Mitigation carbon emissions by microalgae: assessing the viability of culture *Arthrospira platensis* grown on high CO_2 concentrations

Nadezhda Chernova[1, *], *Sophia* Kiseleva[1], *Elizaveta* Chunzhuk[1], *Anatoliy* Grigorenko[2], *and Mikhail* Vlaskin[2]

[1]Lomonosov Moscow State University, Moscow, Russia
[2] Joint Institute for High Temperatures of Russian Academy of Science, Moscow, Russia

Abstract. In this work, experimentally evaluated the viability of a consortium microalgae Arthrospira platensis rsemsu P Bios with heterotrophic bacteria when cultivated in a gas-air mixture with high concentrations of CO2 (from 0.04 to 9%). A laboratory setup was created to test the viability of microalgae strains at high concentrations of CO2. The experiments were carried out using 12 photobioreactors with a culture medium volume of 4 L each, placed in a gas chamber, which makes it possible to create elevated CO2 concentrations in the gas-air medium. The maximum growth rate of biomass of microalgae A. platensis is 170 mg/(l per day), the maximum absolute increase in biomass for 12 days is 1540 mg/l. The relatively low growth rate and absolute increase in the biomass of A. platensis at all concentrations of CO2 in the gas-air mixture may indicate that this culture requires a longer laboratory adaptation to high concentrations of CO2. The high cell viability found in all experiments by cytochemical staining of cells with methylene blue indicates the acquired tolerance of the culture to elevated CO2 concentrations (3–9%). However, after 12 days of the experiment with 9% CO2, morphometric signs of cell suppression are detected, which is expressed in deviations of the cell shape from normal, elongation (lack of division) and an increase in the number of dead cells. Quantitative characteristics of the microalgae consortium viability have been obtained.

1 Introduction

Microalgae (MA) are regarded as one of the most attractive biomass species for mitigation carbon emissions: they have one of the highest photosynthesis efficiencies with CO_2 fixation, are able to grow in a wide range of conditions often unsuitable for terrestrial vegetation, and with the help of thermochemical carbonization reactions/ pyrolysis / gasification / liquefaction, - may be used to provide liquid, gaseous and solid types of biofuels These technologies have many advantages, including the reduction of industrial greenhouse gases, the generation biofuels energy and food from the produced

* Corresponding author: chernova_nadegda@mail.ru

photosynthetic biomass and high value-added by-products. In algae, the light utilization efficiency is 10–50 times higher than in terrestrial plants, they have high growth rates and, consequently, high rates of CO_2 fixation [1, 2]. It has been revealed that one kg of MA biomass can accumulate up to 1.83–1.88 kg of CO_2 [3]. Scientific and patent sources of information often contain conflicting the results about the influence of increased CO_2 concentrations on the microalgae growth and development. For example, it was shown that bubbling 100% by unfiltered coal-fired flue gas with a high concentration of CO_2 increased the microalgae growth rate [4]. González López et al. [5] used a blue-green algae to sequester carbon dioxide. It has been shown that if the experiment was conducted outdoors, carbon dioxide fixation reached 3.0 g per 1 liter of cultural medium per day. However, other authors found inhibition of microalgae growth when exposed to high concentrations of CO_2 [6, 7]. MA are very labile and able to adapt to growth conditions, including stressful conditions, such as high CO_2 concentrations and flue gases [8]. That's why, MA reaction to the effects of increased CO_2 concentrations is specific not only at the level of the species, but also of the strains.

The purpose of this work is cultivation of a consortium microalgae *Arthrospira platensis* with heterotrophic bacteria at different CO_2 concentrations (0.04, 3, 6, and 9%) and laboratory adaptation of microalgae to high CO_2 content and also assessment of the microalgae viability based on cytochemical staining of living and dead cells with methylene blue, followed by cell control by microscopy.

2 Materials and Methods

This work is based on experiments on the cultivation of MA in a gas-air medium with high CO_2 concentrations and the determination of the characteristics of their viability. For these purposes, a laboratory setup was created to test the viability of MA strains at high CO_2 concentrations. Using a gas analyzer MAG-6 T-8-16A (AO Exis, Russia), the composition of the atmosphere inside the chamber was recorded, namely, the concentration of the following gases was determined: carbon dioxide, oxygen, carbon monoxide, ammonia, methane, sulfur dioxide, and nitrogen dioxide. The laboratory setup includes photobioreactors (PBR) with a volume of 6 L each, located inside the atmospheric gas chamber [9] with the ability to maintain and control a given CO_2 concentration inside the chamber. Each PBR includes a reactor designed for a nutrient medium with microalgae inoculum, an LED system, power supply and gas medium supply.

Gas-air mixtures with the following CO_2 content were successively supplied to the chamber: 0.04% (air); 3%, 6%, 9%, with which the culture liquid was bubbled into the PBR using aquarium sprayers, and the MA grown at the initial CO_2 concentration were used for inoculation of the experiment with the subsequent CO_2 concentration. The cultivation of MA was carried out under illumination of 74 μmol/m^2/s for 12 days with microscopic analysis of MA the state and determination of the biomass optical density (photometer Expert-003, Russia), pH of the cultural medium (pH-meter Expert-pH, Russia). Illumination was determined using a Digital Luxmeter MS6610 MASTECH.

As methods for determining the viability of MA strains, a method was tested and substantiated that combines the control of cell viability by the state of the cell permeability barrier based on cytochemical staining of cells with vital dyes followed by microscopy and the microcultural method according to Imshenetsky [10]. In addition to vital staining, MA biomass density analysis was performed to determine the viability of the strains.

The experiments were carried out with the strain of blue-green MA *Arthrospira platensis rsemsu P Bios* (or *Spirulina platensis* according to the old classification) with straight trichomes in a consortium with heterotrophic bacteria of the genera Pseudomonas and Bacterium from the working collection the Scientific Research Laboratory of

Renewable Energy Sources Lomonosov Moscow State University, which is a model object for these tasks [11]. The composition of Zarrouk's medium for cultivating the microalgae *A. platensis*: NaHCO3 - 16.8 g/L; KNO3 - 3.0 g/L; K2HPO4·3H2O - 0.66 g/L; K2SO4 – 1.0 g/l, MgSO4·7H2O – 0.2 g/l, NaCl – 1.0 g/l, CaCl2 – 0.04 g/l, FeSO4·7H2O – 0.018 g/l, EDTA – 0.08 g/l, microelement solution for Zarrouk's medium - 1 ml/l. In experiments distilled water was used.

3 Results and Discussion

The viability of microorganisms is an integral characteristic of all living systems. The development of modern biotechnologies based on the activities of living organisms forces us to look for reliable methods analyzing their viability. As a quantitative characteristic of viability, the viability index (*VI*) is used, which shows the percentage ratio of the number of living cells to the total number of cells of the object under study:

$$VI = Q * 100/A, \quad (1)$$

where Q is the number of living cells, A is the total number of cells. The determining criterion of the living state of microorganisms is primarily their ability to grow and reproduce. There is currently no alternative replacement for the reproductive criterion.

Thus, an important and widely used in research characteristic of viability is the productivity of MA. All currently existing methods for determining viability are divided into direct and indirect. The first group includes methods based on direct accounting of the reproductive capacity of living cells. This method includes, among other things, the determination of the biomass growth rate. The second group includes methods of indirect manifestations of cell vital activity: respiratory and enzymatic activity, mobility, optical and electrical properties, cell wall permeability and the ability of the cell to stain with various dyes, including intravital (vital), etc.

In aqueous solutions, the dyes are dissociated and are thus electrolytes. According to their chemical properties, vital dyes are divided into basic and acidic. The main ones are also called cathodic dyes, in which the chromophore group is associated with a cation. Examples of vital basic dyes are gentian violet, crystal violet, neutral red and methylene blue. In acid dyes such as phenol red and cyanol the chromophore group is bonded to an anion. Predominantly for staining microorganisms, basic dyes are used, which are more intensively bound by the acidic components of the cell.

The essence of the vital staining method is that some dyes are able to penetrate into the cell only through the damaged membrane of dead cells and stain their cytoplasm. Living cells with undisturbed selective permeability of the cell wall or membrane do not pass the dye and remain colourless.

Specific ready-made recommendations and even examples of the use of methods for determining viability on microalgae and cyanobacteria cells have not been found.

It has been established that intravital staining of cells with methylene blue makes it possible to evaluate cell metabolism and identify its early changes and, as a result, characterize the state of cells in culture. Vital dyes were tested, among which methylene blue dye was chosen as the optimal one.

A solution of methylene blue was prepared on a water basis at a concentration of 1:10,000 until the paint was completely dissolved. 1-2 drops of culture liquid with microalgae are placed on a sterile slide; a drop of dye is added on top of it, after which the resulting sample is covered with a coverslip [12]. After 3-5 minutes of incubation at room temperature, microscopic control is carried out using a digital microscope Leica DM 2500 (Leica Microsystems, Germany) and a light microscope - MICMED-5 (LOMO, Russia), as

well as a botanical description of the state of the cells and photography. At least 10 fields of view are viewed, and at the maximum magnification of the microscope, the number of blue-stained (dead) cells or their clusters is counted (Fig.1).

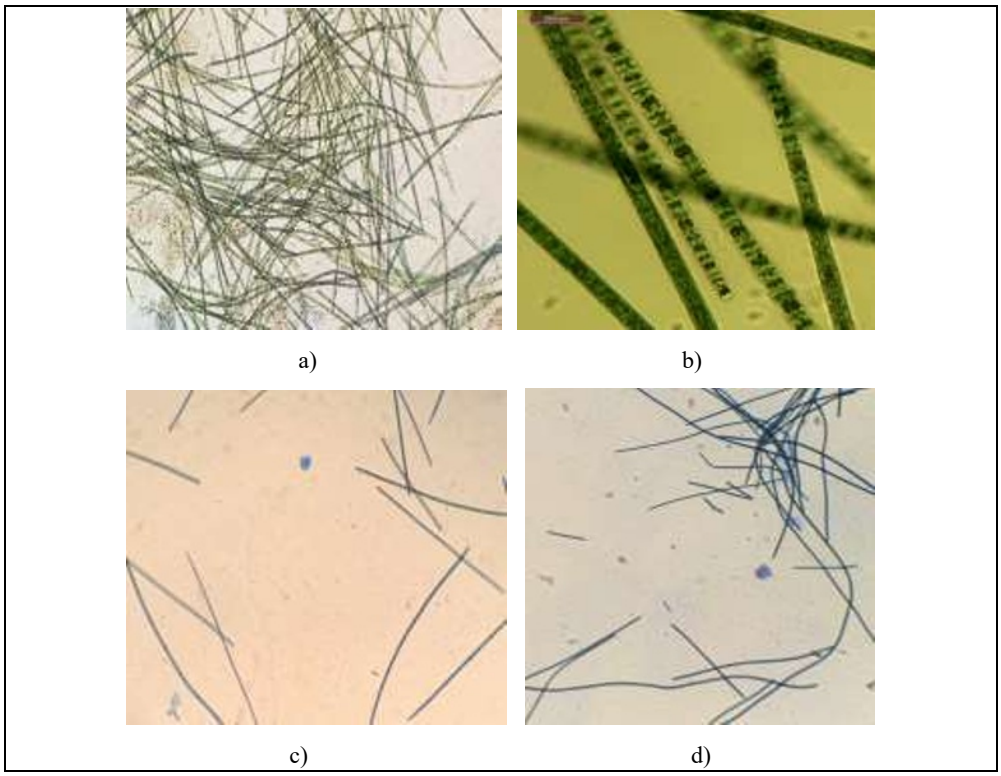

Fig. 1. Trichomes of *A. platensis,* unstained sample at the top: a) – magnification x100; b) - in the photo there is a magnification ruler; c), d) – stained sample, magnification x100; CO2 concentration = 9%, 12 days of experiment.

Figure 1 shows unstained and stained *A. platensis* trichomes from a culture medium sample after 12 days of cultivation with 9% CO2 bubbling. Figures c) and d) show the filaments of *A. platensis*, mostly unstained (only the mucous sheath of trichomes is stained), while very long filaments with a weakly spiral that is uncharacteristic for this strain, and few short hormogonia are noted. These signs indicate a weakening of the process of cell growth and division.

Assessment of viability was based on two characteristics: growth speeds (mg/l by dry matter) and a state of culture, which was established by the microscopy method of the amount living cells in the field of view, which were not stained with methylene blue (Table 1). Based on microscopy, the proportion of living cells in 10 fields of vision was calculated. The combination of these characteristics gave the final indicator of cell viability (modified VI, %). Thus, the modified viability index includes a normalized biomass growth rate factor. We have not discovered similar works in which this method of determining the viability of microalgae was applied.

The table 1 shows that even with high concentrations of CO_2, the proportion of living cells remains quite high. This happened thanks to is to the experimental procedure, which involves gradual laboratory adaptation of strains to CO_2 increase: at each stage of the experiments for inoculation, a biomass was used, grown with lower CO_2 concentrations.

Table 1. Results of assessing the viability of A. platensis P Bios during cultivation with illumination of 74 µmol/m2/s depending on the concentration of CO2

Experience No.	Time, days	CO_2 content, %	Biomass growth rate, mg/l (dry matter)	Absolute increase in biomass, mg/l (dry matter) after 12 days of cultivation	Modified VI, %
1	6	0,04	170	990	97.50
2	12	3	120	1450	82.79
3	12	6	130	1540	85.24
4	12	9	120	1440	81.29

In culture medium samples, was applied pH was measured as one of the most significant characteristics of microalgae cultivation conditions. In all experiments, acidification of culture media due to bubbling with a gas-air mixture with a high content of CO_2 was not detected. Namely, this process, as the most detrimental to microalgae growth,is discussed in many scientific publications on this topic. The constancy and even a slight decrease in pH in experiments with *A. platensis* at 3% CO_2 leads to an increase in the availability of bicarbonates (HCO3-) for MA cells and more efficient use of the nutrient medium.

4 Conclusions

Thus, a laboratory setup was created for microalgae cultivation and testing their viability high concentrations of CO_2, which includes 12 PBR with a culture liquid volume of 4 l:
- experiments were carried out on the cultivation of the *A. platensis P Bios* consortium with heterotrophic bacteria at elevated CO_2 concentrations and its viability was determined. The results of the experiments confirmed cell viability in all experiments (CO_2 concentration from 0.04 to 9% in the gas-air mixture for 12 days), which indicates the acquired cell tolerance to high CO_2 concentrations;
- the obtained results suggest that the detected tolerance of the studied resistant consortium *Arthrospira platensis P Bios* with heterotrophic bacteria to high concentrations of CO_2 (3, 6 and 9%) is due to the experimental procedure, which involves gradual laboratory adaptation of strains to CO_2 increase: at each stage of the experiments, biomass of the consortium based on A. platensis grown at lower concentrations of CO2, was used as an inoculum. Namely, biomass adapted to 3% CO2 was used for PBR inoculation and experiments with CO_2 = 6%. The same algorithm was applied in experiments with CO_2 = 9%;
- a relatively low growth rate and an absolute increase in the biomass of *A. platensis* at all concentrations of CO_2 in the gas-air mixture may indicate that this culture requires a longer laboratory adaptation to high concentrations of CO_2.

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