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Proceeding Paper

A flow photometric cell for in-line analysis of the biomass content of the microalgae Nannochloropsis sp. †

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Abstract: A photometric flow cell for in-line determination of the biomass content in a microalgae *Nannochloropsis sp.* cultivation has been constructed, integrated into the photobioreactor and tested. This process is used for the development of a third-generation biofuel technology. The results obtained have shown a linear dependence of the detected optical density on the biomass content. Therefore, the concept of in-line determination of the biomass content in similar cultivation processes using a photometric sensor based on a single laser light source has been proved.

Keywords: Microalgae biomass determination; online monitoring; photometric sensor; flow cell; cultivation of Nannochloropsis sp., AVR controller

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1. Introduction

In-line process monitoring is increasingly used in the biotechnological production. Modern biotechnology is used in different areas, including the production of biofuels [1]. Microalgae are currently the most promising renewable raw materials for the biofuel. The technology of biofuel production significantly differs from the production technology of traditional fossil fuels. Chemical composition of the biomass is affected by the conditions of its cultivation process. For example, nitrogen deprivation of *Nannochloropsis sp.* culture combined with increasing the brightness of illumination leads to a 51% increase in the concentration of lipids in the biomass composition [2]. The process of cultivation of microorganisms consists in accumulation of their biomass and metabolic products under the conditions of nutrient medium [3, 4, 5]. For successful cultivation of photoautotrophic microalgae cells, the necessary amount of light energy and carbon dioxide, the most favorable temperature and acidic conditions are important. Optimization of the cultivation processes requires an in-line analytical control of its main parameters [6].

One of the critical indicators of biotechnological processes is the biomass content in the reactor. In photosynthesis processes the biomass is represented by microalgae or bacteria. The stages of photosynthesis take from 10^{-5} to 10^{3} seconds; and cell division stages and biomass accumulation is counted in minutes [7]. Therefore, traditional biomass determination methods, such as cell counting chambers (e.g. Goryaev chamber, Fuchs-Rosenthal chamber), are too time-consuming and labor-intensive, and are hardly suitable for in-line control. Existing in-line methods for the determination of biomass in solution use turbidity sensors [8], which can be inaccurate because of the presence of suspended particles, gas bubbles and turbulence in the medium affecting the observed signal [9]. Turbidity sensors are typically equipped with fiber probes, which should be immersed into a reactor and require regular maintenance [10].

The present proof-of-concept study is aimed at testing the possibility of in-line determination of the biomass in the microalgae *Nannochloropsis sp*. cultivation using a photometric flow cell based on a semiconductor laser operating at a particularly chosen wavelength. Microalgae *Nannochloropsis sp*., unlike the majority of cultivated microalgae, contains only chlorophyll *a* in its photosystem, which allowed to minimize the influence of other chlorophylls and carotenes on the measurement results [11].

2. Materials and methods

The photobioreactor was constructed and the biomass of the microalgae *Nannochloropsis sp.* was cultivated in the culture medium Guillard f/2. Tracking of culture growth stages was carried out under visual control on a MIKMED-6 microscope using a Levenhuk C1400NG camera. Cell concentration in the culture medium solution was counted in a Goryaev cell counting chamber model 851.

Taking into account the above described problems of optical determination of biomass concentration, an external photometric flow cell has been constructed (Fig. 1). The flow cell was implemented in a transparent glass tube with two intersecting lateral bends. One side of the tube contained a source of polarized coherent light based on a semiconductor laser. The laser wavelength was chosen at 650 nm, close to the maximum of the absorption peak of chlorophyll *a*, situated at 665 nm. A photodiode for measuring the intensity of light passed through the solution layer was placed on the opposite side of the cell. The use of a monochromatic light source reduces the influence of absorbing components and impurities contained in the solution. The electrical signal from the photodiode was transformed and processed with the analog-to-digital (AD) converter and recorded with a microcontroller Arduino Uno to memory disk [12, 13]. The detected light intensity was measured in millivolts (mV).

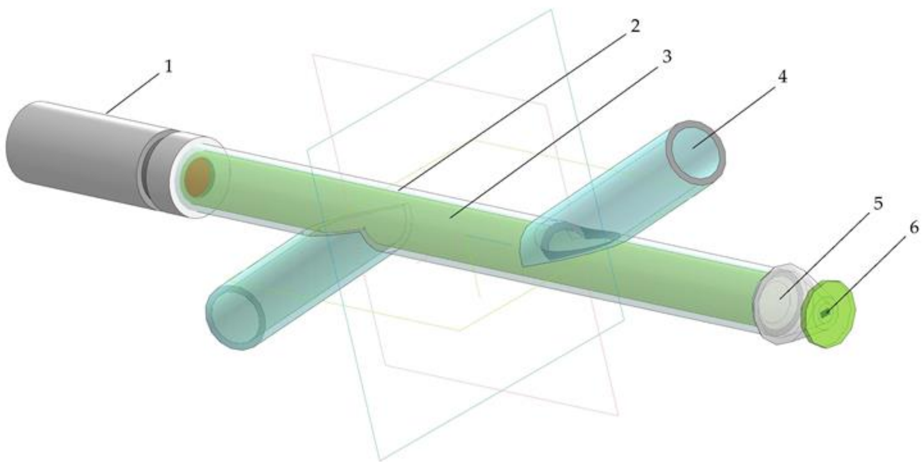

Figure 1. The photometric flow cell: 1 – laser diode module, 2 – flow cell, 3 – sample solution, 4 – inlet/outlet tube, 5 – focal lens, 6 – photosensitive element.

3. Results and discussion

A series of diluted biomass solutions was prepared for the experiment. The culture medium was used as a reference sample for calibration of the reference signal to AD converter. The solutions were measured in the flow cell, and light transmittance and absorption at 650 nm has been calculated [14]. The same series of solutions was examined for biomass cell content using a Goryaev cell counting chamber. The results of the measurements are presented in Table 1.

Table 1. Results of measurements for culture medium and diluted solutions.

Dilution	Cell concentration, units×ml-1	T, transmittance	A, absorbance	Signal from the photo-cell, mV
Culture medium	0	0	0	70
1:4	5,0	10,15	0,0389	64
1:2	8,0	15,94	0,0892	57
1:1	12,4	24,64	0,1208	53
1:0,5	16,1	30,43	0,1730	47
1:0	24,5	42,03	0,2116	43

The observed signal in absorption units was directly proportional to the biomass content. Basing on the obtained data, graphs of transmittance and absorption dependences on the concentration of cells in the studied solutions were plotted, and mathematical dependencies were established according to the obtained graphs.

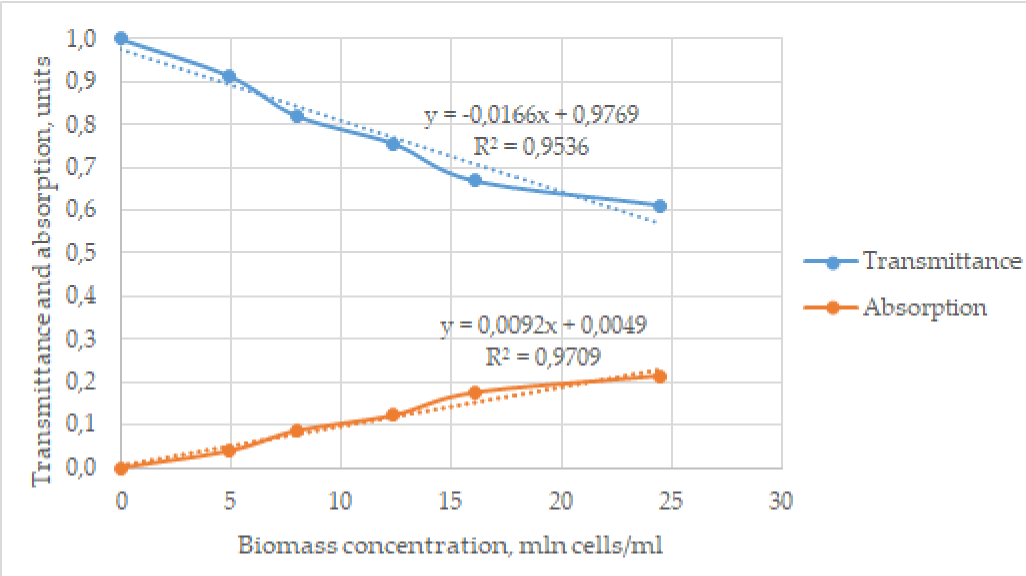

Figure 2. The graphical dependences between transmittance, absorption at biomass concentration.

The biomass solution does not generally obey the Beer's law, and biomass cells are able not only to absorb but also to scatter the light radiation. Nevertheless, the observed coefficient of determination (R^2=0.97) shows a linear dependence of absorption on the biomass content. This result can be explained by an assumption that light scattering effects observed by the flow cell also have a linear character in the considered concentration range.

4. Conclusion

The possibility of in-line quantification of the microalgae *Nannochloropsis sp*. biomass content using a photometric flow cell was proved. A simple calibration model has been obtained, which allow determining the concentration of microalgae cells in the medium based on the detected light intensity in absorption units. The obtained data allow us to conclude that the application of optical sensors for control and optimization of biotechnological processes of microalgae cultivation is promising. To improve the accuracy of measurements, it is necessary to evaluate the contribution of light scattering to the measurement results and to improve the photometric flow cell construction accordingly [15].

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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