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SELECTING A CULTURE MEDIUM AND LIGHT REGIMEN FOR *GYMNODINIUM WULFFII CULTIVATION* IN LABORATORY CONDITIONS

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Introduction

Growing of sea planktonic algae in laboratory conditions has centenary history. Successful cultivation of the Black Sea microalgae provides wide prospects for receiving valuable biomass and extraction of biologically active substances. However only for few species of the Black Sea microalgae the appropriate conditions of the cultivation allowing receiving a sufficient crop of biomass are adjusted. For example, biochemical content of dynophyte algae *Gymnodinium sp.* includes pigments: chlorophylls *a* and *c*, carotenes, peridinin, neoperidinin, dinoxanthin, diadinoxanthin, neodinoxanthin, neodiadinoxanthin [8]; polyunsaturated fatty acids (PUFA), that can reach 57,7% of total fatty acids [9], and other valuable substances. Peridinin-chlorophyll *a*-protein (PerCP) complex is one of the high-value products from dinoflagellates which is commonly used in immunoassays such as FACS and flow cytometry [6].

Gymnodinium wulffii is one of the prevailing planktonic dinoflagellates species in the Black Sea. Since the influence of cultivation conditions on *G. wulffii* growth in culture was not studied before, the purpose of our work was to determine nutrient medium and diurnal light regimen for cultivation *G. wulffii* in laboratory conditions.

Materials and methods

Microalgae *Gymnodinium wulffii* J.Schiller (Dinophyta) culture, obtained from the Department of ecological physiology, IBSS culture collection, was used in our experiment. Microalgae were grown at batch cultivation mode in 250 ml flat-bottomed flasks with 200 ml of culture medium. The temperature was 20 – 22 °C, 24-hour air agitation was made using compressor system at 0,5 l · min^{-1} rate. Cultivation flasks were illuminated by luminescent tubes with 8 klx illuminance

at the surface. Microalgae were grown using culture media f/2, Conway and Trenkenshu A [1, 2, 5]. The media were prepared on the base of filtered and sterilized seawater. For every studied medium microalgal growth was also investigated at two variants, continuous illumination and diurnal light-dark regimen 16 h : 8 h (light : dark). Samples were taken daily at the beginning and the end of the dark period in triplicate for determination of culture density and cell number. Culture density was registered by photometric method using algal suspension optical density at 750 nm (D_{750}) that was measured by photoelectric colorimeter KFK-2 in 5-mm measuring cells and was expressed quantitatively in optical density units (o.d.u.). Cell number was counted in Gorjaev's chamber applying light microscope Axiostar plus (CARL ZEISS, Germany).

Total dark biomass loss (DBL) as fraction of biomass at the end of light period (L_B) was calculated using the formula [7]:

$$L_B = \frac{B_L^{'} - B_D}{B_L^{'}} \cdot 100\% \quad ;$$

DBL (L_P, as fraction of production in the previous light period):

$$L_P = \frac{B_L{}' - B_D}{B_L{}' - B_D{}'} \cdot 100\% \quad ;$$

where B_L' – culture density at the end of the previous light period, B_D – culture density at the end of dark period, B_D'- culture density at the end of the previous dark period.

The maximal productivity and the maximal growth rate were calculated according to models in [3] on the basis of the growth curves. The maximal specific growth rate (μ_m) was figured out by approximating exponential growth phase by equation:

$$B = B_0 \cdot e^{\mu_m \cdot (t - t_0)} \quad ,$$

where B is the current culture density; B_0 is culture density at the beginning of exponential growth phase; t_0 is the time at the beginning of exponential growth phase.

Maximal productivity (P_m) was calculated by approximating growth curve at the linear growth phase area:

$$B = B_l + P_m \cdot (t - t_l) \quad ,$$

where B_l is culture density at the beginning of linear growth phase; t_l is the time at the beginning of linear growth phase.

Results and discussion

Experiment on selection of nutrient medium for reaching the maximal density of microalga *G. wulffii* culture was carried out for 16 days. Growth curves as the dynamics of the optical density in G. *wulffii* batch cultures are presented in fig. 1. Cell number dynamics is presented in fig.2.

At the growth curves it can be seen that exponential growth phase lasted for 112 h for *G. wulffii* grown on f/2 medium, but it was much shorter for Conway and Trenkenshu A media, making 48-64 h.

As the result of the experiment the highest biomass values of *G. wulffii* culture were registered on the Trenkenshu A medium (tab. 1). Productivity values of microalgae were similar on Conway and Trenkenshu A media, but on Conway medium growth slowdown stage started earlier (160 h), while microalgae on Trenkenshu medium continued growing (linear growth phase ended at 280 h).

This culture medium influence can be explained by the fact that Conway and f/2 media contain lower concentrations of nitrogen and phosphorus than Trenkenshu medium. Biological tests [4] showed that the increase in nitrogen and phosphorus leads to a noticeable increase of biomass. At mass cultivation of microalgae temperature and illuminance are other crucial limiting factors, in addition to culture medium.

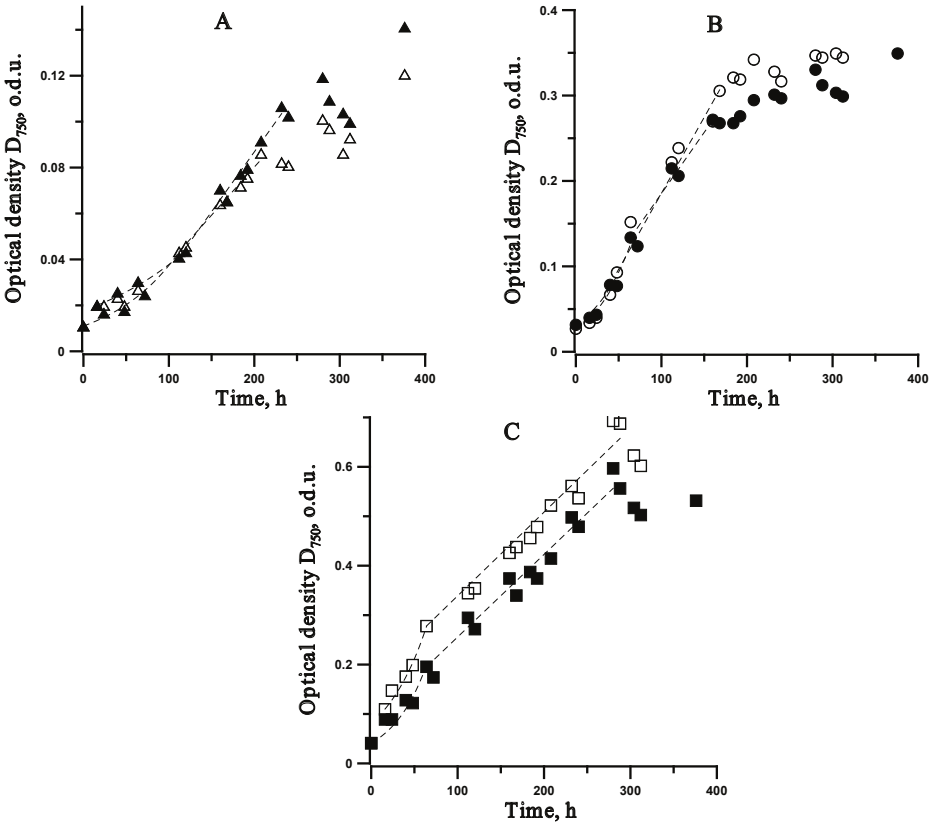

Figure 1. Optical density (D_{750}) dynamics in *Gymnodinium wulffii* batch cultures: A – f/2, B – Conway, C – Trenkenshu A medium; white markers – continuous lighting 24 h, black markers – 16:8 h light-dark regimen. Dashed lines – approximation by model equations (see Methods).

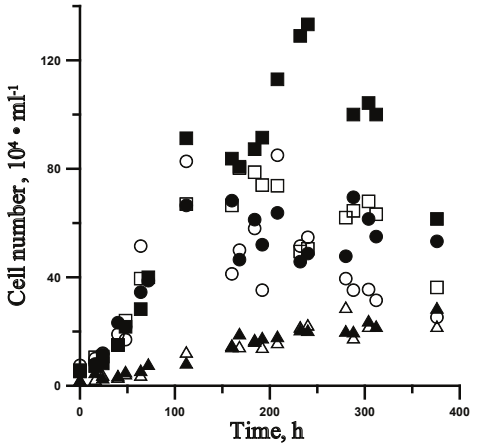

Figure 2. Cell number dynamics in *Gymnodinium wulffii* batch cultures: Δ, ▲- f/2, ○, ● – Conway, □, ▪ – Trenkenshu A medium; white markers – continuous lighting 24 h, black markers – 16:8 h light-dark regimen.

Table 1. *G. wulfii* growth parameters depending on nutrient medium and diurnal light regime

Parameter	Culture conditions					
	Trenkenshu A		Conway		f/2	
	24 h	16 h	24 h	16 h	24 h	16 h
μ_m	1,93	2,47	2,4	2,17	0,76	1,22
g	37,50	32,07	53,19	39,33	57,98	63,07
N_m	$8{,}02 \cdot 10^5$	$1{,}33 \cdot 10^6$	$5{,}48 \cdot 10^5$	$6{,}95 \cdot 10^5$	$2{,}25 \cdot 10^5$	$2{,}08 \cdot 10^5$
P_m	$1{,}69 \cdot 10^{-3}$	$1{,}67 \cdot 10^{-3}$	$1{,}78 \cdot 10^{-3}$	$1{,}43 \cdot 10^{-3}$	$0{,}42 \cdot 10^{-3}$	$0{,}52 \cdot 10^{-3}$
B_m	0,687	0,597	0,347	0,299	0,093	0,119
L_B	–	0 – 11	–	0 – 7,6	–	0 – 31
L_P	–	0 – 29,4	–	0 – 18	–	0 – 87,6

Legend: 24 h and 16 h – light period variants, μ_m – maximal specific growth rate at logarithmic growth stage (h^{-1}), g – doubling time for culture density at logarithmic growth stage (h), N_m – maximal cell number, P_m – maximal productivity at the linear growth stage (o.d.u. $\cdot l^{-1} \cdot h^{-1}$), B_m – maximal biomass (o.d.u.), L_B - DBL as fraction of biomass at the end of light period (%), L_P – DBL as fraction of production in the previous light period (%).

Photoperiod is the important factor affecting the main physiological and biochemical characteristics of microalgae and it needs to be considered when studying productional parameters. So in our research both growth at continuous illumination and at 16 : 8 h light : dark regimen were studied. The 16-h photoperiod simulates day length at summer in the Black Sea region.

At cultivation of *G. wulfii* under the light-dark regime of 16 h: 8 h (light: darkness) natural decrease in culture density during the dark period was observed.

Dark biomass loss varied both on different culture growth stages, and for different media. In general, it can be noted that the larger DBL corresponded with smaller total productivity. For example, when *G. wulfii* was grown on the f/2 medium where the least productivity was registered, DBL made up to 31,1% of biomass and up to 87% of productivity for the previous light period. On the Trenkenshu A medium DBL was to up 11% of biomass and up to 29,4% of productivity. The least values of DBL were observed during microalgal growth on Conway medium, making up to 7,6% of biomass and up to 18% of productivity. At all variants of cultivation the greatest values of DBL were on 2-3 days of microalgae cultivation.

Despite the difference in the amount of totally received light energy by 1,5 times compared with continuous lighting (16 h and 24 h in days), the maximal biomass and cell number at light-dark cycles conditions did not decrease by 1,5 times in comparison with similar values for the continuous light regime. Thus, during microalgae cultivation on the f/2 medium at the 16 h photoperiod and at continuous illumination the maximal cell number was $2{,}87 \cdot 10^5$ and $2{,}9 \cdot 10^5$ cells $\cdot$ ml^{-1}, and the maximal culture density was 0,12 and 0,10 o.d.u., respectively. When *G. wulffii* was grown on Conway medium the maximal values of culture density and cell number at different light modes also did not differed significantly. When cultured on the Trenkenshu A medium at both light regime variants the maximal culture density was reached at the same time by the 12th day, it differed slightly, consisting 0,6 o.d.u. at the photoperiod of 16 h and 0,69 o.d.u. at the continuous lighting. However, the maximum cell number on continuous lighting was $8{,}02 \cdot 10^5$ cells $\cdot$ ml^{-1} by the 7th day of cultivation and did not increase after, but in the light-dark mode conditions cell number increased up to $1{,}33 \cdot 10^6$ cell $\cdot$ ml^{-1} by the 10th day of cultivation.

Conclusion

For cultivation of microalgae *G. wulfii* it is advisable to use Trenkenshu A medium containing balanced amount of nitrogen, phosphorus and trace minerals that promotes fast increase in algal biomass. Maximal biomass in the conditions of our experiment reached 0,69 o.d.u. and maximal productivity $1{,}69 \cdot 10^{-3}$ o.d.u. $\cdot l^{-1} \cdot h^{-1}$.

There were differences in dark biomass loss (DBL) values on different media studied media and at different growth stages. Thus it was noted that in all cultivation variants of microalgae *G. wulfii* the greatest DBL values were registered at 2-3 day of cultivation. It was found that the less was culture productivity, the higher was DBL ratio in daily biomass balance.

G. wulfii seems to be a promising Black Sea microalgae species for cultivation. Further researches considering other growing parameters are needed for optimization and scale-up of cultivation.

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