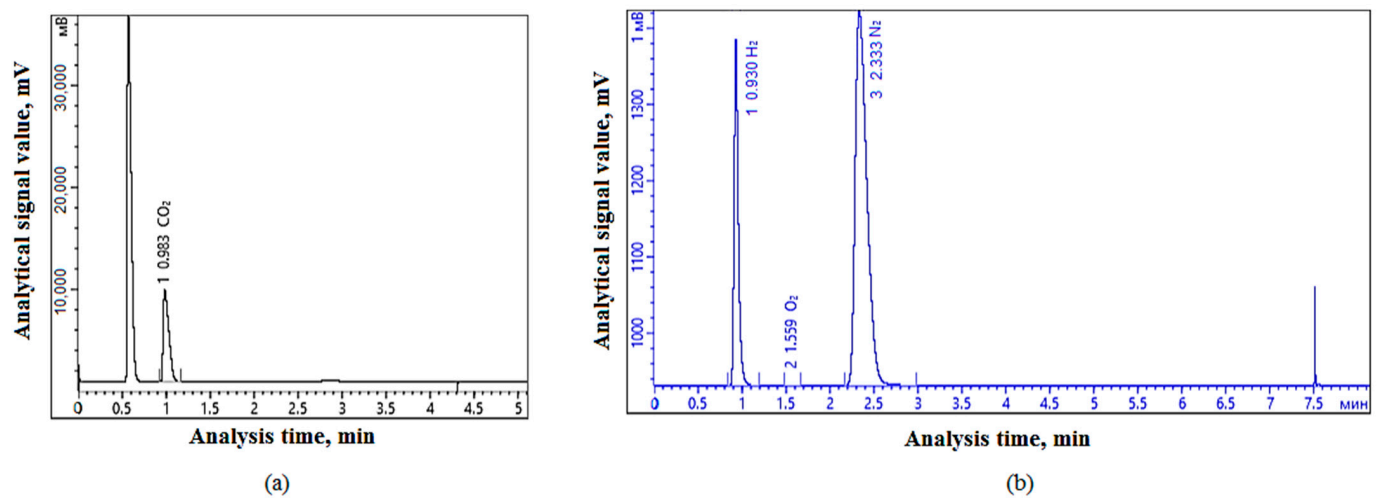


Figure 4. Chromatogram of biogas composition in bioreactor No. 6: (a) detector 1; (b) detector 2.

4. Discussion

Table 2 shows that the highest amount of hydrogen production occurs when using biomass with modified acid and temperature with the addition of starch, as observed in bioreactors 5 and 6. The use of microwave treatment did not give significant results, and the value of biohydrogen does not differ much from the values of biohydrogen obtained from biomass only with acid and temperature treatment (bioreactors 3 and 4 and 7 and 8). The results of biohydrogen production from untreated biomass (bioreactors 1 and 2) and processed biomass indicate that pre-treatment increases biohydrogen yield.

It is known that starch is a polymeric carbohydrate consisting of glucose molecules and can serve as an additional source of carbon for microorganisms during dark fermentation. Starch is a major and common component of agricultural by-products or crop wastes (corn, rice, potato, sweet potato, etc.) [65–70]. Carbohydrates such as glucose and starch are especially good substrates for most of the hydrogen-producing fermentative microorganisms [70]. In microalgae, starch degradation serves as a nutrient source for cell metabolism and growth in the absence of light. Additionally, starch provides a carbon backbone for lipid biosynthesis in adverse conditions [71]. To enhance biohydrogen production, it is essential to manipulate metabolism through genetic or metabolic engineering, aiming to reduce starch degradation [72]. During dark fermentation, the process of biohydrogen formation can be limited by a lack of carbon, which reduces its efficiency. The addition of starch to the culture medium provides the microalgae with an additional source of carbon, which allows them to continue active dark fermentation and therefore increase hydrogen production. Medium with starch as the only carbon source served as a simple model of cheap substrate for algal hydrogen generation [73]. Starch is an additional food for microalgae and facilitates their growth and reproduction, which in turn can increase their total biomass and increase the potential for biohydrogen production.

Due to the fact that bioreactors were purged with nitrogen to create an anaerobic environment, this gas occupies about 70% of the total composition of biogas. In addition to hydrogen, biogas produces CO_2 in the amount of 23%. To obtain pure hydrogen, it is possible to remove nitrogen and carbon dioxide due to sorption or cryogenic purification [74,75]. In the case of cryogenic purification, it is possible to obtain substances with added value: from CO_2 to dry ice at a temperature of $-78\text{ }^\circ\text{C}$; from nitrogen to liquid nitrogen at a temperature of $-195.8\text{ }^\circ\text{C}$. It should be noted that the freezing point of hydrogen is $-259.2\text{ }^\circ\text{C}$.

To solve the environmental problems of air purification from CO_2 and waste water, with the further use of the obtained biomass to produce biohydrogen, we propose a block diagram (Figure 5).

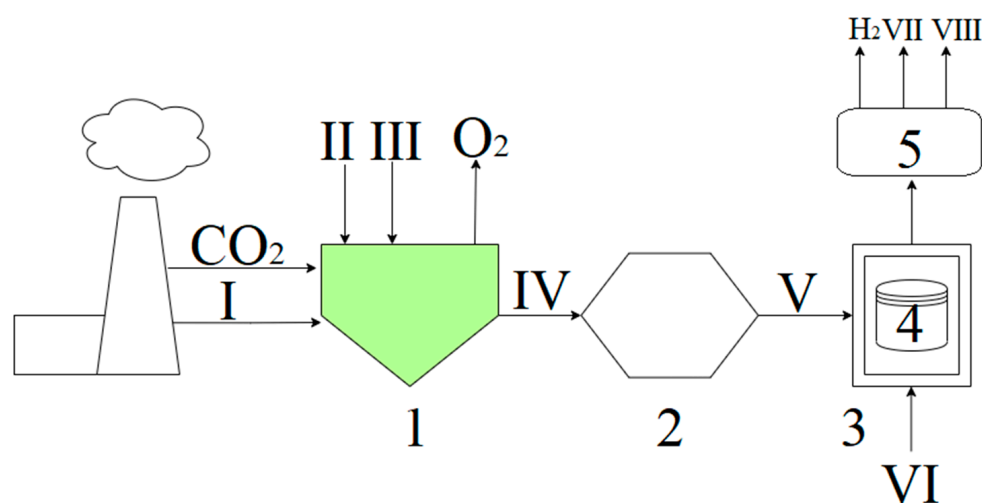


Figure 5. Block diagram of hydrogen production from microalgae biomass: 1—photobioreactor for microalgae cultivation with wastewater and air emissions treatment; 2—biomass pretreatment block; 3—thermobox for creating mesophilic conditions; 4—sealed bioreactor; 5—block of cryogenic cleaning; I—wastewater of the enterprise; II—uterine culture; III—nutrient medium; IV—biomass of microalgae; V—the biomass of microalgae after treatment; VI—inoculum; VII—dry ice; VIII—liquid nitrogen.

This approach has the potential to address multiple environmental and energy challenges while providing valuable resources for the national economy. The purification of water and air from CO_2 and the production of biohydrogen, dry ice, and liquid nitrogen.

Beyond the evident environmental benefits resulting from the implementation of this proposed scheme in various production processes, the primary product of interest is the biohydrogen it generates. The use of this eco-friendly type of fuel is diverse, and every year there are more and more directions for its application. One of the most obvious is the use of biohydrogen as a high-quality efficient energy carrier for refueling fuel cells [76]. It is also proposed to use the resulting biohydrogen as an additive to fossil fuels for combustion [77]. This mixing will reduce the amount of carbon dioxide emissions into the atmosphere [78]. In addition, adding hydrogen to standard diesel fuel will help reduce the amount of vibration in the engine [77]. The use of biohydrogen obtained according to the developed scheme will reduce the cost of obtaining it, thereby bringing additional economic benefits. Also, the application of hydrogen mixing with natural gas in gas network transportation not only improves the utilization rate of hydrogen energy but also reduces the cost of the large-scale updating of household or commercial appliances [79]. Adding hydrogen to the natural gas network for the utilization of factories, gas stations, and urban communities does not only help to relieve greenhouse gas emissions but also facilitates the market's ability to consume hydrogen [80].

The use of biohydrogen has multifaceted and invaluable implications for both ecology and humanity as a whole. The transition to renewable energy sources is now an integral

part of modern technological development. As previously described, biohydrogen opens up new opportunities not only for a smooth transition to green energy but also for achieving the goals of a circular economy. Leveraging years of scientific experience and data from experiments, we can not only avoid common mistakes in renewable energy utilization but also develop tailored strategies for their optimal and sustainable use. That is why it is vital to continue experiments in the field of obtaining biohydrogen and to study the possibilities of its further application.

5. Conclusions

The study showed that microalgae *Chlorella kessleri*, when cultivated, are able to purify wastewater during cultivation with a purification efficiency: from ammonium nitrogen up to 75%, phosphates 52%, nitrites 94%, and sulfates 56%. When cultivated, microalgae use carbon dioxide as a source of inorganic carbon, thereby being able to purify the air. Cleaning from CO₂ with a content of 30% CO₂ in the gas–air flow occurs in 3 days by 100%. It was calculated that 1 L of *Chlorella kessleri* microalgae suspension (initial optical density—0.798) absorbs 0.195 ± 0.001 g of CO₂ on average in 1 day.

The study of the influence of microalgae biomass treatment methods on the biogas yield showed that acid and thermal treatment is sufficient. Processing with microwave radiation at the selected parameters did not give a positive result. An increase in the yield of biohydrogen occurred with the addition of starch. The maximum amount of biohydrogen (44.24 mL/L suspension) was obtained under anaerobic dark fermentation conditions.

Understanding the processes of biohydrogen production from microalgae is an important step in the development of renewable energy sources. These organisms represent a promising source of biomass that can be used to produce hydrogen, a clean and safe fuel. Despite some technical limitations, active research in this area promises to expand the use of biohydrogen in the future and make it more competitive with conventional energy sources.

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