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# A simple method for the cultivation of algae Chlorella vulgaris Bejerinck

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**Abstract.** Chlorella vulgaris Beijerinck stimulates the growth of agricultural plants and suppress the development of pathogenic microorganisms. Original data about on development of culture medium and selection of optimal cultivation conditions for the alga Chlorella vulgaris are presented. The most favorable conditions for algae growth were daylight, temperature 25° C and rotation at the speed 100 rpm. The most effective culture medium was of the Bold basal medium with the addition of vitamins thiamine, cyanocobalamin and soil extract. This method may used for creation the biopesticide and growth stimulators on the basis Chlorella biomass.

## 1 Introduction

*Chlorella vulgaris* is one of the most commercially used species of algae [1-6]. This alga has a stimulating effect on the growth of agricultural plants [7, 8]. Besides, *Chlorella* is also able to suppress the development of pathogenic microorganisms [9]. To obtain chlorella biomass in the shortest time it is necessary to develop optimal methods for its cultivation. Several methods of cultivation *Chlorella vulgaris* in heterothrophic and mixotrophic conditions were described: on industrial dairy waste [10], industrial co-products [11], food waste [12]. For biodisel production *Chlorella vulgaris* was grown in organic fertilizer [13], on wastewater with the high levels of ammonia [14]. This alga was cultivated on recycled aqueous phase nutrients from process of hydrothermal carbonization [15]. In is necessary to note, that described above methods fit for industrial cultivation, but they are complicated for the small scale cultivation.

The aim of the study was to assess the effect of various cultivation conditions on the growth of algae and to develop a simple cost-effective method of its production.

## 2 Method

In study algological and biotechnological methods were used. In experiment authentic strain of *Chlorella vulgaris* BCAC 76 was tested (Fig. 1).

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For developing a modified recipe of culture medium Bold's liquid medium [16] with the addition of soil extract and / or vitamins ($B_1$ - thiamine and $B_{12}$ - cyanocobalamin) were tested. In experiments for identification of optimal cultivation conditions Bold's medium was placed in the required conditions: in refrigerator at +4 ° C Birusa 460 H-1, heating to 25°C without stirring, a mixed device at 100 rpm without heating, under a daylight, under a phytolamp and under a lighting equipment during the light and dark phases of 12:12 h. To assess the influence of conditions two parameters were used: the concentration of cells in 1 ml of suspension (using a Goryaev camera), and the optical density of the suspension (using a KFK-3-01 concentration photocolorimeter).

Observations of algae growth were conducted by a Zeiss Axio Imager A2 microscope with DIC optics and AxioVision 4.9 visualization system.

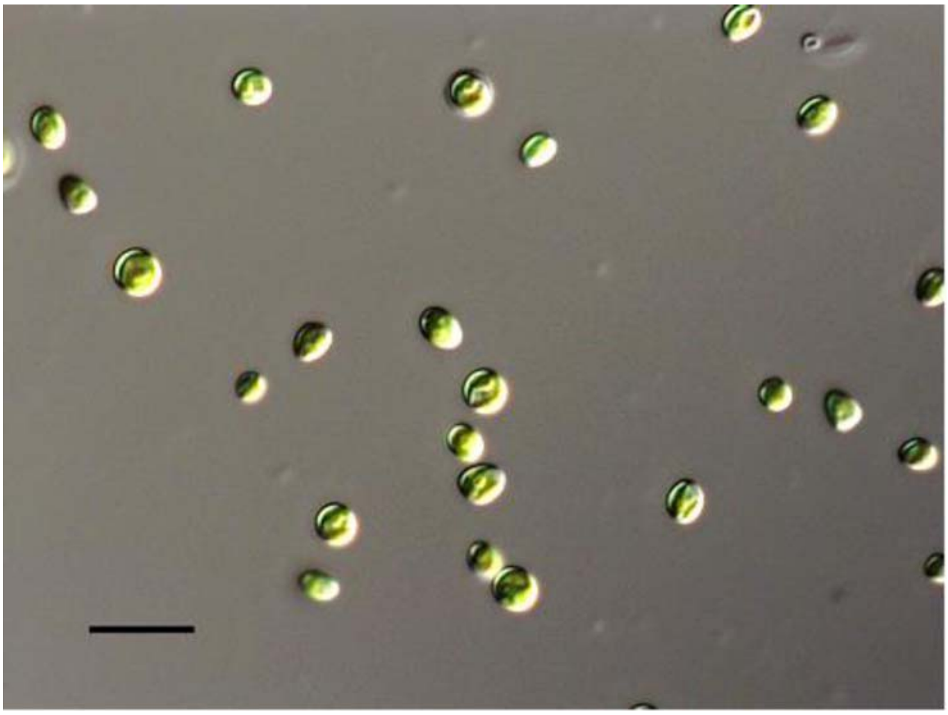

**Fig.1**. Cells of *Chlorella vulgaris* Bejerinck BCAC 76. Scale bar – 10 µm.

Growth rate of algae was calculated according the equation:

$$m = \frac{\ln(N_2/N_1)}{t_2 - t_1} \tag{1}$$

where $N_2$ и $N_1$ – the number of the cell at times $t_1$ и $t_2$ [17].

## 3 Results and discussion

It was found that the combination of Bold's medium with vitamins and soil extract leads to a significant increase in the optical density of *Chlorella vulgaris* (Fig.2). Therefore, this variant of the culture medium is most effective in obtaining a large volumes of a suspension of algae. The microalgae productivity was also determined based on the optical density of the suspension at a wavelength of 670 nm. The highest productivity of *Chlorella* on the 12th day of cultivation with a combination of Bold's medium with vitamins and soil extract was achieved.

In the experiment on estimation the optimal temperature for the cultivation of algae, the maximum increase in biomass was observed when the suspension was heated to 25° C. The previous study of high temperature on *Chlorella vulgaris* reveal, that temperatures from 20 °C to 28 °C caused the increase the growth and mortality of algae cells [18].

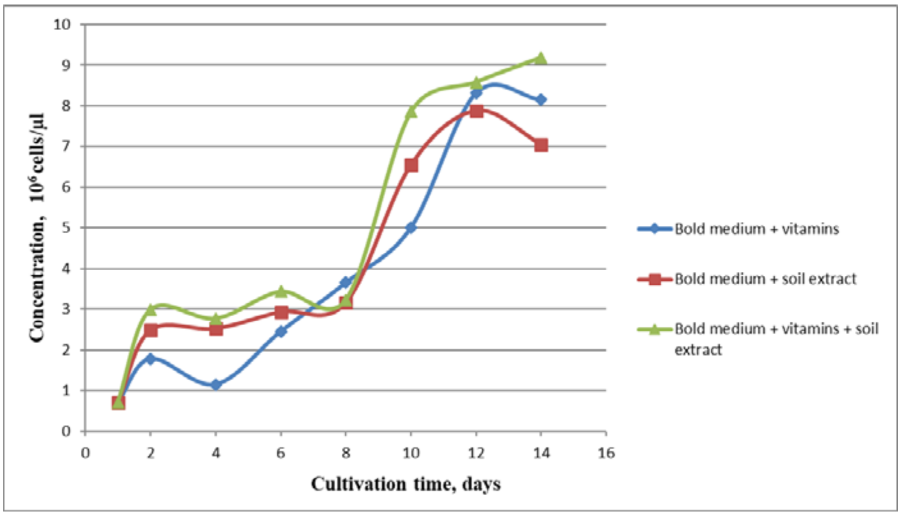


**Fig.2** The optical density of Chlorella vulgaris suspension in different medium

Based on the literature data [19, 20], as well as the results of our experiment, we can conclude that low temperatures negatively affect the growth and development of *Chlorella*. Cultivation at room temperature showed low rates of increase in algae biomass. Thus, to increase the productivity of a suspension of algae, it is necessary to create favorable temperature conditions to 25° C.

In the experiments of influence of the different illumination on *Chlorella* cultivation, it was found that maintaining the natural illumination (daylight) is optimal, which allows the microalgae to achieve maximum growth on the 12th day of cultivation. A relatively small increase in biomass was also shown by cultivation under lighting with a phytolamp.

Another indicator that affects the growth of algae is mixing, during which the movement of cell masses occurs in order to prevent their agglomeration. The results of the experiment showed that mixing at 100 rpm has a positive effect on the growth of algae.

Calculating the growth rate of the algae population under culture conditions revealed that the highest growth rate was observed under conditions of a combination of Bold medium with vitamins and soil extract, as well as algae that were cultivated with stirring and heating showed a good rate also. The lowest growth rate was recorded in algae, which were cultivated in the refrigerator (Table 1).

**Table 1** Chlorella vulgaris growth rate under various conditions

| Condi-tions | Bold medium + vitamins | Bold medium + soil extract | Bold medium + vitamins + soil extract | In refrigerato r at 4°C | Under the phytolamp | Under a lighting equipment | Heating to 25°C | Mixing at 100 rpm |
|---|---|---|---|---|---|---|---|---|
| Growth rate µ | 0,132 | 0,134 | 0,152 | 0,024 | 0,058 | 0,046 | 0,114 | 0,173 |

## 4 Conclusion

Thus, the proposed method of cultivating *Chlorella* vulgaris in conditions of daylight, temperature 25° C and rotation at the speed 100 rpm allows to increase the growth rate of algae without the use of expensive equipment. This method may used for creation the biopesticide and growth stimulators on the basis *Chlorella* biomass.

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## References

1. L. Gouveia, E. Gomes, J. Empis, Zeitschrift für Lebensmittel-Untersuchung und Forschung, **202 (1)** 75 (1996)
2. A. Borowitzka, Progress in Industrial Microbiology, **35** 313 (1999)
3. L. Shizhong, Z. Mingjun , M. Haihua , Y. Jianyun , C. Feng, Journal of South China University of Technology (Natural Science), **28 (12)** 66 (2000)
4. A. Widjaj, C.-C. Chien, Y.- H. Ju, Journal of the Taiwan Institute of Chemical Engineers, **40 (1)** 13 (2009)
5. I. Priyadarshani, B. Rath, Journal of Algal Biomass Utilization, **3 (4)** 89 (2012)
6. C. Safi, B. Zebib, O. Merah, P.-Y. Pontalier, C. Vaca-Garcia, Renewable and Sustainable Energy Reviews, **35** 265 (2014)
7. M.M. Shaaban, Pakistan Journal of Biological Sciences, **4** 628 (2001)
8. R. Dineshkumar, R. Kumaravel, J. Gopalsamy, M. N. A. Sikder, P. Sampathkumar, Microalgae as Bio-fertilizers for Rice Growth and Seed Yield Productivity, **9 (5)** 793 (2018)
9. V. Ördög, W. A. Stirk, R. Lenobel, M. Bancířová, M. Strnad, J. van Staden, J. Szigeti, L. Németh, Journal of Applied Phycology, **16 (4)** 309 (2004)
10. A. P. Abreu, B. Fernandes, A.A.Vicente, J. Teixeira, G. Dragone, Bioresource Technology, **118** 61 (2012)
11. D. Mitra, J.V. van Leeuwen J, B. Lamsal, Algal Research, **1 (1)** 40 (2012)
12. K.Y. Lau, D. Pleissner, C. S. K. Lin Algal Research, **170** 144 (2014)
13. M. K. Lam, K.T. Lee, Applied Energy, **94** 303 (2012)
14. P.J. He, B. Mao, C.M. Shen, L. M. Shao, D.J. Lee, D.S. Chang, Bioresource Technology, **129** 177 (2013)
15. Z. Du, B. Hu, A. Shi, Y. Cheng, P. Chen, Y. Liu, X. Lin, R. Ruan, Bioresource Technology, **126** 354 (2012)
16. H. W. Bischoff, H. C. Bold Phycological studies**, 4** 95 (1963)
17. L.Barsanti, P. Gualtieri, CRC Press, Taylor& Francis Group (2014)
18. R. Serra-Mai, O. Bernar, A. Gonçalves, S. Bensalem, F. Lopes, Algal Research, **18** 352 (2016)
19. D. P. Maxwell, S. Falk, C. G. Trick, NPA. Huner, Environmental and Stress Physiology, **105** 535 (1994)
20. N. P.A Huner, G. Öquist, F. Sarhan, Trends in Plant Science, **3 (6)** 224 (1998)
21. A.V. Moroz, V.V. Davydov, V.Yu. Rud, Yu.V. Rud, V.C. Shpunt, A.P. Glinushkin, Journal of Physics: Conference Series, **1135(1)** 012060 (2018)
22. V.B. Fadeenko, V V Davydov, V Yu Rud', A P Glinushkin, Yu V Rud', V Ch Shpunt, Journal of Physics: Conference Series, **917(9)** 092015 (2017)

23. I.A. Zharikov, R.V. Davydov, V.A. Lyapishev, V.Yu. Rud, Yu.V. Rud, A.P. Glinushkin, Journal of Physics: Conference Series, **917(5)** 052011 (2017)
24. I.S. Kudryashova, V.Yu. Rud, Yu.V. Rud, V.Ch. Shpunt, A.P. Glinushkin, N.N. Bykova, Journal of Physics: Conference Series, **929(1)** 012021 (2017)
25. N. Grebenikova, A. Korshunov, V. Rud, I. Savchenko, M. Marques, MATEC Web of Conference, **245** 11006 (2018)
26. R. Davydov, M. Sokolov, W. Hogland, A. Glimushkin, A. Markaryan, MATEC Web of Conference, **245** 11003 (2018)
27. J. Stenis, W. Hogland, M. Sokolov, V. Rud, R. Davydov, IOP Conference Series: Materials Science and Engineering, **497(1)** 012061 (2019)
28. I.S. Kudryashova, V.Yu. Rud, V.Ch. Shpunt, Yu.V. Rud, A.P. Glinushkin, Journal of Physics: Conference Series, **741(1)** 012106 (2016)
29. V.A. Lyapishev, V.Yu. Rud, M.S. Sokolov, A.V. Cheremisin, Proceedings of the 2018 IEEE International Conference on Electrical Engineering and Photonics, EExPolytech 2018, **8564387** 292-294 (2018)
30. N.M. Grebenikova, K.J. Smirnov, V.V. Davydov, V.Y. Rud, Journal of Physics: Conference Series, **1124(4)** 041011 (2018)