

Conference Paper

Nephelometric Method for Determination of Growth Parameters of Chlorella Culture

A. Mitishev¹, E. Semenova¹, V. Presnyakova¹, E. Presnyakova¹, I. Moiseeva¹, S. Kolesnikova², M. Goncharov¹, Y. Moiseev¹, and S. Presnyakov¹

¹Penza State University, Ministry of Education and Science of the Russian Federation, 40 Krasnaya street, Penza, Russia

²National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Kashirskoe shosse 31, Moscow, 115409, Russia

Abstract

Nephelometric method for the determination of growth parameters of chlorella culture using a photoelectric colorimeter was described. Use of photoelectric colorimeter for cell counting in suspension requires periodic calibration of meter readings using chlorella standard culture (with a certain cell concentration). *Chlorella vulgaris* IPPAS C-66, IPPAS C-111 and IPPAS C-2019 strains served as object of research. Density of initial in vitro suspensions (after inoculation) was 0.9 mln cells/ml. Cultivation was carried out during 12 days on a Hoagland medium with a pH of 7 (temperature of 35°C, illumination of 10 klx). Sample selection for analysis and measurement was carried out daily, three times per day. Based on the obtained data, readings of photoelectric colorimeter KFK-3.01 were calibrated via direct count of chlorella cells quantity in Goryaev's chamber. Use of calibration curve made it possible to reduce significantly time and error in determination of cell number in suspension cultures. The proposed technique allows counting chlorella cells in the growth and development dynamics with sufficient accuracy, high sensitivity, reproducibility and speed. It can be used for comparative determination of the growth parameters of strains in vitro, standardization of suspension cultures, semi-quantitative determination of chlorella biomass in order to predict the yield of desired product.

Corresponding Author:

A. Mitishev

span2361@rambler.ru

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1. Introduction

At the present time, cultivation of microalgae is of great interest all over the world [1–3]. Unicellular algae (green – *Chlorella*, *Scenedesmus*, *Dunaliella* and blue-green – *Spirulina*, *Anacystis*) are used as objects of mass cultivation [4]. Control of the growth and reproduction rates is necessary during cultivation [5]. Control can be exercised on the basis of data on their number and biomass changes. Counting chambers (Goryaev's, Franzev's, Bogorov's) and nephelometric method are used for the determination

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of growth parameters [6]. Nephelometric method of cell counting can be used for microalgae, which grows as homogeneous suspensions e.g., *Chlorella*, *Anacystis*).

Use of photoelectric colorimeter for cell counting in suspension requires periodic calibration of meter readings using chlorella standard culture (with a certain cell concentration). Data, obtained on photoelectric colorimeter, are expressed in relative units, which express total biomass of microalgae rather than cell number. However, error of such determination is significant. In most cases, it is more convenient to express the measured parameter not in the photoelectric colorimeter readings, but in the number of cells per 1 ml of the suspension. For this, the photoelectric colorimeter is calibrated and calibration curves are made for each culture.

Aim of research – to evaluate the possibility of using a nephelometric method for determining the growth parameters of chlorella strains in suspension culture.

2. Materials and Methods

Chlorella vulgaris IPPAS C-66, IPPAS C-111 and IPPAS C-2019 strains served as object of research. Density of initial in vitro suspensions (after inoculation) was 0.9 mln cells/ml. Cultivation was carried out during 12 days on a Hoagland medium with a pH of 7 (temperature of 35°C, illumination of 10 klx). Sample selection for analysis and measurement was carried out daily, three times per day.

Number of cells was determined in Goryaev's chamber (double-grid) under the microscope BIOMED-3 (magnification of 10x). Nephelometry was carried out using photoelectric colorimeter KFK-3.01 (Figure 1). Experiment was conducted using the 1-mm thick cuvettes. The chlorella suspension was shaken and poured into one of the cuvettes. Transparent liquid medium for chlorella cultivation served as a control. Turbidity of suspension with precisely known number of cells was measured at a wavelength of 560 nm (green optical filter).

Calibration curve for the photoelectric colorimeter was prepared in the following way.

1. *Chlorella vulgaris* IPPAS C-66, IPPAS C-111 and IPPAS C-2019 strains were cultivated in the aforementioned conditions.
2. Fourteen standard solutions with different, precisely known number of cells were prepared from the algae suspension.
3. Measurements of diluted suspensions were carried out using the photoelectric-colorimeter KFK-3.01 in 1-mm thick cuvettes. The cuvettes were absolutely clean

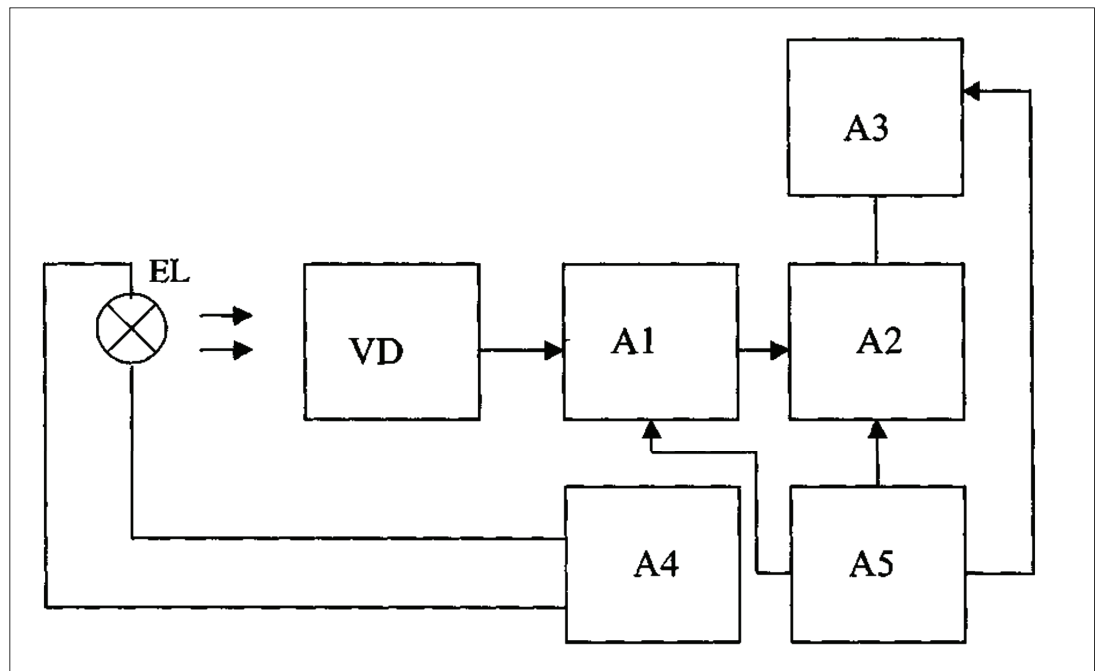


Figure 1: Circuit diagram of photoelectric colorimeter KFK-3.01: EL – lighter; VD – converter of optical radiation; A1 – DC amplifier; A2 – microcomputer; A3 – angle sensor of diffraction grating; A4 – stabilizer of lighter voltage; and A5 – power supply.

and were in the same position in cuvette holder during measurement. For more accurate results, formation of air bubbles on the walls of cuvette and in suspension was carefully avoided.

4. In parallel with measuring on photoelectric colorimeter, density was counted in Goryaev's chamber (double-grid) in three replicates.
5. Calibration curve was prepared on the basis of obtained data (Figure 2).

Statistical processing of the results was carried out using the program STATISTICA 10.0 Trial Version [7].

3. Results and Discussion

Number of cells on 1, 2, 3, 4, 6, 8, 10, 11, 12 days was determined according to data on optical density (Table 1) of chlorella suspension (*C. vulgaris* IPPAS C-66, IPPAS C-111 and IPPAS C-2019 strains) during cultivation (Table 2). The calibration curves (Figure 3) were used.

Research showed that life cycle of IPPAS № C-111 strain cells consists of the following phases. During the daytime, an active process of photosynthesis takes place. As a result, cells increase in size from 1.7 to 9.2 μm (from 6 μm to 9 μm). Biomass is also

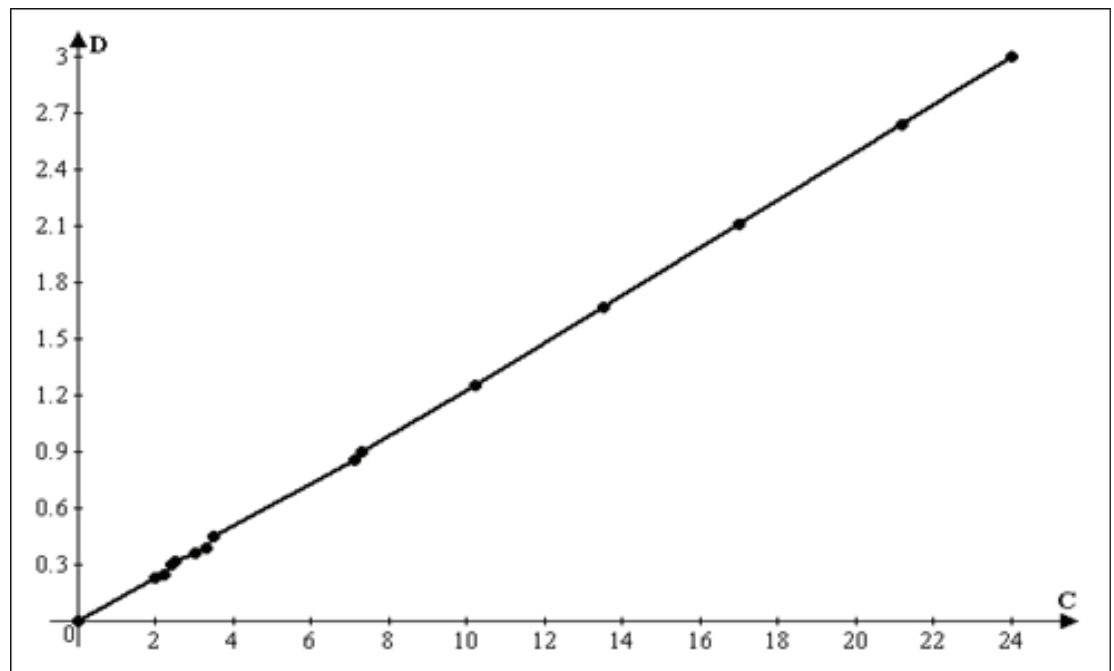


Figure 2: Calibration curve for determination of chlorella cells density in suspension culture (D – optical density; C – number of cells, 10^6 cells/ml).

TABLE 1: Optical density in dynamics of cultivation of IPPAS C-66, IPPASC-111 and IPPAS C-2019 strains.

Day St. ^a	Optical Density									
	0	1	2	3	4	6	8	10	11	12
IPPAS C-66	0.10 ±0.00	0.17 ±0.01	0.27 ±0.02	0.36 ±0.01	0.39 ±0.02	0.80 ±0.01	1.16 ±0.03	1.24 ±0.01	1.29 ±0.01	1.28 ±0.02
IPPAS C-111	0.10 ±0.00	0.19 ±0.01	0.36 ±0.00	0.51 ±0.04	0.70 ±0.04	1.48 ±0.02	2.24 ±0.01	2.49 ±0.06	2.62 ±0.01	2.50 ±0.00
IPPAS C-2019	0.10 ±0.00	0.23 ±0.01	0.49 ±0.06	0.73 ±0.04	0.90 ±0.00	1.74 ±0.02	2.58 ±0.01	2.75 ±0.04	2.88 ±0.04	2.85 ±0.01

^aStrain

TABLE 2: Density of cells of IPPAS C-66, IPPAS C-111 and IPPAS C-2019 strain sin growth and development dynamics.

Day St. ^a	Number of cells during cultivation, 10^6 cells/ml									
	0	1	2	3	4	6	8	10	11	12
IPPAS C-66	0.90 ±0.00	1.40 ±0.40	2.30 ±0.16	2.90 ±0.10	3.30 ±0.02	6.60 ±0.02	9.50 ±0.07	10.10 ±0.09	10.50 ±0.01	10.40 ±0.01
IPPAS C-111	0.90 ±0.00	1.60 ±0.20	3.00 ±0.18	4.00 ±0.16	6.00 ±0.11	12.00 ±0.12	18.00 ±0.08	20.00 ±0.10	21.00 ±0.01	20.80 ±0.30
IPPAS C-2019	0.90 ±0.00	1.90 ±0.01	3.79 ±0.10	5.90 ±0.01	7.30 ±0.10	14.00 ±0.12	20.67 ±0.22	22.00 ±0.00	23.00 ±0.41	22.80 ±0.20

^aStrain

intensively accumulated in the light phase. Adult spherical chlorella cells, which are 5-8

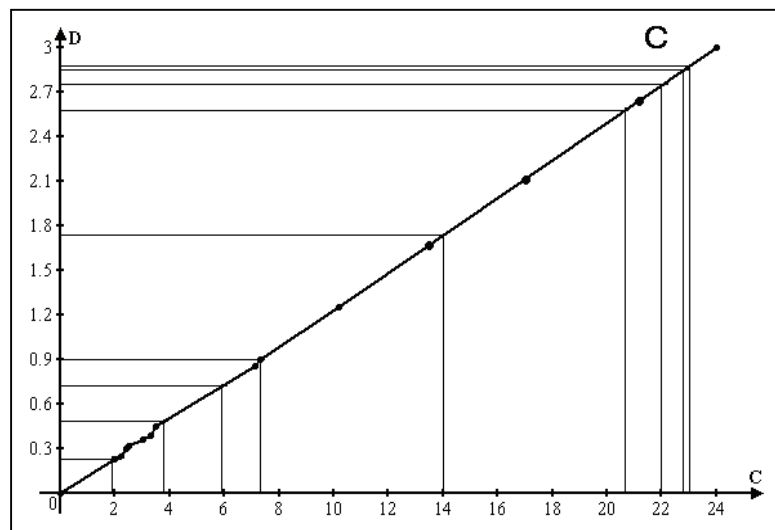
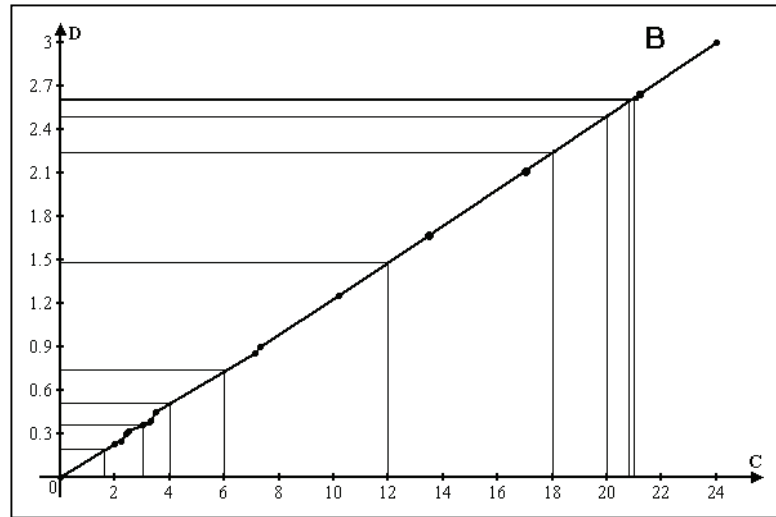
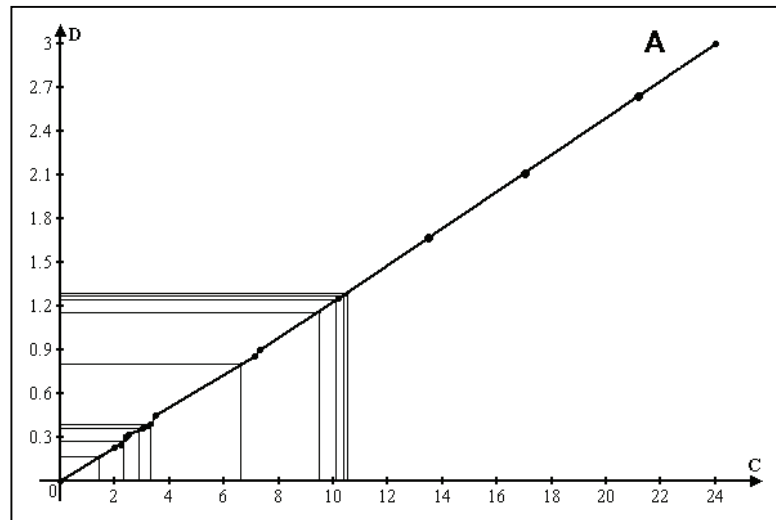


Figure 3: Density of cells of chlorella strains in cultivation dynamics: 1 – IPPAS C-66; 2 – IPPAS C-111; 3 – IPPASC-2019 (D – optical density; C – number of cells, 10^6 cells/ml).

μm in diameter, form the basis of biomass. Basically, cells divide into 2-8 autospores,

very seldom into 16. Spherical or slightly ellipsoidal in shape young cells, ranging in size from 2 to 4 μm , are ready for photosynthesis. Development does not depend on season of the year or light source. Stable cell growth is observed on the third day of cultivation (Figure 4). On the 10th day, culture density was 20 mln cells/ml.

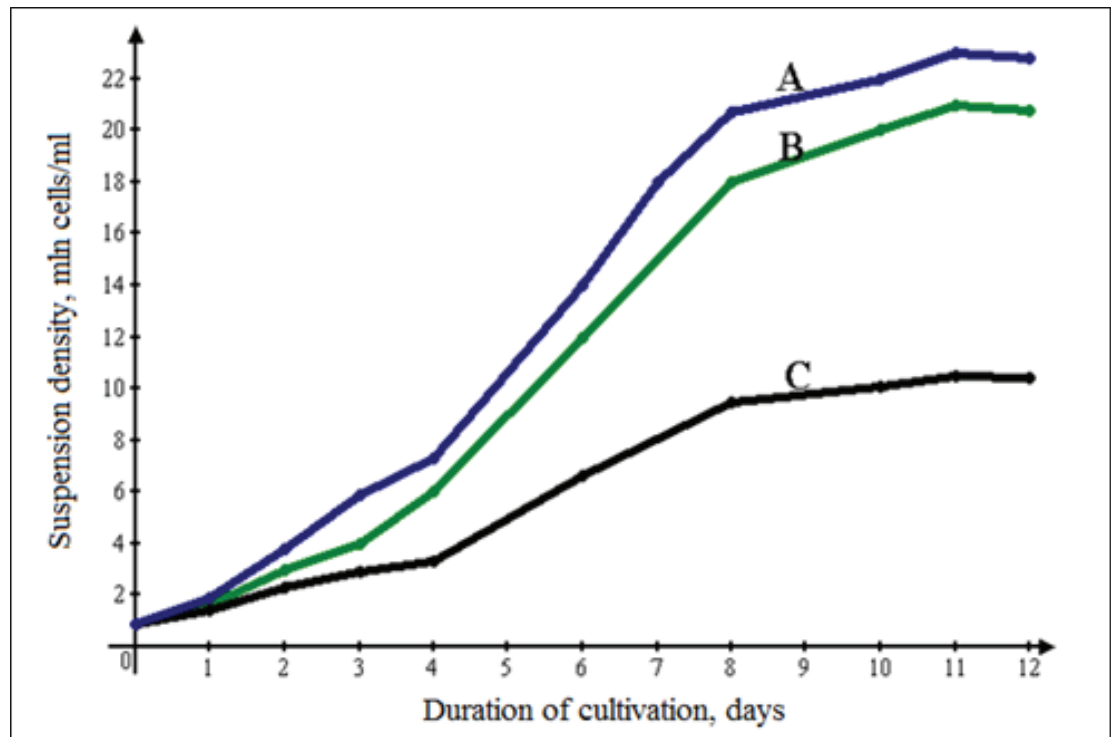


Figure 4: Growth curves of suspension cultures (A – C-2019; B – C-66; C – C-111).

The IPPASN^o C-66 strain is autotrophic. Chlorella cells have different sizes during ontogenesis: at the beginning of the phase of accelerated growth they are small and dense, 1.5–2.0 μm , during the stationary phase they greatly increase in size and can reach 8 μm . They contain a wide, belt-like, non-closed chloroplast, which is a cup-shaped chromatophore. In vitro on the fourth day cell density reaches 3 mln/ml (Figure 4). On the ninth day, cell density reaches 10 mln/ml.

Increase in number of cells of IPPAS S-2019 strain is observed on the second day of cultivation. Young cells have slightly ellipsoidal shape, range in size from 1.5 to 2.0 μm . Adultspherical cells, 5–8 μm in size, have pyrenoid. Chromatophore is cup-shaped, wide, belt-like, non-closed, sometimes reticulated-perforated, green or dark green in color. Flagellum is absent. In glass vessels on the fourth day, cell density reaches 7 mln/ml. On the ninth day, cell density reaches 22 mln/ml.

4. Conclusion

Research showed that nephelometric method is fairly accurate, simple in performing measurements of optical density. Relatively simple equipment is used. Use of calibration curve made it possible to reduce significantly time and error in determination of cell number in suspension cultures. The proposed technique allows counting chlorella cells in the growth and development dynamics with sufficient accuracy, high sensitivity, reproducibility and speed. It can be used for comparative determination of the growth parameters of strains in vitro, standardization of suspension cultures, semi-quantitative determination of the chlorella biomass in order to predict the yield of desired product.

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