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Features of the Microalgae and Cyanobacteria Growth in the Flue Gas Atmosphere with Different CO_2 Concentrations

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Abstract: Nowadays, it is important to create the optimal technology for the absorption of flue gases with high CO_2 content. In this regard, the aim of the investigation is to study the five different microalgae strains (*Chlorella vulgaris*, *Chlorella ellipsoidea*, *Elliptochoris subsphaerica*, *Gloeotila pulchra*, and *Arthrospira platensis*) under the influence of flue gases. The cultivation of microalgae was carried out in the atmosphere of flue gases with a gas flow rate of approximately 1 $L \cdot min^{-1}$ at high CO_2 concentrations (3, 6, or 8%—from lower to higher concentrations), under continuous (24 $h \cdot d^{-1}$) illumination intensity of 200 μmol quanta$\cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 27 ± 1 °C. The duration of the experiments was 12 days. *Chlorella vulgaris* and *Chlorella ellipsoidea* demonstrated the highest biomass growth rate at CO_2 = 6% (0.79 and 0.74 $g \cdot L^{-1} \cdot d^{-1}$, respectively). The lowest growth rate (0.21 $g \cdot L^{-1} \cdot d^{-1}$) was achieved for *Arthrospira platensis* at CO_2 = 3 and 6%. There was no significant drop in pH in the entire series of experiments. The results of microscopy showed a lack or a minimal number of dead cells in the strains under selected conditions. The obtained results can be used for further development of CO_2 capture and storage technologies.

Keywords: CO_2 capture; flue gases; microalgae; adaptive laboratory evolution; cultivation methodology

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1. Introduction

Effective environmental management is an important topic of our time to maintain an ecological balance in the field of environmental protection. In this regard, the field of application of biologically based materials, which are obtained from various photosynthetic micro-organisms, is growing. In recent years, with regard to the climate change trends, the problem of reducing greenhouse gas emissions has become particularly important [1,2] for which, carbon dioxide, one of the main sources in thermal power plants. The choice of the optimal technology for greenhouse gas absorption is an urgent topic of scientific research at present. The cultivation of various biomass types of autotrophic micro-organisms as a way to reduce the concentration of CO_2 in the air and reduce its content in the emissions of industrial facilities, primarily thermal energy facilities, is promising. Cultivation provides carbon storage through the production of various biomass products [3]. One of the widely studied CO_2 capture methods is the use of microalgae as energy resources [4,5]. Due to several advantages of microalgae, such as high productivity, the possibility of growing using wastewater from the domestic and industrial sectors [6,7], and the availability of developed and tested technologies for converting microalgae biomass into useful products (biofuels, feed, food additives, pharmaceutical products, etc.) [8,9], they (microalgae) are put forward for CO_2 removal from the environment as part of flue gases from energy and

other sources. However, impurities in flue gases can harm the microalgae's growth. The analysis of the published articles shows that the main problems of microalgae cultivation, to which the research is devoted, are: achieving the highest possible microalgae growth rates when growing them in conditions of high flue gas concentration; maintaining the pH of culture media in optimal ranges for cultures; and assessing the effect on the microalgae growth of such toxic flue gas components as nitrogen and sulfur oxides (NO_x, SO_x).

Among the microalgae capable of reducing the CO_2 content in flue gases, *Chlorella* strains usually show the best results in terms of growth and adaptation to cultivation conditions [10,11]. Therefore, various *Chlorella* strains are considered in many works as objects of research on the absorption of CO_2 from flue gases. Thus, the work of Na' et al. [12] shows the results with *Chlorella pyrenoidosa* Chick, for which the maximum growth rate in the flue gas atmosphere at the levels of 0.51 and 0.13 $g \cdot L^{-1} \cdot d^{-1}$ and the efficiency of CO_2 utilization from flue gases of 0.95 and 0.25 $g \cdot L^{-1} \cdot d^{-1}$ were obtained, respectively, in a laboratory and pilot installation. For the *Chlorella fusca LEB* 111 strain, in an experiment with flue gas containing 10 vol% CO_2, SO_2 = 200 ppm, and NO = 200 ppm, a specific biomass growth rate μ of 0.18 ± 0.01 d^{-1} was obtained ($\mu = \left(ln\frac{m_1}{m_2}\right)\frac{1}{\Delta t}$, where m_1, m_2—biomass at time t_1 and t_2, $\Delta t = t_2 - t_1$) in the work of Duarte et al. [13]. At the same time, the content of sulfur and nitrogen oxides up to 400 ppm in the above study did not limit the CO_2 absorption, and the biochemical composition of microalgae was similar in experiments with different CO_2 contents and toxic components NO_x, SO_x in the flue gas composition: carbohydrates—19.7%, lipids—15.5%, proteins—50.2%. *Chlorella* sp. MTF-15 is indicated as a highly effective strain [14,15], which showed a degree of CO_2 absorption close to 50% from the flue gas of steel mills with a content of CO_2 = 25%, NO_x = 70–80 ppm, and SO_2 = 80–90 ppm. Based on the analysis of *Chlorella* strains cultivation, generalized data on the efficiency of CO_2 absorption by various *Chlorella* strains are presented in the work of Latypov et al. [16]. The strains *Chlorella sorokiniana*, *Chlorella pyrenoidosa* M18, and *Chlorella* sp. M4 exhibit maximum biomass growth and carbon dioxide uptake. Comparative analysis permitted the identification of those CO_2 concentration ranges in gas–air mixtures at which the productivity of microalgae and the efficiency of their CO_2 absorption are maximum (at 7–9% CO_2).

Several published scientific papers have proposed and implemented strategies for adapting microalgae to avoid inhibiting their growth. Thus, experimental studies use various adaptation methods, including some studies on the methods and results of adaptation to both single stressors (including high CO_2/flue gas concentrations) and multiple stressors. One of the important results is the revealed property of exposure to multiple stressors. Namely, using complex stressful conditions simultaneously, such as wastewater [17,18] or filtrate from landfills [19], can increase the tolerance of microalgae to various components of these mixture stressors simultaneously. Conversely, experiments with a single stressor can be used to increase the microalgae's resistance to multiple stressors at the same time. In the works of Chen et al. and Mavrommati et al. [20,21], the concept and method of adaptive laboratory evolution (ALE) were proposed to expand the microalgae resistance to multiple environmental stresses and maintain quick cell growth. The ALE technology was successfully applied and developed in subsequent studies [22–24]. Since flue gases from industrial sources contain approximately 100–300 ppm of NO_x and SO_x (depending on the type of fuel), many algae strains reported in the literature cannot be used for direct fixation of CO_2 from flue gases and require special adaptation or other approaches to overcome the inhibitory effect of toxic gas impurities [25]. Thus, in the work of Jiang et al. [26], the authors used an intermittent supply of flue gas during the cultivation of the microalgae *Scenedesmus dimorphus* to control pH, which avoids inhibition of cell growth. As a result of using the same method [27] for the cultivation of *Chlorella* sp. with intermittent aeration of the culture liquid by flue gas, an average efficiency of CO_2 removal from the flue gas of up to 60% was achieved. Another method of achieving effective CO_2 absorption is the use of microbe strains from aquatic or soil objects located near flue gas sources; therefore,

these micro-organisms may have higher resistance to toxic impurities that are part of flue gas [28]. It was also found that the reaction of microalgae to the presence of NO_x and SO_x depends on various factors. Thus, in the work of Yoshihara et al. [25], it was shown that the tolerance of microalgae to NO depends on the biomass density in the culture liquid. When the model flue gas with 100 or 300 ppm NO was supplied to a tubular photobioreactor where microalga cell concentration was lower than 1.0 $g \cdot L^{-1}$, cell growth was completely suppressed, and NO was not eliminated from flue gas. However, when the model flue gas aerated the same microalgae when the cell concentration was approximately 1.5 $g \cdot L^{-1}$ evident inhibition of cell growth resulting from the presence of NO in the model flue gas was not observed. The results of SO_x effect on the microalgae growth are ambiguous. On the one hand, as mentioned above, various *Chlorella* strains showed high growth rates even at significant SO_x concentrations in flue gases. On the other hand, it was reported in the work of Yanagi et al. [29] that *Chlorella* microalgae (HA-1 strain tolerant to high concentrations of CO_2) were inhibited by the content of SO_2 = 50 ppm in the gas mixture. It was assumed that the toxic effect was associated either with a decrease in pH during bubbling of the culture liquid with a gas mixture or with direct inhibition of microalgae growth directly by SO_2 itself.

In our earlier article, microalgae strains were adapted to grow in conditions of high CO_2 concentrations in a gas–air mixture by stepwise cultivation with a change in the CO_2 content from 0.04 to 9% [30]. The strains showed sufficiently high growth rates and stable viability and therefore were selected for this study. Thus, the distinguishing feature of our research was: to study the growth characteristics of microalgae, tolerant to high CO_2 concentrations, in the atmosphere of flue gas, with consistent adaptation to increasingly high flue gas concentrations in the gas–air mixture. To achieve these goals, experiments were conducted on the cultivation of microalgae strains for 12 days in a flue gas atmosphere with a concentration of CO_2 = 3, 6, and 8%. The novelty of our study also includes the research of five different microalgae strains that consume various carbon sources: *C. ellipsoidea*, *C. vulgaris*, *E.subsphaerica*, and *G. pulchra*, assimilating gaseous CO_2; *A. platensis*—carbon from the culture liquid in the form of HCO_3^- anions. There was also a comparative analysis of the reaction of the most significant biomass characteristics (growth rate, biochemical parameters, uptake rate of the main components of nutrient media, and morphological characteristics of microalgae) and an evaluation of the microalgae viability using the express method of cytochemical cell staining with a vital dye followed by light microscopy control. This method was as close as possible to the conditions of industrial production for CO_2 utilization. The microalgal biomass obtained during the experiments can be used for the production of various types of biofuels using previously developed and tested methods [31,32].

2. Materials and Methods

2.1. Microalgae Strains

In this research microalgae strains *Chlorella ellipsoidea* rsemsu Chl-el, *Chlorella vulgaris* rsemsu Chv-20/11-Ps, *Elliptochloris subsphaerica* rsemsu N-1/11-B, *Gloeotila pulchra* rsemsu Pz-6, and a resistant consortium of cyanobacterium *Arthrospira platensis* rsemsu P Bios with heterotrophic bacteria (heterotrophic bacteria are the representatives of the genera *Pseudomonas* and *Bacillus*) were studied. Strains are taken from the collection of the Renewable Source Energy Laboratory at Lomonosov Moscow State University (RSE LMSU). These strains have a quite high biomass productivity and are resistant to changes in environmental conditions. A detailed description of microalgae cultures, as well as the rationale for choosing the listed microalgae for conducting experiments with flue gas at high CO_2 concentrations, are presented in our earlier work [30].

For experiments with flue gas, microalgae strains underwent the adaptation procedure to high CO_2 concentrations. For these purposes, microalgae were grown in the photobioreactors, placed in a gas chamber, in several successive series of experiments, when the CO_2 concentration in the gas–air mixture increased sequentially from 0.04%

to 9%. At the same time, the microalgae biomass at the end of each experiment was used for sowing photobioreactors and cultivation at higher CO_2 concentrations. Each experiment lasted for 12 days. For most strains (*Chlorella vulgaris*, *Chlorella ellipsoidea*, and *Elliptochloris subsphaerica*), an increase in growth rate was observed as CO_2 concentration in the gas–air mixture increased. A slight decrease and further stabilization of the growth rate was found for strains *Gloeotila pulchra* and *Arthrospira platensis*. At the same time, microscopy did not show a noticeable change in cell morphology. Subsequently, microalgae strains that underwent the adaptation procedure were maintained in an atmosphere with an increased CO_2. Thus, for the experiments with flue gas presented in this paper, strains tolerant to high CO_2 concentrations were used.

2.2. Composition of Nutrient Media

Composition of Tamiya medium for cultivation of *Chlorella ellipsoidea* and *Chlorella vulgaris*: KNO_3—5.0 $g \cdot L^{-1}$, KH_2PO_4—1.25 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$—2.5 $g \cdot L^{-1}$, $FeSO_4 \cdot 7H_2O$—0.009 $g \cdot L^{-1}$, EDTA—0.037 $g \cdot L^{-1}$, H_3BO_3—2.86 $mg \cdot L^{-1}$, $MnCI_2 \cdot 4H_2O$—1.81 $mg \cdot L^{-1}$, $ZnSO_4 \cdot 7H_2O$—0.22 $mg \cdot L^{-1}$, MnO_3—0.018 $mg \cdot L^{-1}$, NH_4VO_3—0.023 $mg \cdot L^{-1}$.

Composition of BG-11 medium for cultivation of *Elliptochloris subsphaerica* and *Gloeotila pulchra*: $NaNO_3$—1.5 $g \cdot L^{-1}$, $K_2HPO_4 \cdot 3H_2O$—0.04 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$—0.075 $g \cdot L^{-1}$, $CaCl_2 \cdot 2H_2O$—0.04 $g \cdot L^{-1}$, Na_2CO_3—0.02 $g \cdot L^{-1}$, citric acid—0.006 $g \cdot L^{-1}$, Na_2EDTA—0.001 $g \cdot L^{-1}$, ammonium iron citrate—0.006 $g \cdot L^{-1}$, microelement solution—1 $mL \cdot L^{-1}$; composition of trace elements: H_3BO_3—2.86 $g \cdot L^{-1}$; $MnCl_2 \cdot 4H_2O$—1.81 $g \cdot L^{-1}$; $ZnSO_4 \cdot 7H_2O$—0.22 $g \cdot L^{-1}$; $Na_2MnO_4 \cdot 2H_2O$—0.4 $g \cdot L^{-1}$; $CuSO_4 \cdot 5H_2O$—0.08 $g \cdot L^{-1}$; $Co(NO_3)_2 \cdot 7H_2O$—0.05 $g \cdot L^{-1}$.

Composition of modified Zarrouk's medium for cultivation of *Arthrospira platensis*: $NaHCO_3$—16.8 $g \cdot L^{-1}$, KNO_3—3.0 $g \cdot L^{-1}$, $K_2HPO_4 \cdot 3H_2O$—0.66 $g \cdot L^{-1}$, K_2SO_4—0.5 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$—0.2 $g \cdot L^{-1}$, NaCl—1.0 $g \cdot L^{-1}$, $CaCl_2$—0.04 $g \cdot L^{-1}$, $FeSO_4 \cdot 7H_2O$—0.018 $g \cdot L^{-1}$, EDTA—0.08 $g \cdot L^{-1}$, microelement solution for Zarrouk's medium—1 $mL \cdot L^{-1}$.

2.3. Experimental Facility

To conduct experiments on microalgae cultivation in the atmosphere of flue gas at different CO_2 concentrations, a laboratory installation was created, which includes the following main elements: flue gas generation plant (FGGP), the atmospheric gas chamber (AGC), and photobioreactors (PBRs) (10 pcs). A full description and design of the AGC and PBRs are presented in [30]. Flue gas mixtures with air were prepared in the FGGP, which was located next to the gas chamber and was connected to it by a gas supply system. The range of changes in the main components (SO_2 and NO_2) of flue gas in experiments with different CO_2 concentrations was: at CO_2 = 3% the content of NO_2 varied within 1–16 ppm, at CO_2 = 6% the content of NO_2 varied within 17–18 ppm, at CO_2 = 8% the content of NO_2 varied within 12–18 ppm; the content of SO_2 was 0.0–0.7 ppm in each experiment with all CO_2 concentrations. Unlike other studies (including those discussed in the introduction), the content of gas components in our experiments was low, although not exclusive. Our analysis of the flue gas composition from the Caterpillar G3520C gas generator showed NO_2 content from 6 to 46 ppm, SO_2 from 0 to 4.8 ppm. Photos of the FGGP and AGC are shown in Figure 1. The scheme of the FGGP is shown in Figure 2.

Ten PBRs were placed in the AGC during the experiments. The main components of the reactor were a glass cylindrical flask with a height of 40 cm, an external diameter of 15 cm, with a wall thickness of 3 mm, a cover of gauze, and an air aerator with the size of 80 × 50 mm, placed at the bottom of a glass flask. Equable constant illumination ($24\ h \cdot d^{-1}$) was provided through all the PBR by using strip LED lights. The flue gas mixture was supplied to the PBRs through air aerators, while the gas flow rate was $1\ L \cdot min^{-1}$.

(**a**) (**b**)

Figure 1. (**a**) General view of the atmospheric gas chamber with PBRs placed in it, (**b**) general view of the flue gas generation plant.

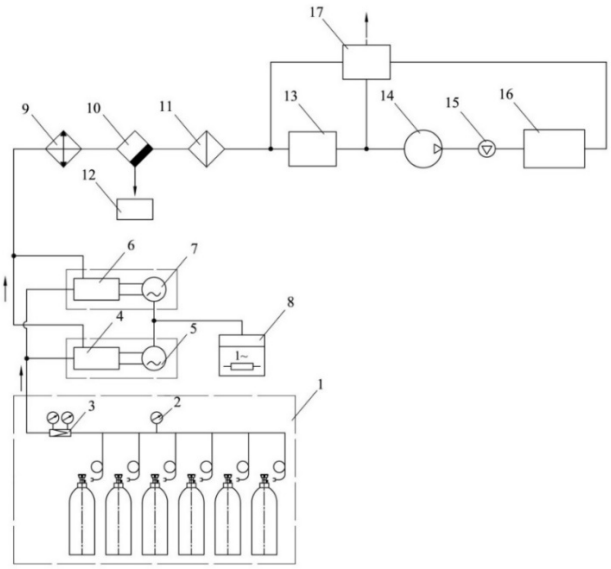


Figure 2. The scheme of FGGP. 1—methane discharge ramp, 2—manometer, 3—gas reducer, 4,6—internal combustion engine, 5,7—generator, 8—heater, 9—cooler, 10—condensate collector, 11—filter, 12—condensate container, 13—mixing device, 14—compressor, 15—rotameter, 16—photobioreactor, 17—buffer container.

2.4. Cultivation Methodology

The experimental algorithm for microalgae cultivation was as follows: at first, preparation of nutrient medium on distilled water in a volume of 8 L for each strain, seeding the medium with an inoculum of each strain to the original concentration of microalgae biomass (0.2–0.25 $g \cdot L^{-1}$), and placing this culture liquid into two PBRs in a volume of 4 L for each strain; second, placing the PBRs in the AGC, switching on illumination and bubbling, injection of flue gas mixture (0.25 vvm) to a specified CO_2 concentration in the AGC, and sealing of the chamber; third, microalgae cultivation during 12 days at specified CO_2 content. Since the ending of the experiment, microalgae biomass with culture liquid was filled into 5-L containers for subsequent use in the following experiment.

2.5. Experimental Procedure

Here are the experimental conditions: the duration of experiments—12 days, water for nutrient media preparation—distilled water, illumination—200 µmol quanta$\cdot m^{-2} \cdot s^{-1}$, temperature in the AGC—27 ± 1 °C, and CO_2 content in each experiment: 3, 6, or 8%. Preliminary experiments with air bubbling (CO_2 = 0.04%) provided average growth rates for the strains, respectively: *Chlorella ellipsoidea*—0.29 $g \cdot L^{-1} \cdot d^{-1}$, *Chlorella vulgaris*—0.31 $g \cdot L^{-1} \cdot d^{-1}$,

Elliptochloris subsphaerica—0.25 $g \cdot L^{-1} \cdot d^{-1}$, *Gloeotila pulchra*—0.31 $g \cdot L^{-1} \cdot d^{-1}$, and *Arthrospira platensis*—0.17 $g \cdot L^{-1} \cdot d^{-1}$.

The timetable of biomass and culture liquid analysis:

- On the 0th and 12th day: optical density (OD), pH of the medium, microscopy, and composition of the culture medium;
- On the 3rd and 9th day: OD and pH of the medium;
- On the 6th day: OD, pH of the medium, and microscopy.

And after each experiment, a biochemical analysis of the biomass was performed. The general procedure followed the analytical methods described in the work of Efremenko et al. [33] with minor modifications. Briefly, the biomass samples were treated by thermolysis and then lipids were extracted by methanol–chloroform. The extract was washed by NaCl solution and evaporated to dryness, and the residue was weighted, giving the mass of lipids. After lipid extraction, the remaining sediment was dried in a Binder VD53 at 50 °C for 20 h. Proteins were extracted 3 times by 40 $g \cdot L^{-1}$ Sodium dodecylsulfate (SDS) in TRIS-HCl buffer while monitoring the efficiency of extraction at wavelengths of 260 and 280 nm. Concentrations of proteins in combined extracts were determined with Bradford assay [34] using bovine serum albumin to obtain a calibration curve. Concentration of SDS within all analyzed samples was adjusted to 0.2 $g \cdot L^{-1}$. Protein quantities were calculated using determined concentrations and known volumes of extracts. The biomass sediment remaining after the protein extraction was then dried in a Binder VD53 desiccator at 60 °C for 20 h, followed by its hydrolysis in concentrated sulfuric acid. To determine the amount of carbohydrates phenol-sulfuric acid method was used [35] to analyze separately the protein extracts (i.e., soluble carbohydrates and peptidoglycans) and acid hydrolysate (i.e., insoluble carbohydrates). A calibration curve was obtained using aqueous glucose solution. The total quantity of carbohydrates was a sum of two values determined above. Each analysis was repeated in 2 independent experiments.

In our study the following methods and measuring instruments were applied to measure biomass and culture liquid characteristics: photometer Expert-003 (Russia) (OD determination); pH meter Expert-pH (Russia) (pH determination); titration (bicarbonates determination); ICS-1600 ion chromatograph (California, USA) with conductivity detector (nitrates and phosphates determination). Microscopic monitoring of the state of microalgae cultures and their viability was carried out by using a light microscope Mikmed-5—LOMO (Russia). To determine the viability of microalgae cells, a method of cytochemical staining was used. A vital dye methylene blue was applied in this method [30], which stained dead microalgae cells. At least 10 fields from each sample of experiments taken on the 0th, 6th, and 12th day were viewed during microscopy; at the optimal microscope magnification the number of blue-stained cells (or their) clusters was counted.

3. Results and Discussion

In this work, strains of microalgae tolerant to high CO_2 concentrations were used, and in our previous works [30,36] the optimal growth conditions were found. Here, Table 1 shows the results of the microalgae growth rate for experiments on microalgae cultivation in a flue gas atmosphere with CO_2 concentrations of 3, 6, and 8%.

The maximum growth rate was observed for *Chlorella* strains, and for both strains, the highest biomass growth rate was at CO_2 = 6%: for *Chlorella vulgaris* (0.79 $g \cdot L^{-1} \cdot d^{-1}$) and for *Chlorella ellipsoidea* (0.74 $g \cdot L^{-1} \cdot d^{-1}$). It should be noted that in [16], where the analysis of the biomass growth rate of various *Chlorella* strains (as the most effective microalgae for CO_2 absorption) was carried out under conditions of high CO_2 concentrations, only 7 out of 21 strains showed a growth rate from 0.5 to 1.0 $g \cdot L^{-1} \cdot d^{-1}$. *E. subsphaerica* and *G. pulchra* strains showed lower and similar growth rates, with a slight maximum at CO_2 = 3% (0.56 and 0.49 $g \cdot L^{-1} \cdot d^{-1}$, respectively), and for *E. subsphaerica* with a gradual decrease as the concentration of CO_2 in the flue gas increases. The growth rate of *A. platensis* was the lowest of all the strains considered, with an almost equal growth rate at CO_2 = 3 and 6% (0.21 $g \cdot L^{-1} \cdot d^{-1}$) and a slight increase at CO_2 = 8% (0.27 $g \cdot L^{-1} \cdot d^{-1}$).

Table 1. The growth rate of microalgae biomass ($g \cdot L^{-1} \cdot d^{-1}$) in the atmosphere of flue gases at CO_2 content (3, 6, and 8%). Average growth rate of microalgae biomass over 12 days.

Microalgae	The Growth Rate of Microalgae Biomass, $g \cdot L^{-1} \cdot d^{-1}$		
	3%	6%	8%
C. vulgaris	0.64 ± 0.03	0.79 ± 0.05	0.74 ± 0.04
C. ellipsoidea	0.58 ± 0.03	0.74 ± 0.04	0.68 ± 0.03
E. subsphaerica	0.56 ± 0.03	0.51 ± 0.03	0.51 ± 0.02
G. pulchra	0.49 ± 0.02	0.42 ± 0.02	0.48 ± 0.03
A. platensis	0.21 ± 0.01	0.21 ± 0.01	0.27 ± 0.02

The main difference between the flue gases used in the experiments and the mixture of air and CO_2 is the presence of NO_2 impurities, which are also a stressor for microalgae. However, according to the results obtained, the NO_2 concentration present in the flue gases (not exceeding 18 ppm) did not have a significant inhibitory effect on the growth of microalgae: the growth rate was 1.1–1.4 times higher (for different strains) than the growth rate in experiments on the cultivation of microalgae in mixtures of air and CO_2 (Figure 3). Note that only for *E. subsphaerica* and *A. platensis*, the growth rate in experiments with air–gas mixtures (CO_2 = 6%) and illumination of 200 µmol quanta$\cdot m^{-2} \cdot s^{-1}$ was higher than in experiments with flue gas (Figure 3.). Statistically significant changes in the growth rate were recorded for all strains except *C. ellipsoidea*.

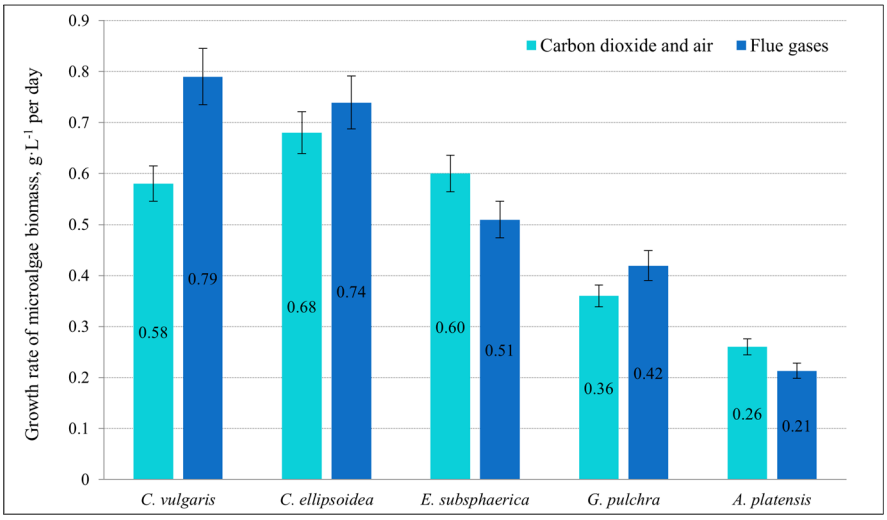


Figure 3. The growth rate of microalgae biomass ($g \cdot L^{-1} \cdot d^{-1}$) in the experiments with carbon dioxide and air (from our previous article [36]) and flue gas (this work). CO_2 = 6% and illumination 200 µmol quanta$\cdot m^{-2} \cdot s^{-1}$. The error bars represent standard deviation.

Apparently, in experiments with flue gas, a high and stable growth rate was obtained due to the long-term microalgae adaptation to stressful conditions (high concentrations of CO_2) as well as more optimal illumination conditions (200 µmol quanta$\cdot m^{-2} \cdot s^{-1}$). As can be seen, the presence of NO_2 in low concentrations has not produced a noticeable inhibitory effect. From one side, it is confirmed that adaptation to a single stressor can expand the overall adaptation of microalgae strains to various environmental stressors. On the other hand, this provides grounds for carrying out the procedure of adaptation to increasingly higher NO_2 concentrations against the background of high CO_2 concentrations. Experimental cultivation of microalgae in the atmosphere of flue gas, containing NO_x, including those shown in Table 2, was carried out at a wide range of NO_x concentrations: from 50–70 ppm [37] to 400 ppm [13]. However, it was not possible to identify any dominant

trend reflecting the influence of this gas impurity at this stage when analyzing the literature data. Table 2 shows the growth rates of microalgae strains that were achieved in the atmosphere of flue gas (the results of this study, from the review article by Yen et al. [38] and from other sources).

Table 2. Comparison of the biomass productivity ($g \cdot L^{-1} \cdot d^{-1}$) of various microalgae strains when grown in a flue gas atmosphere.

Microalgae	CO_2, %	Light Intensity, μmol Quanta·m^{-2}·s^{-1}	Biomass Productivity, $g \cdot L^{-1} \cdot d^{-1}$	Reference
Chlorella vulgaris	10–13	1150	2.500	[39]
Chlorella fusca Leb 111	10	41.6	0.140	[13]
Spirulina platensis	15	120	0.170	[40]
Nannochloropsis oceanica KA2	13	Outdoor (variable)	0.013	[41]
Monoraphidium minutum	13.6	200	0.189	[42]
Scenedesmus dimorphus	15	100	0.485	[26]
Nannochloropsis salina	10–11	Outdoor (variable)	0.032	[37]
Chlorella vulgaris	8	200	0.740	This work
Elliptochloris subsphaerica	8	200	0.510	This work
Arthrospira platensis	8	200	0.270	This work

The obtained growth rates are second only to the results of the article by Brányiková et al. [39] at extremely high illumination values. However, high illumination (if it is not provided by natural sunlight) requires additional energy costs, which reduces the efficiency of CO_2 capture, leading to an additional intake of carbon dioxide into the environment.

At the first stages of experimental work, quantitative estimates of the specific CO_2 absorption by microalgae were carried out, for which the increase in biomass of microalgae (cyanobacterium *Arthrospira platensis*) was accurately estimated during their long-term (14 days) cultivation in a large-capacity photobioreactor (70 L) placed in a hermetically sealed gas chamber [43]. By measuring the increase in biomass over the entire period of the experiment and changes in CO_2 concentrations in a sealed AGC, the values of the specific CO_2 absorption per 1 g of biomass were determined. At the same time, a thorough check of the tightness of the chamber was carried out in advance. The CO_2 concentration was determined throughout the experiment with a frequency of 1–5 min. The results of our work generally confirmed the previously determined efficiency of CO_2 absorption [44] and amounted to 1.8–2.0 g of $CO_2 \cdot g^{-1}$ of microalgae biomass. On the basis of specific indicators, an assessment of CO_2 absorption in the experiments described above was carried out depending on the microalgae strains (Table 3).

Table 3. CO_2 mass absorbed by microalgae suspension with a volume of 1 L in 12 days of the experiment.

Microalgae	CO_2 Absorbtion by Microalgae (from Flue Gases), $g \cdot L^{-1}$		
	3%	6%	9%
C. vulgaris	15.4	19.0	17.8
C. ellipsoidea	13.9	17.8	16.3
E. subsphaerica	13.4	12.2	12.2
G. pulchra	11.8	10.1	11.5
A. platensis	5.0	5.0	6.5

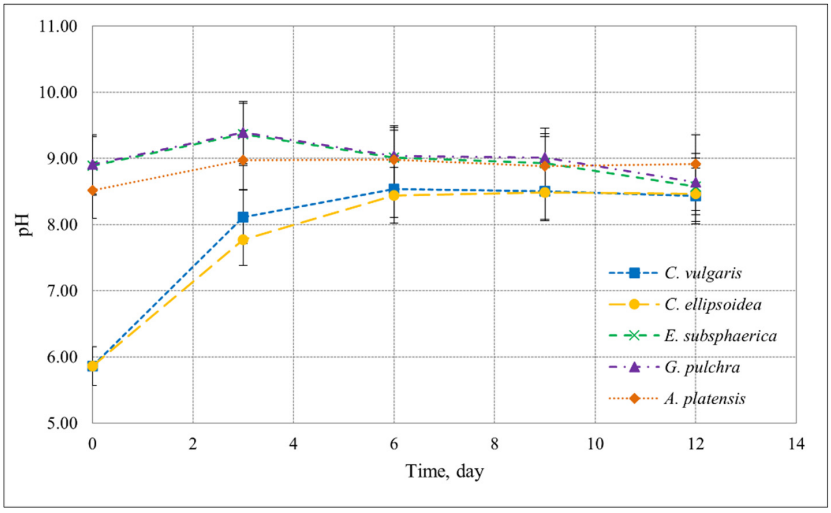
Such a different effect of increased concentrations of flue gas and CO_2 on various microalgae types and cyanobacteria is partially explained by their species specificity and even strain specificity, which we found when studying the effects of different stressors on a wide range of microalgae strains in the study of lipid induction in algae cells [45,46].

It is important to consider changes in the characteristics of the culture liquid in experiments, since they largely determine the conditions of vital activity of microalgae cells. Figure 4 shows the change in the pH values of the culture liquid for experiments on cultivating microalgae strains in a flue gas atmosphere with a concentration of CO_2 = 3, 6, and 8%.

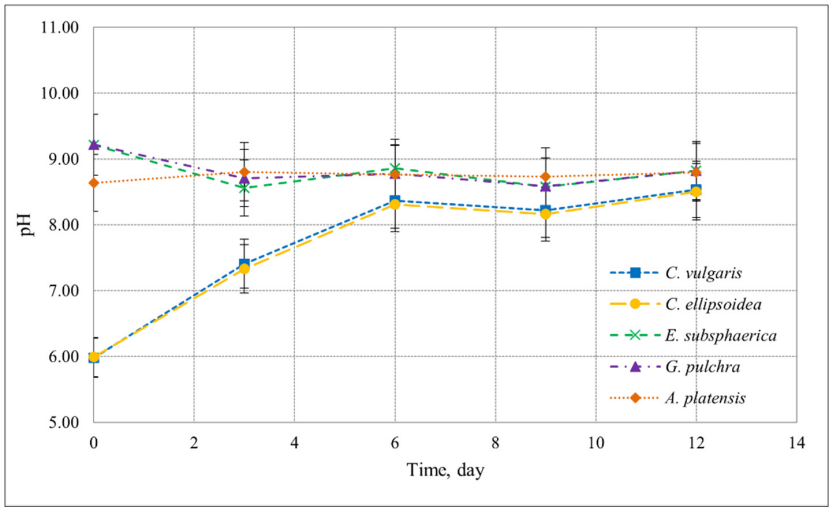
The change in culture liquid pH in experiments with CO_2 = 3 and 6%, as one of the most significant characteristics of microalgae cultivation conditions, was heterogeneous: slight fluctuations (in the range of 8.5–9.3); for strains *Elliptochloris subsphaerica*, *Gloeotila pulchra*, and *Arthrospira platensis*; a sharp increase during the first three days of the experiment (from 5.86 to 8.45) and further reaching a constant value (8.47) for both *Chlorella* strains. This dynamic can be explained by the peculiarities of microalgal metabolism and the buffering properties of nutrient media. Qualitatively, the same pH dynamics were observed in experiments with a mixture of air and CO_2 [30]. However, the pH increase in the nutrient media of *Chlorella* strains in previous experiments reached lower values (at a maximum—7.50–7.75). The change in the pH values, which is a crucial characteristic of the microalgae growth conditions during bubbling with flue gas in the experiment at CO_2 = 8%, was also qualitatively identical to the results obtained in other experiments with CO_2 = 3 and 6%. At the same time, a significant drop in pH, which is reported in many similar studies [9,44,47] and which leads to the inhibition of microalgae, was not observed in the entire series of experiments.

The dynamics of culture liquid components in experiments with biomass cultivation in a flue gas atmosphere were as follows. In all experiments, for all studied strains, an increase in the concentration of hydrocarbonates (HCO_3^-) was recorded due to continuous bubbling with carbon dioxide (CO_2), its subsequent dissolution and dissociation. It was not compensated by the microalgal CO_2 absorption. Minor changes in the content of potassium and magnesium were noted, as well as a decrease in the content of phosphates, sulfates, and nitrates by the 12th day of the experiments. Nitrates showed the most significant reduction: by more than 50% for *Chlorella* strains and almost 100% for other strains. For all strains in experiments with CO_2 = 3, 6, and 8%, an increase in chemical and biological oxygen consumption (COD, BOD_5) was noted. This indicates the regular formation of dying and decomposing cells with chemical and biological absorption of oxygen by the end of the experiment (12th day). Thus, only the absorption of NO_3^- can be considered as a factor potentially leading to a decrease in the growth rate. To neutralize the influence of this factor and search for more optimal growth conditions (and CO_2 absorption), experiments should be conducted with the introduction of medium components (especially nitrates) after the first 6 days of the experiment.

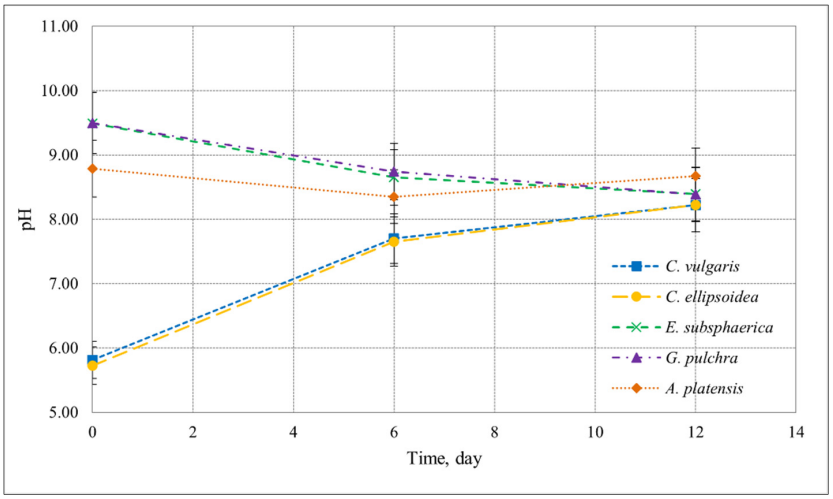
In the context of this study, the response of the biochemical composition of microalgae, as indicators of metabolism, to the cultivation of flue gas in the atmosphere is significant. Figure 5 shows the results of a biochemical analysis of microalgae biomass grown in a flue gas atmosphere.



(**a**)

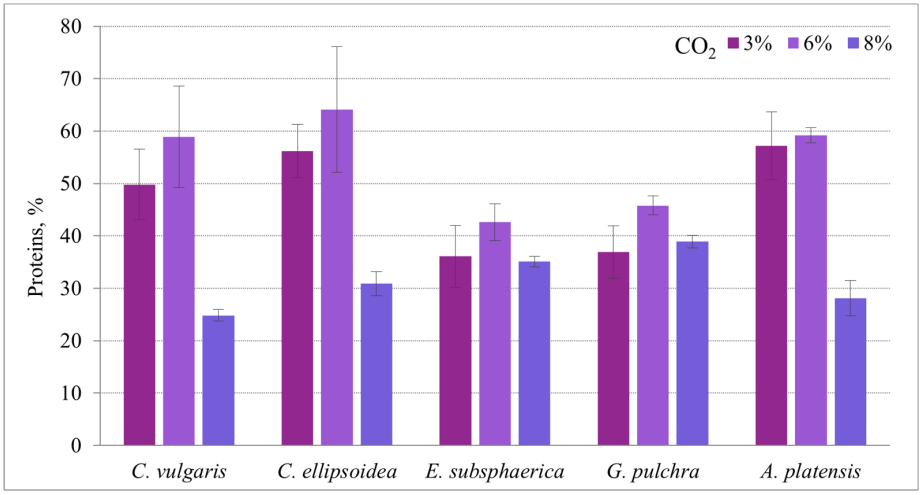


(**b**)

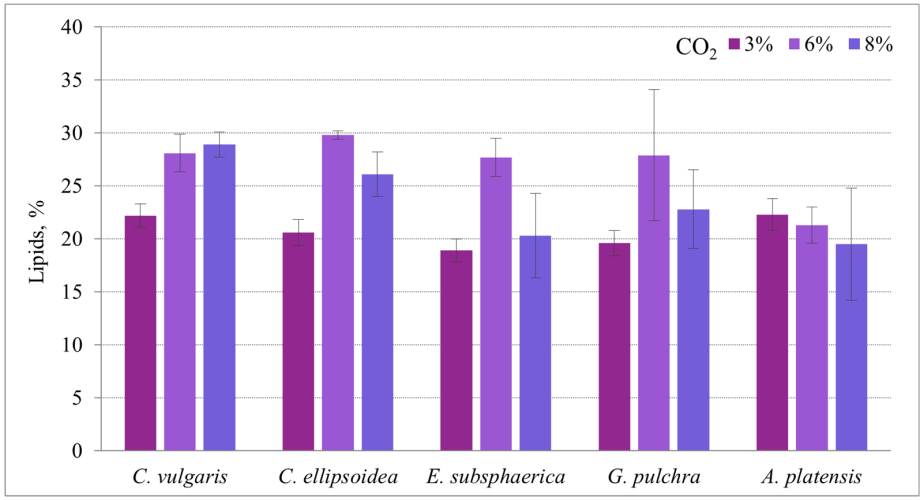


(**c**)

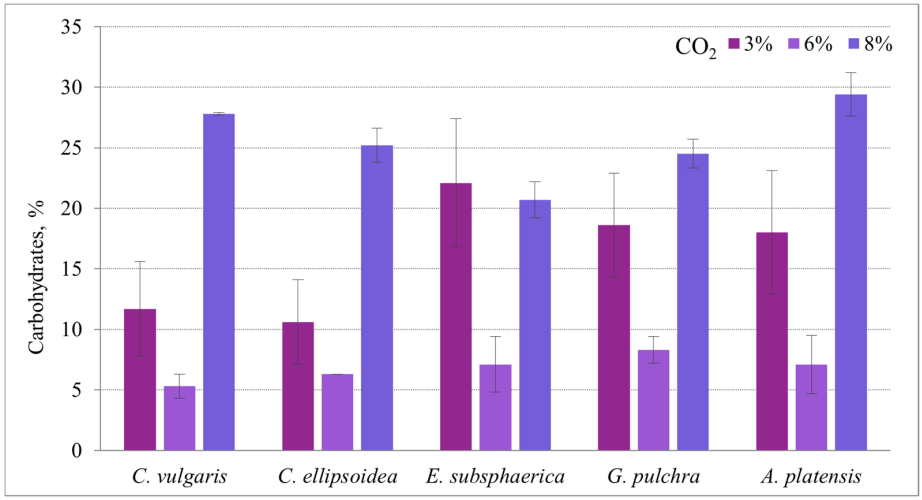
Figure 4. Change in pH during the experiments with flue gas at CO_2 concentrations: (**a**) 3%, (**b**) 6%, and (**c**) 8%. The error bars represent standard deviation.



(**a**)



(**b**)



(**c**)

Figure 5. The results of biochemical analysis in the experiments with flue gas at CO_2 content (3, 6, and 8%): (**a**) proteins, (**b**) lipids, and (**c**) carbohydrates. The error bars represent the error of the method.

With an increase in the concentration of flue gas (from 3 to 6% CO_2), the amount of protein within the margin of error remained unchanged, although the average values show a tendency to increase their content; the lipid content increased significantly for all strains except *A. platensis*, for which this content remained constant (within the margin of error); carbohydrates fell significantly (especially in the *E. subsphaerica*). With an increase in the concentration of flue gas (from 6 to 8% CO_2), there is a reduction in the amount of protein in all strains, especially in *Chlorella* and *A. platensis* strains; the lipid content remained unchanged in the strains of *C. vulgaris*, *G. pulchra*, and *A. platensis*, and decreased slightly in the strains *C. ellipsoidea* and *E. subsphaerica* (within the margin of error); carbohydrates increased significantly, and for all strains except *E. subsphaerica*, their amount has become more than at 3% CO_2. Since flue gases are stressors for microalgae, it is generally seen that at the stage of increasing the concentration of CO_2 from 3 to 6%, the strains are resistant to exposure: there is no dramatic change in the most significant biochemical components—lipids and proteins. With an increase in CO_2 concentration from 6 to 8%, the protein content in *Chlorella* and *A. platensis* strains decreased.

Let us also compare the results obtained by growing microalgae strains on mixtures of air and CO_2 with the results of the biochemical analysis of this study (Table 4). We will compare the results (taking into account the error limits) for equal/similar CO_2 concentrations.

Table 4. Biochemical analysis of microalgae cultivation on mixtures of air and CO_2 [30], and flue gas.

CO_2	Microalgae Strains	Proteins, %		Lipids, %		Carbohydrates, %	
		CO_2 and Air	Flue Gases	CO_2 and Air	Flue Gases	CO_2 and Air	Flue Gases
3%	*C. vulgaris*	44.4 ± 11.1	49.8 ± 6.7	22.8 ± 2.1	22.2 ± 1.1	25.4 ± 7.3	11.7 ± 3.9
	C. ellipsoidea	48.1 ± 8.3	56.2 ± 5.1	21.8 ± 1.5	20.6 ± 1.2	25.4 ± 7.3	10.6 ± 3.5
	E. subsphaerica	35.8 ± 7.3	36.1 ± 5.9	22.0 ± 1.6	18.9 ± 1.1	33.2 ± 7.9	22.1 ± 5.3
	G. pulchra	41.4 ± 6.8	36.9 ± 5.0	21.8 ± 1.9	19.6 ± 1.2	33.3 ± 5.7	18.6 ± 4.3
	A. platensis	22.8 ± 7.2	57.2 ± 6.5	16.3 ± 1.6	22.3 ± 1.5	23.0 ± 7.1	18.0 ± 5.1
6%	*C. vulgaris*	45.5 ± 8.3	58.9 ± 9.7	20.8 ± 1.8	28.1 ± 1.8	41.4 ± 9.8	5.3 ± 1.0
	C. ellipsoidea	34.6 ± 10.5	64.1 ± 12.0	20.3 ± 2.2	29.8 ± 0.4	42.8 ± 9.6	6.3 ± 1.0
	E. subsphaerica	46.2 ± 11.2	42.6 ± 3.5	22.9 ± 2.0	27.7 ± 1.8	27.4 ± 7.4	7.1 ± 1.3
	G. pulchra	43.8 ± 12.1	45.8 ± 1.8	20.8 ± 2.6	27.9 ± 6.2	34.5 ± 7.8	8.3 ± 1.1
	A. platensis	19.7 ± 10.4	59.2 ± 1.5	13.2 ± 1.6	21.3 ± 1.7	21.0 ± 5.8	7.1 ± 2.4
8–9%	*C. vulgaris*	39.6 ± 12.3	24.8 ± 1.1	20.3 ± 2.7	28.9 ± 1.2	32.8 ± 8.2	27.8 ± 0.1
	C. ellipsoidea	49.8 ± 12.1	30.9 ± 2.3	23.4 ± 3.1	26.1 ± 2.1	34.0 ± 7.2	25.2 ± 1.4
	E. subsphaerica	42.2 ± 10.9	35.1 ± 1.0	22.2 ± 3.0	20.3 ± 4.0	34.9 ± 7.5	20.7 ± 1.5
	G. pulchra	41.8 ± 9.4	38.9 ± 1.2	21.2 ± 2.4	22.8 ± 3.7	30.3 ± 5.4	24.5 ± 1.2
	A. platensis	16.3 ± 8.1	28.1 ± 3.4	13.1 ± 1.7	19.5 ± 5.3	26.5 ± 6.9	29.4 ± 1.8

At CO_2 = 3%, the carbohydrate content for *Chlorella*, *E. subsphaerica*, and *G. pulchra* strains is noticeably reduced in the case of flue gas experiments, while the protein and lipid contents are the same as in experiments on mixtures of air and CO_2. For *A. platensis*, the situation is reversed, the number of proteins and lipids is higher in the case of flue gas experiments, and the amount of carbohydrates is the same in both studies. At CO_2 = 6%, in flue gas experiments, the lipid content is higher for all strains, and the carbohydrate content is lower, while the protein content is higher only for *Chlorella* and *A. platensis*, for the other strains, the amount of protein is the same in both studies. At CO_2 = 8–9%, the comparison provides the same results as at CO_2 = 3%, only in this case, in experiments with flue gas for *Chlorella* and *E. subsphaerica*, the protein content is lower, as well as for the *C. vulgaris*, the number of lipids is higher than in experiments on mixtures of air and CO_2.

The state of microalgae cells, which was monitored by microscopy, is important for detecting the reaction to flue gas. Figures 6 and 7 show the results of microscopy in experiments with flue gas at CO_2 contents of 3, 6, and 8%. These photos were taken in conditions close to production, directly at the experimental site, where an atmospheric gas chamber with photobioreactors was installed (by using a light microscope).

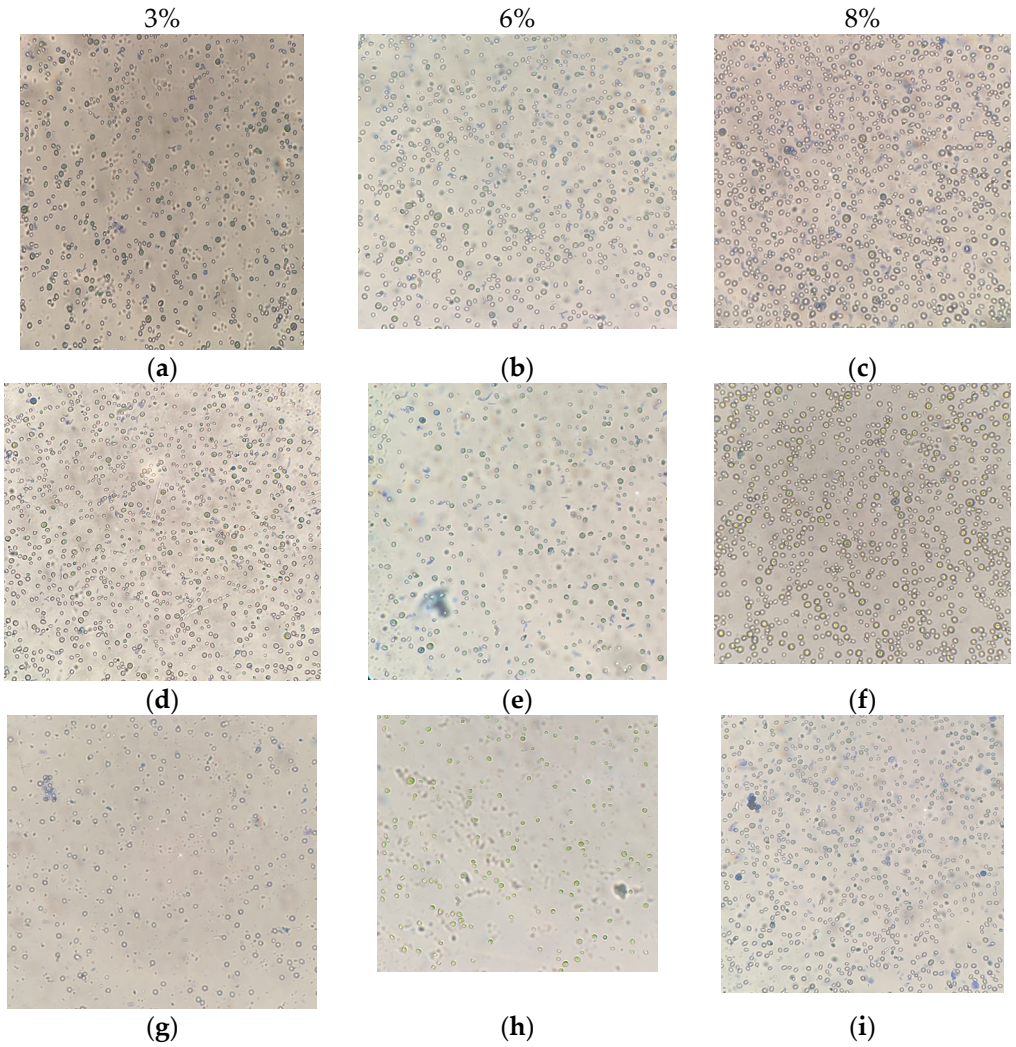


Figure 6. Flue gas. (**a**–**c**) *C. vulgaris*, (**d**–**f**) *C. ellipsoidea*, and (**g**–**i**) *E. subsphaerica*. CO_2 concentrations for each strain are 3, 6, and 8%. Samples with staining, magnification ×400.

The photo analysis taken of the strains at CO_2 = 3% shows a lack or a minimal number of dead (stained) cells. In the photo of *G. pulchra* (Figure 7a), the cells of the *E. subsphaerica* microalgae are visible. As a result of contamination, they were present in a small amount already on the 6th day of the experiment. The living filaments of *G. pulchra* are long, and unstained, which indicates favorable conditions for culture growth. Figure 7d shows *Arthrospira platensis* trichomes—live (only the cell mucosal is stained) and long. For experiments with flue gas at CO_2 = 6%, microscopy provides similar results.

For comparison, the following are the microscopy results of microalgae strains in experiments with a gas–air mixture at CO_2 = 6% and an illumination of 200 µmol quanta·m^{-2}·s^{-1} (Figure 8). Note that small clusters of methylene blue-stained culture cells can be seen in photographs of *Chlorella* strains, and several short trichomes of *A. platensis* strain are also visible in Figure 8e, which may indirectly indicate a weakening of the process of cell growth and division. In the case of microscopy of strains grown in a flue gas atmosphere at

CO_2 = 6%, such features were not observed, which may be due to the acquired resistance of cultures to CO_2.

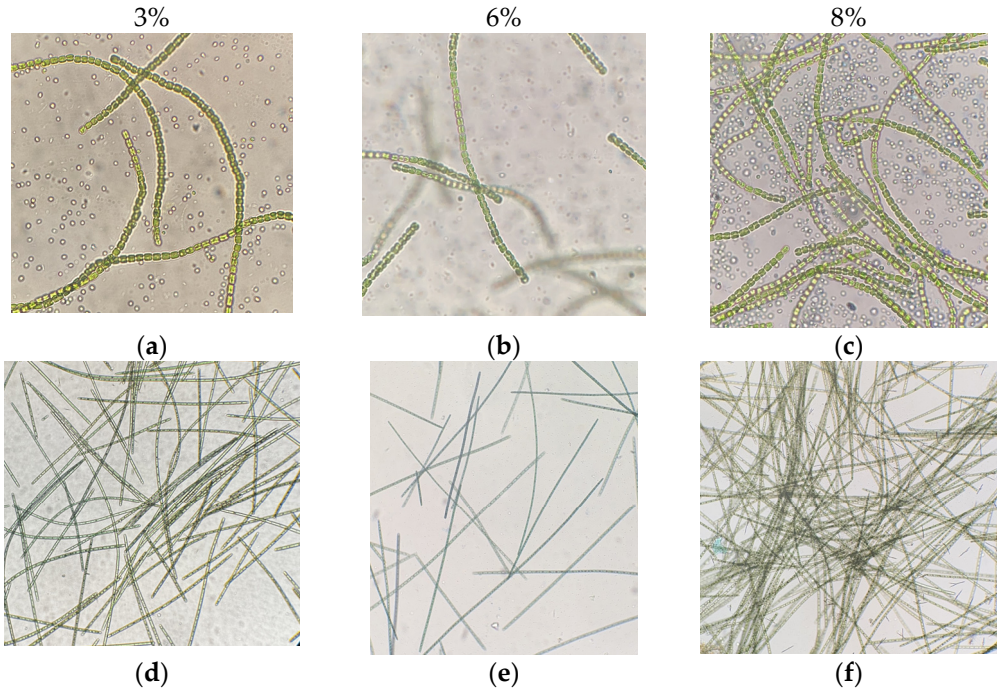


Figure 7. Flue gas. (**a**–**c**) *G. pulchra* (magnification ×400) and (**d**–**f**) *A. platensis* (magnification ×100). CO_2 concentrations for each strain are 3, 6, and 8%. Samples with staining.

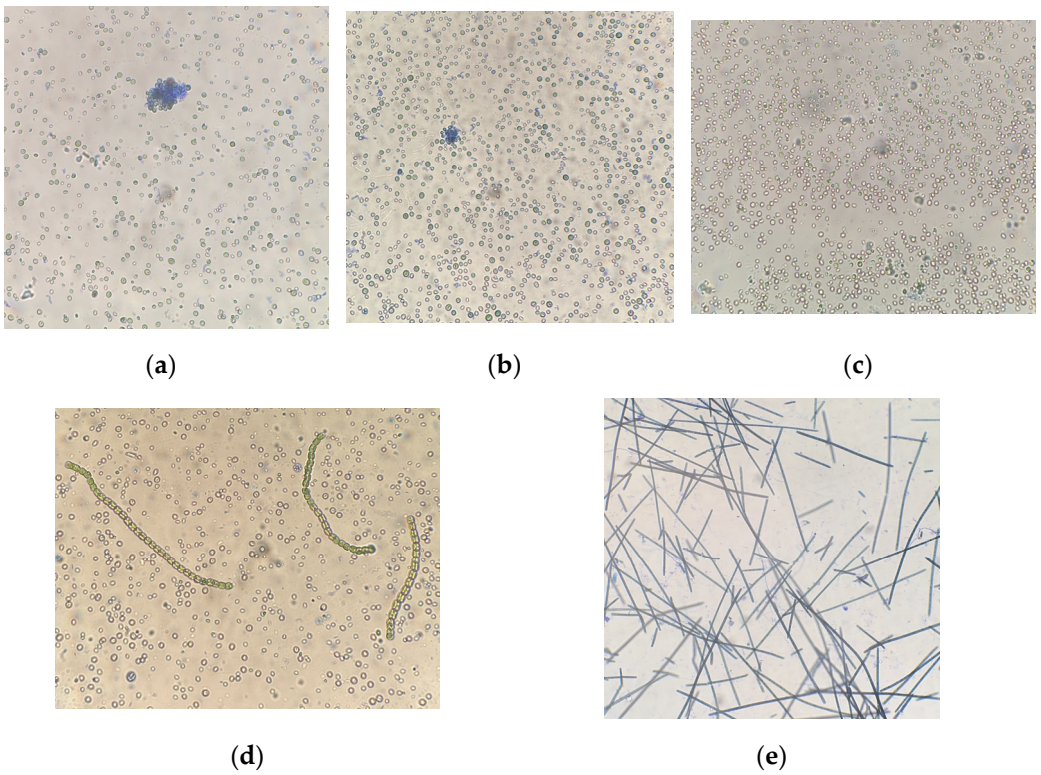


Figure 8. (**a**) *C. vulgaris*, (**b**) *C. ellipsoidea*, (**c**) *E. subsphaerica*, (**d**) *G. pulchra* (magnification ×400), (**e**) *A. platensis* (×100). Samples with staining. Gas–air mixture of air and CO_2 (CO_2 = 6%, 200 µmol quanta·m^{-2}·s^{-1}, [36]).

Thus, the results of microscopy and in vivo staining of microalgae strains showed a lack or minimum number of dead strain cells under all experimental conditions in a flue gas atmosphere. Almost the whole microalgae mass of strains grown at high concentrations of flue gases (CO_2 = 3, 6, and 8%) during experiments lasting 12 days remains alive, which indicates the viability and preservation of the cultures. At the same time, in the experiment with flue gas and CO_2 = 8%, an increased proportion of dead cells is detected, which indicates the possible launch of the cellular mechanism of apoptosis in these conditions.

4. Conclusions

Experimental studies were carried out to assess the condition of microalgae grown in the atmosphere of flue gases with CO_2 contents of 3, 6, and 8%. Microalgae strains (*A. platensis*, *C. ellipsoidea*, *C. vulgaris*, *E. subsphaerica*, and *G. pulchra*) previously adapted to high CO_2 concentrations were selected for experiments to increase the resistance of microalgae strains and the efficiency of CO_2 utilization. Cultivation was conducted for 12 days under controlled laboratory conditions with ten photobioreactors (working volume of 4 L) placed in the atmospheric gas chamber, and flue gases were supplied using a specially designed flue gas generation plant. The maximum growth rate (and, consequently, the rate of removal of CO_2 from the gas mixture) was achieved at CO_2 concentrations of 6 and 8% for *Chlorella vulgaris* rsemsu Chv-20/11-Ps and at CO_2 = 6% for *Chlorella ellipsoidea* rsemsu Chl-el when grown on Tamiya medium.

According to the results of experiments, the obtained growth rate of microalgae biomass density is at the level of the average values presented in scientific publications: the maximum for *Chlorella vulgaris* and *Chlorella ellipsoidea* strains (up to 0.8 $g \cdot L^{-1} \cdot d^{-1}$), the minimum for *Arthrospira platensis* (up to 0.27 $g \cdot L^{-1} \cdot d^{-1}$). The remaining strains had a growth rate of no more than 0.3 $g \cdot L^{-1} \cdot d^{-1}$. At the same time, none of our experiments achieved a zero-growth rate in 12 days (a constant value of biomass density). Significant pH changes were observed in the first 6 days of each experiment, and for *Chlorella* strains—an increase (by 2.5 pH), for other strains—a slight decrease (by 0.5 pH). In the future, the pH of the culture liquid will remain almost constant. At the stage of increasing the CO_2 concentration from 3 to 6%, there is no dramatic change in the most significant biochemical components—lipids and proteins. With an increase in CO_2 concentration from 6 to 8%, the protein content in *Chlorella* and *A. platensis* strains decreased, the lipid content decreased slightly in the strains *C. ellipsoidea* and *E. subsphaerica*, and carbohydrates increased significantly, which may be the result of exposure to stressful conditions when growing them in a flue gas atmosphere with a sufficiently high CO_2 content. Microscopy of microalgae strains showed the absence or minimum number of dead cells under all experimental conditions in a flue gas atmosphere.

Thus, microalgae strains have demonstrated resistance to flue gas, while obtaining higher results than when growing them in a mixture of air and CO_2 at the same CO_2 concentrations. We can conclude that preliminary adaptation to various stressors increases the resistance of cultures to the effects of flue gas components and contributes to an improvement in the biomass growth rate. The obtained results can be used for further development of CO_2 capture and storage technologies, as well as for the creation of industrial flue gas utilization plants to reduce carbon dioxide emissions from the atmosphere.

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