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Impact of various physical exposures on *Chlorella Sorokiniana* microalgae cultivation

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Abstract

This work presents investigation of impact of the following physical factors: aeration, mixing operation, daylight fluorescent lamp illumination and infrared irradiation on *Chlorella Sorokiniana* biomass production. Analysis of biomass production under these exposures was carried out using optical density. The calculation of cell number in ml was performed using hemocytometer (Goryaeva chamber). Suspension pH values were controlled during cultivation. It was shown that pH value strongly affects *Chlorella Sorokiniana* biomass production. Optimum values of medium pH were determined and pH shift towards alkaline region was explained. Morphological studies of *Chlorella Sorokiniana* population before and after various physical exposures were carried out by microscopy of intravital cells (colored and non-colored). Magnification power was 640 times. It was shown that agglutination and cell precipitation of microalgae *Chlorella Sorokiniana* took place without aeration. The optimum conditions for microalgae *Chlorella Sorokiniana* cultivation were chosen aiming at the highest biomass production.

Keywords: microalgae *Chlorella Sorokiniana*, biomass, pH, infrared radiation, daylight fluorescent lamp, aeration.

INTRODUCTION

Chlorella Sorokiniana microalgae biomass is widely used in various branches of the national economy, namely: as a source for third-generation biofuel, for valuable components (lipids, carotenoids, pectin substances) obtaining, as a biological additive for livestock and poultry feed, and others. So, the vital task is choosing of optimal parameters for biomass cultivation without high energy and material expenses aiming at maximum biomass obtaining in a short time period. To increase cultivation rate it is possible to stimulate energy potential of the plant cell by various physical treatments. External physical exposures (EPE): infrared (IR), daylight fluorescent lamp (DFL), create additional electrical current at biological objects. These exposures vary the membrane potential and can govern the organism growth and evolution processes having both stimulative and inhibitory impacts. This impact depends on exposure characteristics: wavelength (λ), power and time [1,2].

Membranes, as natural barriers, are the first ones to be affected by stress factors. Being dynamic structures, membranes are able to be quickly responsive on living conditions variations. However, the changes in membranes cause a cascade of shifts in metabolic process of cell as a whole. Membrane permeabilty increases, depolarization of plasmalemma membrane potential takes place, cytoplasm pH becomes acid, hydrogen ions H^+ activity becomes higher. Shifts in membranes functional activity are accompanied by reconstruction of its structure, which in the initial stage express their stability increase until stressor activity will come to exertion. In phospholipide molecules the most important elements are strictly qualitative and quantitative determinated [3-5].

It is known that infrared irradiation stimulates growth and life activity of living organisms and plants. It causes thermal effect when absorbed in substance. Infrared region of electromagnetic waves optical spectrum was discovered in 1800 by astronomer W. Herschel while studying energy distribution for sun radiation spectrum. Infrared wave region is located between visible and radio waves and extends from 0.75 to 750 μm [6]. In view of biological impact the IR-radiation can be divided into the following regions: Short-wavelength: λ = 0.74–2.5 μm; Mid-wavelength: λ = 2.5–50 μm; Long-wavelength: λ = 50–2000 μm. Near-infrared radiation action mechanism is governed by low energy of its quanta (0.9-1.0 eV), which cannot cause a pronounced photochemical effect. When absorbed by biological tissues, it is almost totally converted into oscillating energy of endogenous oxygen. This energy is sufficient for activation of ferments, which act as triggers for physiological response activation at a biotissue level. In the organism IR radiation is predominantly absorbed by molecules of water, oxygen and some ferments. Relatively powerful (1-10 W) and short (70-150 nm) pulses of IR laser radiation make bioobject tissues more transparent for low-intensity (10-100 mW) continuous IR-radiation [7]. IR radiation plays an important role in heat exchange. Thermal exposure impact on organism depends on the following: flux density, irradiation duration, area of impact, and wavelength, which denotes radiation penetration

depth. Plant life processes may occur only at certain thermal background, which is denoted by heat quantity and action time. Earth atmosphere in the wavelength range from 3 to 5 and from 8 to 14 μm is transparent for IR-radiation [8].

The aim of this work was to choose optimum conditions for intensification of *Chlorella Sorokiniana* biomass production by means of various physical treatments (IR, DFL, aeration).

MATERIALS AND METHODS

Firstly we investigated impact of various physical exposures on biomass cultivation rate. The implemented physical exposures were:

- infrared lamp of 220 V and 250 W, illuminance is 14100 lx.

- daylight fluorescent lamp, illuminance is 2800 lx.

- control (cultivation without physical exposure).

All experiments were carried out accompanied by periodical mixing of the following regime: 15 min of mixing, 120 min of rest. Mixing rate was 500 rpm. The aeration was performed using aerator with flow rate 1.5 l/min. Cultivation took place at photobioreactor at "day/night regime". Photobioreactor is a cylindrical glass vessel of 500 ml volume, Fig.1. Its height is 380 mm, its diameter is 500 ml. Suspension solution temperature was 26-30 °C, depending on physical factor exposure.

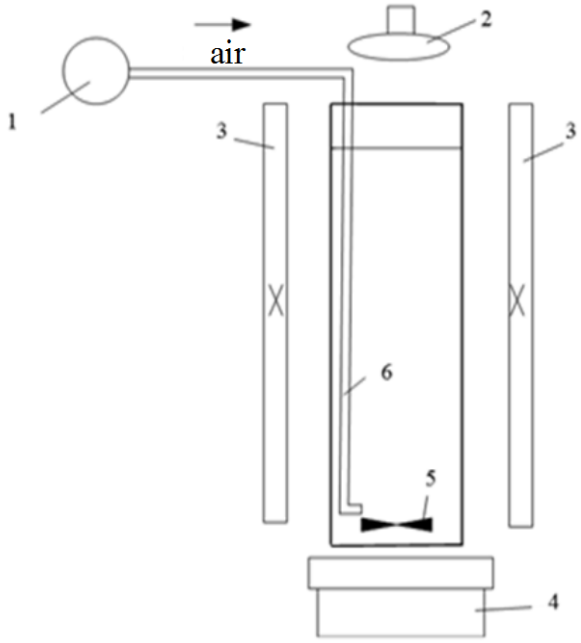


Figure 1: Photobioreactor scheme: 1 - pump-aerator, 2 - radiation source (IR, UV), 3 - daylight fluorescent lamps, 4 - magnetic mixing machine, 5 - anchor of magnetic mixing machine, 6 - air supply pipe

Population growth was estimated according to optical density of Chlorella suspension. It was performed using spectrophotometer UNICO 1208 at 750 nm wavelength with further recalculation at hemocytometer (Goryaeva chamber) for counted cell number in ml. Suspension temperature and pH were controlled by laboratory device HANNA HI2212 PH-ORP.

Morphological study of *Chlorella Sorokiniana* population was carried out by microscopy of intravital cells (colored and non-colored). Magnification power was 640 times. For describing shape of chlorella cells we made microphotograps using digital camera IS-500 and "Microanalysis FOTO" software for 10 fields of vision. Microphotographs analysis (colored and non-colored photographs) was performed using Levenguk software, which allows one to adjust contrast and brightness and additionally magnify the image.

RESULTS AND DISCUSSION

In order to choose optimum conditions for biomass growth, we prepared suspension of microalgae with nutrient broth. Its composition is presented in Table 1.

Table 1: Nutrient broth composition

Substance	Concentration, mg/l
$ZnSO_4 \cdot 7H_2O$	100
$CuSO_4 \cdot 5H_2O$	10
$CoSO_4 \cdot 7H_2O$	100
$MnCl_2 \cdot 4H_2O$	500
$H_3BO_3 \cdot WF$	50
$Na_2MoO_4 \cdot 2H_2O$	100
$FeCl_3 \cdot 6H_2O$	4,000
$Na_2EDTA \cdot 2H_2O$	6,000
KNO_3	3.03
KH_2PO_4	0.32
$MgSO_4 \cdot 7H_2O$	2.4

The initial optical density of the microalgae suspension was 0.20 at 750 nm wavelength. Recalculation at hemocytometer (Goryaeva chamber) for counted cell number showed 5 mln cell per ml at the initial suspension. At IR-irradiation intense growth is observed for the first 4 days of cultivation: biomass increases for 3 times and becomes equal to 13.7. After 4 days the stabilization phase takes place. At daylight fluorescent lamps illumination, the intense population growth is observed during 8 days. The amount of cells increases for more than 10 times and reaches 53.82 mln cells per ml (Figure2). After this, stabilization phase begins, agglutination and cell precipitation is visually observed.

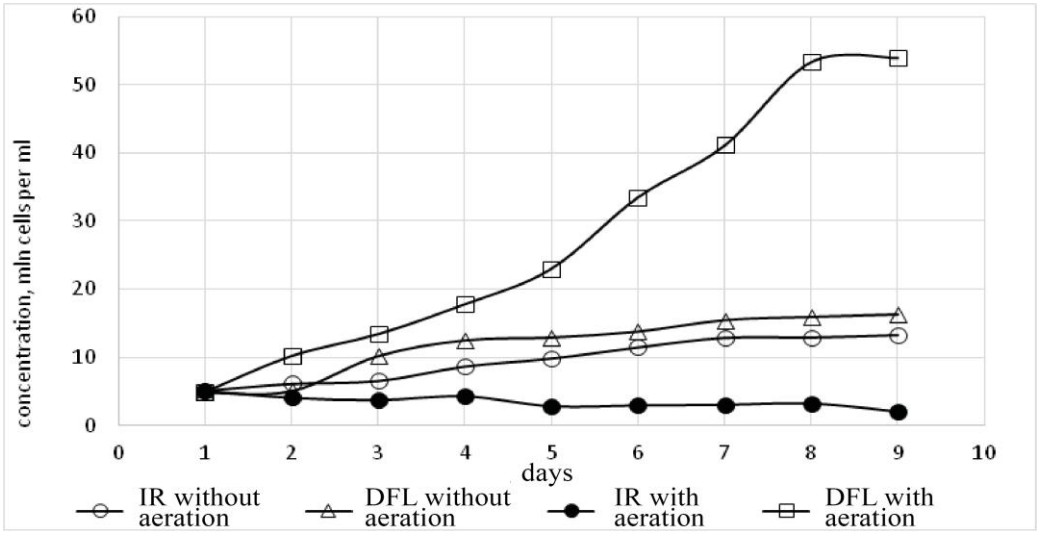


Figure 2: Relation between *C. Sorokiniana* cell concentration and various physical exposures (IR, DFL, aeration)

Aeration is an important factor which affects biomass growth. Cell breakage and its saturation by oxygen and carbon dioxide take place during aeration. Regular aeration allows one to maintain the appropriate for microalgae biomass growth pH value for suspension environment. It is known from literature, that optimum pH values [9] are from 6 to 8. During cultivation, environment pH value increases. This is due to cell absorption of nutrient broth salt anions and ammonia accumulation. The major mechanism, which serves for ammonia inhibition of microalgae, is "intoxication" of its photosynthetic system. During cultivation without aeration pH value might be up to 11. The initial suspension pH value is in the range 6.5-7 (Figure3). A drastic increase of medium alkalinity is a negative factor for population growth. In the presence of aeration and intermixing, we see a significant increase of cell number, at this medium alkalinity is growing slowly (Table 2). Consequently, aeration and intermixing are mandatory factors for high-speed cultivation of *Chlorella Sorokiniana* microalgae.

Table 2: Suspension *C. Sorokiniana* pH value for the 9th day at various exposures

Cultivation conditions		pH value for the 9th day of cultivation
IR radiation	with aeration	8.96
	without aeration	10.79
Daylight fluorescent lamp	with aeration	8.88
	without aeration	11.04

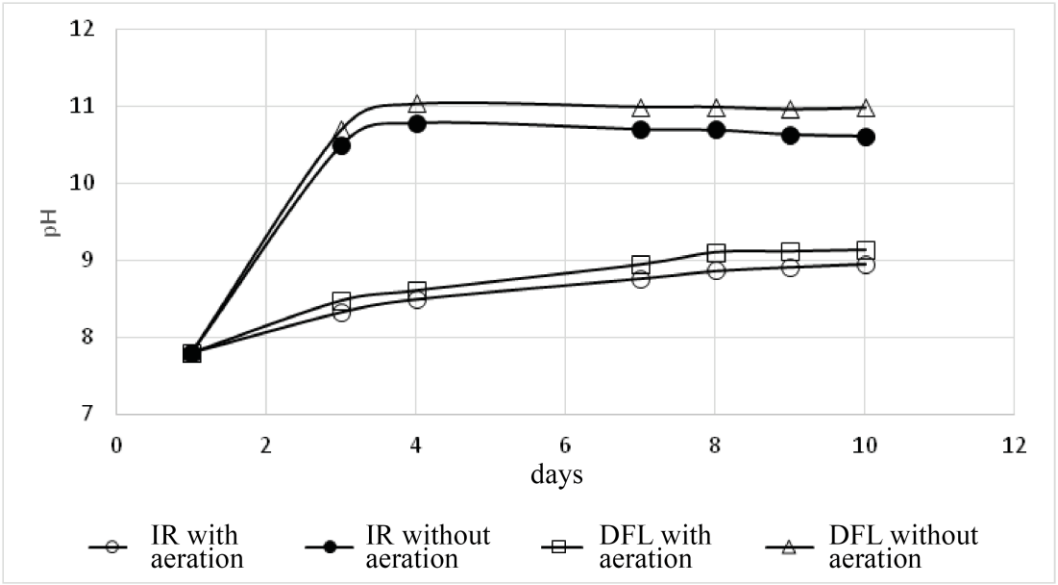
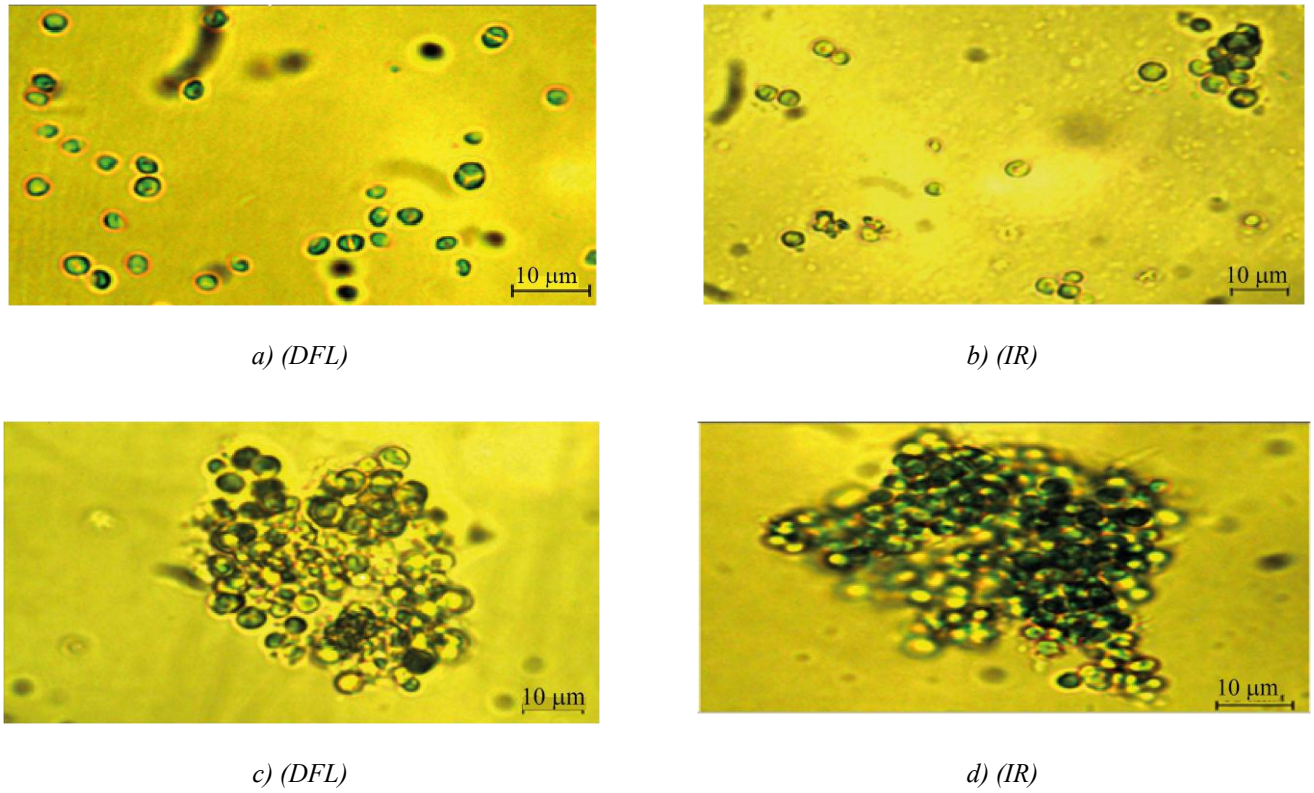


Figure 3: Medium pH values for *C. Sorokiniana* cultivation with various physical exposures (IR, DFL, aeration)

At IR irradiation with aeration the critical pH value is 8.96. At daylight fluorescent lamps illumination with aeration the critical pH value is 8.88 (Table 2).

Microscopic images of *C. Sorokiniana* cells showed that without aeration cells are flocculating and form agglomerates (Figure 4 c,d), where cell division process is slower. It is known that microalgae biomass growth occurs by autosporulation. The wall of mother cell, where new spores are produced, is highly resistant to chemical exposure, but it is easily damaged at mechanical exposure. Thus during aeration and intermixing, which can be regarded as mechanical exposures, mother cell wall is damaged faster. Autospores are released, and new cells are produced quicker.

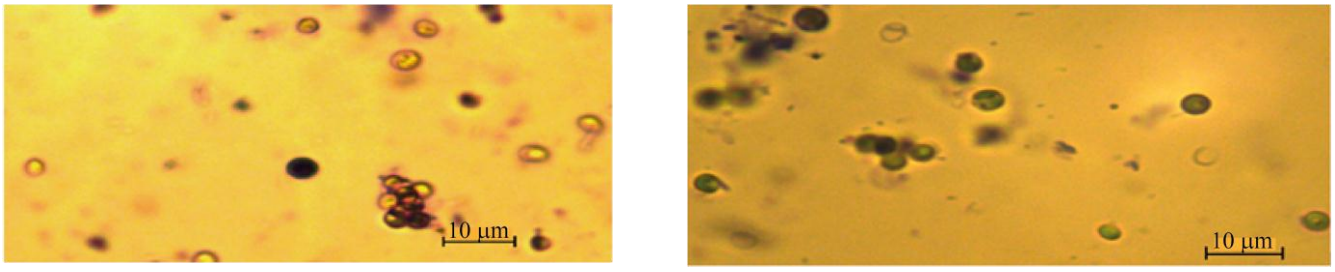
Methylene blue coloration of *C. Sorokiniana* suspension cells after IR and DFL exposure with aeration allow one to find dead cells and cells with inactivated ferments. The microscopic image of methylene blue colored compounds revealed dead cells in the amount of up to 30% at IR exposure during 6 hours (Figure 5, b). At 6 hours DFL exposure the amount of dead cells not exceeded 10% (Figure5, a).



a) (DFL) *b) (IR)*

c) (DFL) *d) (IR)*

Figure 4: Microscopic image of *C. Sorokiniana* cells at various physical exposures: without barbotage a-at DFL exposure, b-at IR irradiation and with barbotage: c-at DFL exposure, d-at IR irradiation



a) (DFL) *b) (IR)*

Figure 5: *C. Sorokiniana* cell compounds, colored by methylene blue: a-at DFL exposure, b-at IR irradiation

CONCLUSIONS

Our experiments showed that the highest biomass production of *Chlorella Sorokiniana* cells takes place at daylight fluorescent lamps illumination combined with aeration and intermixing. Cell treatment without aeration results in cell agglutination with subsequent precipitation. Without aeration the cells stop their reproducing. At this we observe pH value increase, which is due to significant loss of nitrate-anions.

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