Aquabiotechnology of directed cultivation of microalgae Chlorella sorokiniana biomass

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Abstract. We carried out cultivation of Chlorella sorokiniana microalga using various irradiation sources: daylight lamp with spectral range 530-610 nm and filament lamp with spectral range 3.5-5.0 μm. Specific growth rates were evaluated for these conditions at exponential phase. From the obtained biomass we extracted lipids and determined its' fatty-acid content. Also we analyzed triacylglycerol (TAG) content and unsaturated fatty acids, as well as oleinic acid content. Biomass irradiation by infrared source causes enlarging of total lipid content in alga biomass, as well as saturated fatty acids and oleic acid portions. It is viable to use infrared irradiation mode for accumulation of non-polar lipids (TAG) at stationary growth phase, which might be used for biofuel obtaining after fractionating. Polar lipid fraction, extracted from biomass at linear growth stage, might be used for obtaining essential polyunsaturated fatty acids.

Introduction

Chlorella sorokiniana microalga is able to rapidly increase its biomass during cultivation and efficiently transform solar energy into chemical one. Biomass of C. sorokiniana might be considered as a renewable biofuel source of the third-generation (biodiesel, bioethanol, biohydrogen, biobutanol) [1-6]. Despite this, there is still no technology for biofuel obtaining from microalgae of adequate competitiveness price [7].

Biomass of this microalga type is also a perspective raw material for production of feed and high value-added products: essential lipids, pigments, carotenoids, proteins, carbohydrates [8].

Biomass of Chlorella family microalga is characterized by high specific growth rate, and for alga cultivation minimum requirements are needed for creation of optimum cultivation conditions [9]. For Chlorella cultivation wastewater may be used [10-12].

Development of aquabiotechnologies for directed cultivation and cascade processing of Chlorella microalgae biomass are of immediate interest. Impact of various factors allows one to perform directed synthesis of lipids and phytochemical substances of Chlorella microalgae [13]. It is known that stress factors connected with shortage of nutrient broth components (for example, nitrogen)[7,13], result in lipid accumulation in biomass. Also chemical stressors of lipid accumulation may be used [7,14]. However, directed usage of stress factors for lipid accumulation must not be accompanied with reduction of biomass output [15]. It is known, that lipid accumulation generally takes place in alga cover [16].

The aim of the present investigation is to study influence of light spectral characteristics during cultivation on lipid content in biomass of single-cell alga C. sorokiniana and its fatty-acid content.
Materials and methods

The objects under investigation were *Chlorella sorokiniana* preculture (strain 211-8k) from Culture Collection of Algae at Göttingen University, international acronym SAG.

Cultivation of microalgae *Chlorella sorokiniana* biomass was performed in the laboratory photobioreactor [17], its dimensions are the following: height 380 mm, diameter 50 mm, volume 500 ml.

For microalga illumination we used daylight fluorescent lamp (DFL) with intensity 2500±300 lx, T=400 K, spectral range 530-610 nm; and filament lamp of 14100 lx intensity, spectral range 3.5-5.0 μm (IR).

Cultivation temperature was (23±1) °C; mixture aeration intensity was 1.5 l/min; mixing mode was periodical (15 minutes once a day); mixing speed was 500 rpm.

For microalga cultivation we used cultural medium, which was well-balanced in macro- and microelements content [18]. Duration of cultivation before reaching maximum cell concentration in the suspension was 9-10 days in the laboratory conditions.

Microalga cell shapes were studied by microphotographs from digital camera IS-500 (AO "LOMO", Saint-Petersburg). Microphotograph processing was implemented using Microanalysis FOTO software (AO "LOMO", Saint-Petersburg) and Levenguk ("Levenhuk LabZZ").

Microalgae samples were preliminary lyophilized at 1 mbar at temperature -55 °C in lyophilic drying installation Alpha 1-2 LDplus (Martin Christ Gefriertrocknungsanlagen GmbH). At cell wall disintegration the sample of 3 g weight was mixed with 10 ml of n-hexane and homogenized using high-speed homogenizer (IKA® Werke, T25 Basic) at 10 000 rpm for 5 min. Lipid extraction was carried out at Buchi Extraction Unit e-812 SOX installation. Mixture of 96%-ethanol and n-hexane 1:9 was used as an extracting agent. Composition of higher fatty acids in lipid extract of *Chlorella sorokiniana* microalga was determined using gas chromatograph with flame-ionization detector Agilent Technologies Sales & Services GmbH & Co.KG, at column BPX70 (60m×0.25mm×0.25 μm), SGE Analytical Science, VWR International GmbH; carrier gas is nitrogen.

Results and discussion

Research of influence of various irradiation conditions on *C. sorokiniana* biomass accumulation.

Analysis of cultivation curves (Fig.1) shows that the most favorable conditions for biomass growth are at DFL illumination mode.

![Fig.1 Cultivation curves for *C. sorokiniana* microalgae for various illumination modes.](image-url)
It was determined, that in accumulation culture of single-cell alga biomass its growth is well described by S-shape curve [9], at which lag-phase, exponential growth phase, stationary phase are clearly observed. It was stated that maximum concentration of cells in biomass equal to $41 \times 10^6$ cells/ml was achieved at the 9-th day of cultivation. Specific rate of linear phase of biomass growth was $\mu = 0.26$ days$^{-1}$. At IR irradiation mode we observed less intensive population growth. Maximum growth of population was achieved at the 14th day, whereas specific biomass growth rate was $\mu = 0.15$ days$^{-1}$.

It was revealed, that linear phase of microalga biomass growth at IR mode was accompanied by cell flocculation, which is driven by pH shift to alkali region. Increase of alkalinity is definitely a stress factor. Flocculation might be considered as a response cell reaction to alkalinity increase, which reduces influence of this factor on single cells (Fig. 2b). At DFL mode increase of flocculation cell amount is observed only at the 9th day of cultivation, which corresponds to stationary phase.

Fig.2. Microscopic image of C. Sorokiniana population while cultivation at various illumination conditions

Investigation of influence of illumination spectral characteristics on accumulation of lipids and higher fatty acids in biomass.

Cultivation of C. Sorokiniana at IR-irradiation results in increase of lipid content in biomass as compared to DFL irradiation mode for 5% in average (Fig.3).

Fig. 3 Influence of illumination on lipid content in C. Sorokiniana biomass
At IR irradiation accumulation of saturated higher fatty acids takes place (Fig.4a) while DFL irradiation mode activates synthesis of unsaturated fatty acids (FA) (Fig.4b)

![Graph A: Saturated FA content](image)

**Fig. 4.** Influence of irradiation conditions on saturated and unsaturated FA content in lipids of C. Sorokiniana biomass

In literature there are some data allowing one to distinguish three aspects of adaptive function of triacylglycerol (TAG) of microalgae [19]. Neutral lipids have a function to be a source of long-chain fatty acids, which are construction blocks for membranes. Simultaneously with that, TAG biosynthesis provides runoff for photoassimilants, which prevent development of photooxidative damages during stresses, which reduce cell ability to utilize photosynthesis products. And, finally, TAGs which are deposited in the form of lipid globules, form storage area for secondary carotenoids, which provide protection from photodamage. After interruption of stress conditions the biochemical composition of cells restores[20].

Conditions for microalgae biomass illumination significantly affect oleinic acid content (C18:1) in total lipid fraction (Fig.5). At thermal exposure metabolization of polyunsaturated fatty acids takes place and accumulation of monounsaturated ones.

![Graph B: Oleinic acid content](image)

**Fig.5.** Oleinic acid content in a lyophilized biomass of C. Sorokiniana.
According to one of the hypothesis [21], reduction of light intensity in terms of one microalga cell is considered as a signal for termination of exponential growth phase. This leads to redirection of usage of surplus of absorbed light energy predominantly to synthesis of reserved TAG. TAG composition of these types is characterized by prevalence of saturated and unsaturated FA in them.

Thus, one of the important conditions for directed cultivation is usage of stimulators of lipid synthesis at preservation of high biomass output. It is valuable to use IR-radiation sources for illumination of the accumulated biomass in stationary growth phase. This leads to increase of nonpolar lipids (TAG) content, which will be used for biofuel obtaining after fractioning. Polar lipid fraction, extracted from microalga biomass at linear growth phase is aimed to be used for obtaining essential unsaturated fatty acids.

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References

[1] Lal A and Das D 2016 Biomass production and identification of suitable harvesting technique for Chlorella sp. MJ 11/11 and Synechocystis PCC 6803 3 Biotech 6 1–10
[2] Kamyab H, Lee C T, Din M F M, Mohamad S E, Mohanadoss P, Khudhair A B and Roudi A M 2014 Biodiesel production from microalga-Chlorella sorokiniana. Aust. J. Basic Appl. Sci. 86,140-145
[3] Chader S, Mahmah B and Al E 2011 Biodiesel production using Chlorella sorokiniana a green microalga Rev. des Energies Renouvelables 14 21–6
[4] Vishnyakova M A, Seferova I V and Samsonova M G 2017 Genetic sources required for soybean breeding in the context of new biotechnologies: (Review) Sel’skokhozyaistvennaya Biol. 52 905–16
[5] Politaeva N A, Kuznetsova T A, Smyatskaya Y A, Trukhina E V and Atamanyuk I 2018 Energy Production from Chlorella Algae Biomass Under St. Petersburg Climatic Conditions Chem. Pet. Eng. 1–5
[6] Politaeva N, Smyatskaya Y, Slugin V, Touni A and Bouabdelli M 2018 Effect of laser radiation on the cultivation rate of the microalga Chlorella sorokiniana as a source of biofuel IOP Conf. Ser. Earth Environ. Sci. 115
[7] Dvoreckij D S, Dvoreckij S I, Temnov E I and Peshkov E V. 2015 Tekhnologiya poluchenii lipidov iz mikrovodoroslej [Technology of lipids obtaining from microalga] (Tambov: Izdatel’stvo FGBOU VPO «TGTU»)
[8] Skjånes K, Lindblad P and Muller J 2007 BioCO 2 – A multidisciplinary , biological approach using solar energy to capture CO 2 while producing H 2 and high value products Biomol. Eng. 24 405–13
[9] Vladimirova M G and Semenenko V E 1977 Mass microalgal cultivation [Massovoe kul’tivirovanie mikroskopicheskikh vodoroslej] Plant life [Zhizn’ rastenij] (Moscow: Izd-vo «Prosveshenie») pp 367–76
[10] Yao L, Shi J and Miao X 2015 Mixed wastewater coupled with CO2 for microalgae culturing and nutrient removal PLoS One 10
[11] Franco M C 2014 Batch cultivation of microalgae in the Labfors 5 Lux Photobioreactor with LED Flat Panel Option InforsHT
[12] Alexeev D V, Shagivaleev A A, Urazbakhtina L R and Napoykina E A 2015 Improving the efficiency of water recycling systems at wastewater treatment power plants from insoluble suspensions Proc. High. Educ. institutions. ENERGY Sect. Probl. 1–2 11–7
[13] Rai V, Muthuraj M, Gandhi M N, Das D and Srivastava S 2017 Real-time iTRAQ-based proteome profiling revealed the central metabolism involved in nitrogen starvation induced lipid accumulation in microalgae Sci. Rep. 7

[14] Wase N, Tu B, Allen J W, Black P N and DiRusso C C 2017 Identification and metabolite profiling of chemical activators of lipid accumulation in green algae Plant Physiol. pp.00433.2017

[15] Ngangkham M, Ratha S K, Prasanna R, Saxena A K, Dhar D W, Sarika C and Prasad R B N 2012 Biochemical modulation of growth, lipid quality and productivity in mixotrophic cultures of chlorella sorokiniana Springerplus 1 1–13

[16] Watanabe K, Imase M, Sasaki K, Ohmura N, Saiki H and Tanaka H 2006 Composition of the sheath produced by the green alga Chlorella sorokiniana Lett. Appl. Microbiol. 42 538–43

[17] Politaeva N, Kuznetsova T, Smyatskaya Y, Elena T and Ovchinnikov F 2017 Impact of various physical exposures on Chlorella Sorokiniana microalgae cultivation Int. J. Appl. Eng. Res. 12 11488–92

[18] Czarena L Crofcheck, Michael Monstross, Xinyi E, Aubrey P Shea, Mark Crocker and Rodney Andrews 2012 Influence of media composition on the growth rate of Chlorella vulgaris and Scenedesmus acutus utilized for CO2 mitigation J Biochem Tech 4 589–94

[19] Solovchenko A E 2012 Physiological role of accumulation of neutral lipids by eucariotic (in Russian) Physiol. plants 59 192–202

[20] Klyachko-Gurvich G L and Rudova T S 1973 Influence of imidazol on fatty acid exchange at reconstruction of Chlorella cells after nitrogen depletion Physiol. plants 20 326–31

[21] Cohen Z and Khozin-Goldberg I 2010 Searching for Polyunsaturated Fatty Acid-Rich Photosynthetic Microalgae Single Cell Oils: Microbial and Algal Oils: Second Edition pp 201–24