Capture CO_2 from flue gases during the cultivation of cyanobacteria/microalgae *Arthrospira platensis*

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Abstract. Experimental studies and assessment of the state of cyanobacteria/microalgae *Arthrospira platensis* in a sustainable consortium with heterotrophic bacteria were carried out grown by bubbling culture medium with microalgae by a mixture of flue gases and air. The investigations were carried out in an experimental sample of a gas chamber with photobioreactors, which was supplemented by a designed flue gas generation system. The CO_2 content in the gas mixture was 3, 6, and 8%. The resulting growth rate of microalgae biomass density was 0.21 g/l per day at CO_2 concentrations of 3 and 6%, 0.27 g/l per day at 8%. The present growth rate was 1.75 – 2.25 times higher than the growth rate in experiments conducted when growing this microalga in mixtures of air and CO_2. An increase in the amount of carbohydrates in biomass to 29.4% and the lipid content at the level of 21 – 23% was recorded, which makes biomass attractive for biofuel production.

1 Introduction

Over the past decade, the topic of decarbonization has come to the fore not only in the energy sector as a whole, but also in the algal energy sector. In the context of climate change and increasing energy demand, carbon sequestration methods and the production of carbon-neutral fuels are needed. Algal technologies are considered among the existing strategies for CO_2 capture and storage (CCS - Carbon capture and storage), based on the experimentally proven efficiency of carbon dioxide consumption by microalgae (MA): 1.83 – 1.88 kg CO_2/1 kg biomass [1, 2]. However, the CCS strategy refers to a linear economy, while the transition to a circular economy or a closed–loop economy is relevant, which involves not only CO_2 capture, but also the production of demanded products and materials from it (CCU - Carbon capture and utilization). Thus, CCU, unlike CCS, eliminates expensive long-term storage of carbon dioxide and provides, along with the utilization of CO_2 by microalgae, wastewater treatment and the production of biofuels from grown biomass.

In the production of biofuels from algae by thermochemical methods, a significant amount of inert biochar is formed, which can be considered not only as a source of raw materials (for example, in chemical industries and electrical engineering), but also as a way

of long-term deposition of CO_2 in the natural environment (for example, in soil). The use of cultivated biomass for the production of food, feed, and biomaterials can also be considered as a method of implementing low-carbon circular bioeconomics [3].

Currently, MA are grown on an industrial scale mainly to obtain high-value market products and partially for wastewater treatment [4, 5]. The capture and industrial utilization of flue gases by growing microalgae has already been used in pilot projects in breweries, cement plants and gas power plants, since these enterprises produce gases with relatively low concentrations of SO_x and NO_x [6]. Flue gases from the combustion of hydrocarbon fuels (coal, fuel oil, to a lesser extent gas), in addition to CO_2, contain toxic compounds (SO_x, NO_x, etc.), high concentrations of which can negatively affect the growth of MA [7]. The flue gas obtained from coal combustion was used to cultivate MA, but with its dilution with air or minor additives in photobioreactors (PBRs), where MA cultivation was carried out [8]. The direct supply of flue gases to the PBR can lead to a change in the pH of nutrient media and remove the growth conditions of microalgae beyond the optimal limits [9]. Therefore, for the effective utilization of CO_2 from flue gases, experimental studies are needed to identify strains of microalgae resistant to such stressful conditions and their consortia with bacteria.

2 Materials and Methods

The experiments were carried out in a sealed gas-air chamber, in which an atmosphere was created with a given concentration of flue gases, temperature and lighting conditions. Microalgae were cultivated in glass cylindrical PBRs equipped with a continuous bubbling system of culture medium by a gas-air mixture. Previously, experiments were conducted in this facility to assess the growth rate and viability of microalgae cells during bubbling with mixtures of air and pure CO_2 in concentrations 0.04, 3, 6, and 9% [10]. The experimental installation was supplemented with a designed and manufactured flue gas generation system. The concentration of flue gases in the gas-air mixture was indicated by the concentration of CO_2, which in experiments was 3, 6, and 8%, which generally corresponds to the concentration of CO_2 in the flue emissions of power plants (in particular, from the Caterpillar G3520C generator). Cyanobacteria/microalgae *Arthrospira platensis rsemsu P Bios* in a sustainable consortium with heterotrophic bacteria of the genera *Pseudomonas*, *Bacterium*, *Bacillus* was used as an object of research from the collection of the Renewable Source Energy Laboratory at Lomonosov Moscow State University (RSE LMSU), which earlier underwent long-term cultivation with bubbling by gas-air mixtures at high CO_2 concentrations (up to 9%). At the same time, the strain showed a quite high growth rate, stable viability and weak inhibition even at the highest CO_2 concentrations (9%). The preliminary stage of experiments is a gradual laboratory adaptation of microalgae to stressful conditions and is very important for growing microalgae in an atmosphere with an increased concentration of flue gases. The illumination level of PBRs was increased to 202.7 μmol/m^2/s compared to 74.3 μmol/m^2/s in previous experiments, the temperature was maintained at 27 C^o [10].

The algorithm of the experiments was as follows: cultivation of microalgae under the conditions, which described above, for 12 days and sampling (on the 0, 3, 6, 9, and 12 day of the experiment) for analysis of growth rate, viability of microalgae cells and pH dynamics of culture medium; analysis of culture medium by basic nutrients (bicarbonates, carbonates, phosphates, nitrates, potassium, magnesium) to determine the intensity of their absorption, analysis of the biochemical composition of the grown biomass of microalgae (on the 0 and 12 days). To assess the viability of microalgae cells, a complex indicator was used that takes into account both the rate of biomass growth in each experiment and the results of cell staining with methylene blue followed by microscopy to determine the

specific number of living cells in the microscope's field of view (quantitative characteristics), the morphological state of cells (expert qualitative assessment). After the end of each experiment with an initial concentration of CO_2 in flue gases, part of the biomass was used for seeding in the next experiment with higher CO_2 concentrations. A detailed description of this step-by-step adaptation method of cultivation microalgae at elevated CO_2 concentrations is given in [10, 11].

3 Results and Discussion

In all experiments, a steady increase in the biomass of *A. platensis* microalgae was recorded, which confirms the viability of microalgae when growing them in a flue gases atmosphere. The growth rate of microalgae biomass density obtained in experiments corresponds to the average values presented in scientific publications: 0.21 g/l per day at CO_2 concentrations of 3 and 6% in flue gases, 0.27 g/l per day at CO_2 = 8%. It should be noted that the given growth rate is 1.75 – 2.25 times higher than the growth rate in experiments conducted during the cultivation of MA in mixtures of air and CO_2 (Table 1) [10]. Apparently, the reason for such a high and stable growth rate is the longer laboratory adaptation of microalgae to a gradual increase in CO_2 concentrations as a stressor, as well as more optimal conditions for experiments in this series in terms of illumination (202.7 μmol/m^2/s compared with 74.3 μmol/m^2/s in [10]).

Changes in the pH of the culture medium, which is an important characteristic of the growth conditions of microalgae during bubbling by flue gases in experiments at CO_2 = 3, 6, and 8%, were insignificant in magnitude: from 8.5 to 9.0. This is due to the buffering of the Zarrouk nutrient medium [12], on which microalgae were cultivated. It was found that even bubbling by a gas-air mixture at CO_2 concentration of 8% in flue gases during the 12 days of the experiment did not shift the pH to the acidic side, although, as it is known, weak carbonic acid (H_2CO_3) is formed when CO_2 is dissolved.

The results of the analysis of the chemical composition of culture media at the beginning (day 0) and at the end (day 12) of experiments with different concentrations of flue gases showed the following results: 1) an increase in the content of hydrocarbonates (HCO_3^-) due to bubbling of the culture medium with flue gases at high CO_2 concentrations, its subsequent dissolution and dissociation, which is not compensated by the absorption of CO_2 by the biomass of microalgae; 2) a decrease in the content of phosphates (from 0.24 g l to 0.06 g/l at CO_2 = 8%), sulphates and nitrates by 12 days of experiments due to their absorption during biomass growth. At the same time, the most significant reduction was in the nitrate content at all CO_2 concentrations – by almost 100% (from 1.6 g/l to 0.002 g/l). This should be taken into account in further experiments and practical implementation with using the method of CO_2 utilization from flue gases in order to avoid limitations of the microalgae growth in nitrogen; 3) reduction of magnesium content in the culture medium by 25 – 30% for all experiments with flue gases;

To control the state of *A. platensis* biomass at the end of the experiments (CO_2 concentrations 3, 6, and 8% in the composition of flue gases), a biochemical analysis of the obtained microalgae biomass was carried out, the main purpose of which was to identify the dynamics of the content of proteins, lipids and carbohydrates. The protein content in biomass cultivated during bubbling by flue gases at CO_2 = 3 and 6% was 57.2 and 59.2%, at CO_2 = 8% the amount of proteins decreased markedly to 28.1%. At the same time, the amount of carbohydrates did not show a stable dynamics: at CO_2 = 6% it was 2.6 times lower compared to the experiment with CO_2 = 3%, and at CO_2 = 8% it was 1.6 times higher than the initial level and amounted to 29.4%. Since the biomass of this strain *A. platensis*, grown without carbon dioxide bubbling, is characterized by a carbohydrate content of 5.3 – 7.1% [13], the results obtained in this experiment are attractive for the production of

bioethanol and biobutanol from biomass. The amount of lipids in biomass at all CO_2 concentrations in flue gases remained almost at the same level of 21 – 23%, despite the fact that the lipid content at the level of 12% or less is common for them [13]. These indicators show that biomass is enriched with lipids and might be interested for the production of biodiesel from it by lipid transesterification.

The viability assessment took into account the growth rate of biomass density, the proportion of living cells in samples using the lifetime staining method, as well as the morphological characteristics of *A. platensis* cells revealed during microscopy (Fig. 1).

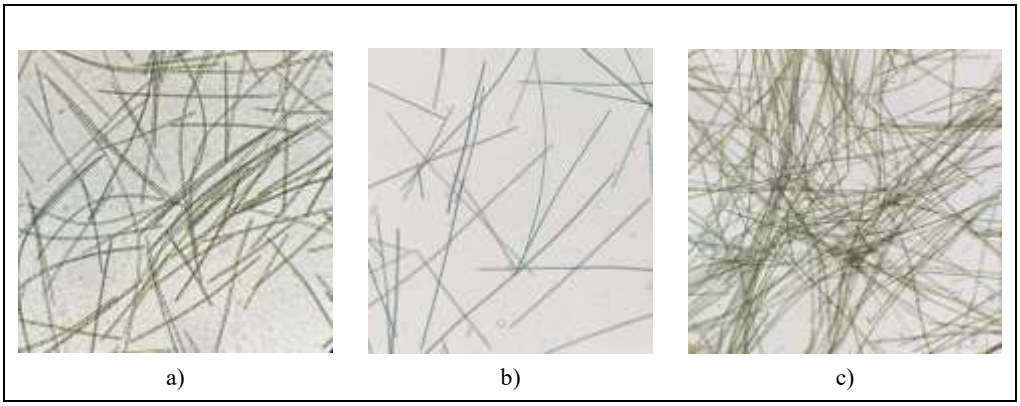

Fig. 1. Trichomes of *A. platensis*, stained samples, magnification x100. Flue gases with concentrations: a) – 3% CO_2, b) – 6% CO_2, c) – 8% CO_2.

Table 2 shows a comparative assessment of the viability indicators of *Arthrospira platensis* and *Chlorella vulgaris* grown in the atmosphere of flue gases, according to the results of intravital staining of cells and calculating the proportion of living cells in samples. Table 3 shows the viability indicators for all experimental conditions in comparison with the work [10] with the same strain (*A. platensis*), where cultivation was carried out by bubbling the culture medium with a mixture of air and CO_2. When calculating the viability indicator, the proportion of living cells was assigned a weight coefficient of 0.6, and the growth rate biomass density was assigned a weight coefficient of 0.4. When the weight coefficients vary, the absolute values change, but the relative position of the strains in terms of viability remains constant (Table 3).

Table 1. Comparative assessment of indicators of *A. platensis* biomass density growth cultivated in flue gases atmosphere and in a mixture of CO_2 and air. CO_2 concentrations 3, 6, and 8%. The duration of cultivation is 12 days.

	Flue gases		**Mixture of air and CO_2**	
CO_2 content in flue gases, %	Absolute increase in MA density, g/l	Growth rate of MA density, g/l per day	Absolute increase in MA density, g/l	Growth rate of MA density, g/l per day
3	2.47	0.21	1.45	0.12
6	2.56	0.21	1.54	0.13
8	3.28	0.27	1.44	0.12

Table 2. Comparative assessment of the viability of *Arthrospira platensis* and *Chlorella vulgaris* strains based on the results of intravital cell staining and calculation of the proportion of living cells in the samples.

CO_2 content in flue gases, %	***C. vulgaris***	***A. platensis***
3	0.95	0.92
6	0.82	0.90
8	0.89	0.82

Table 3. Comparative assessment of viability indicators of *Arthrospira platensis* and *Chlorella vulgaris* strains (average for flue gases experiments).

Microalgae strains	**Generalized viability indicators**	
	Weight coefficients 0.4/0.6	Weight coefficients 0.5/0.5
Chlorella vulgaris	91.9	91.9
Arthrospira platensis	63.3	57.2

4 Conclusions

Experiments on the cultivation of cyanobacteria/microalgae *Arthrospira platensis rsemsu P Bios* in a sustainable consortium with heterotrophic bacteria of the genera *Pseudomonas*, *Bacterium*, *Bacillus* in a flue gases atmosphere with CO_2 concentrations 3, 6, and 8% were carried out and the characteristics of growth and viability of culture cells were determined. According to the results of the experiments, the growth rate of microalgae biomass density was obtained, which is at the level of the average values presented in scientific publications on this topic: 0.21 g/l per day at CO_2 concentrations 3 and 6%, 0.27 g/l per day at CO_2 = 8%. The indicated growth rate was 1.75 – 2.25 times higher than the growth rate in experiments conducted during the cultivation of these microalgae in mixtures of air and CO_2. At the same time, none of the experiments achieved a zero growth rate in 12 days (a constant value of biomass density). Apparently, the reason for such a high and stable growth rate is the longer laboratory adaptation of microalgae to a gradual increase in CO_2 concentrations as a stressor, as well as more optimal conditions for experiments in this series in terms of illumination (202.7 μmol/m^2/s compared with 74.3 μmol/m^2/s).

When bubbling the culture medium with flue gases, a change in the biochemical composition was detected: when bubbling with CO_2 = 8%, the amount of carbohydrates in the grown biomass increased 1.6 times and amounted to 29.4%, which becomes promising for the production of bioethanol and biobutanol from biomass. The amount of lipids in biomass at all CO_2 concentrations in flue gases was almost at the same level of 21 – 23%, but these indicators show that biomass is enriched with lipids and might be interested for the production of biodiesel from it by lipid transesterification. With hydrothermal liquefaction of such biomass, the yield of bio-oil and bio-coal will increase.

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