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Influence of Infrared Radiation on the Growth Rate of Microalgae *Chlorella sorokiniana*

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***Abstract*—**Nowadays, the progressive decrease of primary natural resources and ongoing upward trend in terms of energy demand, have resulted in development of new generation technological processes which are focused on step-wise production and residues utilization. Thus, microalgae-based 3rd generation bioeconomy is considered one of the most promising approaches that allow production of value-added products and sophisticated utilization of residues biomass. In comparison to conventional biomass, microalgae can be cultivated in wide range of conditions without compromising food and feed production, and thus, addressing issues associated with negative social and environmental impacts. However, one of the most challenging tasks is to undergo seasonal variations and to achieve optimal growing conditions for indoor closed systems that can cover further demand for material and energetic utilization of microalgae. For instance, outdoor cultivation in St. Petersburg (Russia) is only suitable within rather narrow time frame (from mid-May to mid-September). At earlier and later periods, insufficient sunlight and heat for the growth of microalgae were detected. On the other hand, without additional physical effects, the biomass increment in summer is 3-5 times per week, depending on the solar radiation and the ambient temperature. In order to increase biomass production, scientists from all over the world have proposed various technical solutions for cultivators and have been studying the influence of various physical factors affecting biomass growth namely: magnetic field, radiation impact, and electric field, etc. In this paper, the influence of infrared radiation (IR) and fluorescent light on the growth rate of microalgae *Chlorella sorokiniana* has been studied. The cultivation of *Chlorella sorokiniana* was carried out in 500 ml cylindrical glass vessels, which were constantly aerated. To accelerate the cultivation process, the mixture was stirred for 15 minutes at 500 rpm following 120 minutes of rest time. At the same time, the metabolic needs in nutrients were provided by the addition of micro- and macro-nutrients in the microalgae growing medium. Lighting was provided by fluorescent lamps with the intensity of 2500 ± 300 lx. The influence of IR was determined using IR lamps with a voltage of 220 V, power of 250 W, in order to achieve the intensity of 13 600 ± 500 lx. The obtained results show that under the influence of fluorescent lamps along with the combined effect of active aeration and variable mixing, the biomass increment on the 2nd day was three times, and on the 7th day, it was eight-fold. The growth rate of microalgae under the influence of IR radiation was lower and has reached $22.6 \cdot 10^6$ cells·mL^{-1}. However, application of IR lamps for the biomass growth allows maintaining the optimal temperature of microalgae suspension at approximately 25-28°C, which might especially be beneficial during the cold season in extreme climate zones.

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I. Introduction

MICROALGAE are a group of microorganisms that conduct oxygen-related photosynthesis, and due to unique biochemical cell composition, which is mainly represented by proteins, lipids and saccharides, have become a focus in numerous research activities all over the world [1]-[3].

Freshwater green microalgae are reach on vitamins and protein composition that is comparable or exceed soybean and egg, thus they can effectively be used as feed and food additives [4], [5]. Moreover, as a viable source microalgal protein contains all essential amino acids (EAA) that cannot be biosynthesized by organisms and shall be included in a diet [5]. Since Chlorella microalgae might contain more than 50% of protein (in comparison, the indicator for wheat is about 12%) in Japan, it is added to bread, confectionery and ice cream [6]. In addition, some species of microalgae can also be cultivated for commercial astaxanthin production. It needs to be mentioned that for this purpose freshwater (*Chlorella sorokiniana*, *Haematococcus pluvialis* etc.) and marine (*Tetraselmis* sp.) microalgae can be used [7], [8]. In this case, the effect of light and the presence of inorganic carbon as well nitrogen source in a system has to be contentious monitored in order to ensure suitable cultivation conditions and high quality of obtained products [8], [9].

Under the influence of light, microalgal cell will be incurred to dynamic changes of biochemical cell composition as a response to applied environmental stress [10]. For instance, decrease of light intensity will generally lead to increase of chlorophyll a, chlorophyll b and chlorophyll c, whereas secondary pigment such as lutein, β-carotene and astaxanthin will decrease. At the same time, higher light intensity enhances saccharides and lipids production within the algal cell [9]. It has also been reported that the effect of light, and in particular its source, is one of the most important factors that affects microalgal autotrophic growth. In a research study [11], the optimal light intensity f of 9 W·m^{-2} using fluorescent lamp was demonstrated for Chlorella vulgaris, which resulted in biomass productivity of 0.0287 g·(L·d)$^{-1}$. Alternatively, laser beam with a diameter of 5 cm was proved to give a positive effect on the growth rate of *Chlorella sorokiniana* [12]. On the other hand, modification of biochemical composition might be achieved by the temperature and nutritional factors, particularly nitrogen availability. Temperature variations affect partitioning of photosynthetic

carbon, which can be fixed into different types of macromolecules, for instance proteins, saccharides and lipids [9]. Therefore, microalgae cultivation in the temperature range that is below or above the optimal diapason shall lead to higher demand on carbon source to produce the same volume or mass compared to recommended conditions [13]. Withal, as a vital functional and structural component of all cells, nitrogen has to be available in cultivation media to maintain continuous growth and reproduction of microalgae species [14], [15].

The cultivation of *Chlorella sorokiniana* in climatic conditions of Saint Petersburg (Russia) is described in [16], [17]. Due to low light intensity as well as day and night temperatures, the cultivation under the natural conditions is only possible within a relatively narrow timeframe from mid-May to mid-September. However, even in this case the biomass increment is determined to be 3–5 times per week, depending on the solar radiation and ambient temperature [17]. Therefore, the main objective of this study is to investigate the effect of infrared (IR) radiation on the growth of microalgae *Chlorella sorokiniana*.

II. Materials and Methods

A. Microalgae Cultivation and Control of the Growth Rate

In order to evaluate the effect of IR irradiation on microalgal biomass growth, a cultivation process was carried out in photobioreactor (PBS) with a total volume of 500 mL (see Fig. 1). For this type of PBS, fluorescent lamps (OSRAM DULUX S 11W / 21-840 with the intensity of 2200-2800 lx) are exposed to the outside, whereas IR lamp is located at the top of the reactor. The voltage of 220 V and the power of 250 W were maintained for IR lamps in order to achieve the intensity of 13 600 ± 500 lx. To accelerate the cultivation process and ensure homogeneous distribution of nutrients along the medium, the microalgal culture was stirred for 15 minutes at 500 rpm following 120 minutes of the rest time.

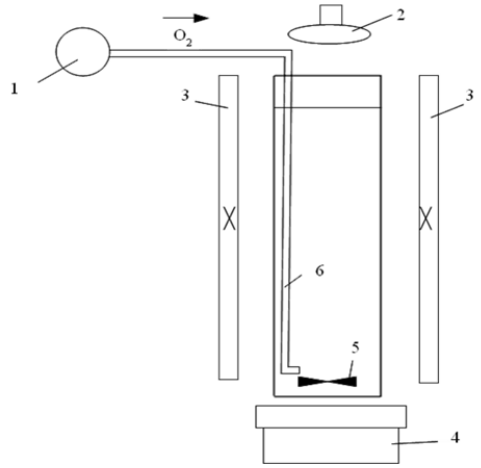

Fig. 1 Photobioreactor for microalgae cultivation with external light source 1 - pump-aerator; 2 - IR lamp; 3 - fluorescent lamps; 4- magnetic stirrer; 5 - anchor of the magnetic stirrer, 6 - air supply tube

The composition of the medium used for the cultivation of microalgae *Chlorella sorokiniana* (strain SAG 211-8k) is presented in [17].

The rate of growth of biomass was determined photometrical using UNICO 2101 spectrophotometer at a wavelength of 750 nm. Each sample was analyzed in triplicate and the results are presented as an average value. Obtained values of the optical density were recalculated using Goryaev chamber and expressed in million cells per 1 mL.

To describe the shape of microalgal cells, photomicrographs were made using the IS-500 digital camera and FOTO Microanalysis software. Analysis of photomicrographs was conducted by Levenguk computer program that allows to contrast and change the brightness of photos, and additionally, to enlarge an image.

B. Samples Preparation and Determination of Protein Concentration

To determine protein content in microalgal samples, biomass was concentrated by means of centrifugation at the room temperature at 6000 rpm for 10 minutes. Obtained pellet was kept at -20°C for 24 h and further freeze-dried. Determination of total proteins in microalgae was conducted by two methods, namely Kejadahl Nitrogen (TKN) and Bradford assay.

TKN analyses were conducted using KJELDATHERM® (Gerhardt GmbH und Co. KG) block digestion unit. Digestion is carried out in sulfuric acid/ peroxide/ catalyst mixture at 400°C. For each of the samples, all analyses were done in duplicate. For the recalculation of Total Kejadahl Nitrogen to protein concentration, a coefficient of 5.95 was used [18].

For determination of soluble protein fraction, acid lysis with 0.5 M NaOH in combination with high speed homogenization (21500 rpm for 15 minutes) was used. Re-suspended biomass was incubated at 55°C for 60 minutes and then centrifuged (14500 rpm at 4°C for 20 minutes). After acid lysis by 0.5 M HCl, a supernatant was centrifuged again under the same conditions that are described above. A pellet was re-suspended in 0.01 M NaOH and pH was adjusted to neutral values of 7.2-7.4.

Soluble protein fraction was subsequently analyzed according to Bradford assay. All analyses were carried out using the Thermo Scientific Pierce™ Coomassie Protein Assay Kit in duplicate for each sample according to the specification. As a standard, bovine serum albumin (BSA) was prepared at different concentration rates from 25 $\mu g \cdot mL^{-1}$ to 2000 $\mu g \cdot mL^{-1}$.

III. Results and Discussion

As has been mentioned in Section II, the effect of light on the growth rate of microalgae *Chlorella sorokiniana* were studied using fluorescent as well as IR lamps. Analysis of the cultivation curves indicates that the most favorable effect is provided by fluorescent lamps (see Fig. 2). The maximum concentration of cells ($41 \cdot 10^6$ cells$\cdot mL^{-1}$) and optical density of 1.5 were reached on the 9th cultivation day. IR radiation resulted in less intense population growth with the cell

concentration of $22.6 \cdot 10^6$ cells·mL^{-1}, (optical density 0.79) on the 10th day.

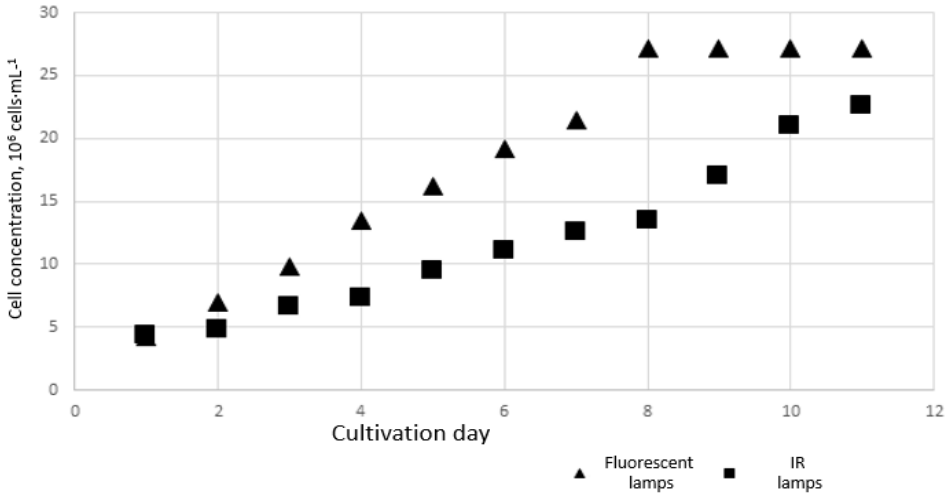

Fig. 2 Growth dynamics of microalgae *Chlorella sorokiniana* under the influence of fluorescent as and IR lamps

From Fig. 2, it can be seen that during the cultivation under the influence of fluorescent lamps, the growth rate shows an up-ward exponential growth over the cultivation time of 9 days till a stationary phase (stabilization phase) has been reached. In contrast, the influence of IR radiation has caused a lag phase over the first days, whereas from the 3rd to the 10th day the growth of the number of cells occurred linearly.

An important indicator of population growth is an increase of pH values in the cultivation medium due to the consumption of anions of salts of the nutrient medium by cells and accumulation of ammonia. In the case of high autotrophic production without supply of CO_2 as an inorganic source of carbon, pH can significantly exceed the optimal range of 7.4-7.6. In the system with no addition of CO_2, pH values of the microalgae culture were 7.4 ± 0.1 during the first days (optical density ~ 0.200). The most intensive growth of the alkalinity (pH ~ 8.42) in the medium was observed on the 4th day when IR radiation was applied.

Under the influence of fluorescence lamps the increase of pH values was less significant. Only at the last cultivation days, pH values have reached 8.6-8.9, which became one of the limiting factors that reduced further intensity of cell multiplication. In order to overcome identified problem, C, N and P were added to the cultivation medium to insure autotrophic microalgae growth. In addition, optimal pH and nutrients availability might dramatically increase light utilization efficiency [19].

The increase in alkalinity during the cultivation is undoubtedly a stress factor that results in agglutination of cells. The increase in the number of agglutinating cells was observed within the stationary cultivation phase using micrographs of *Chlorella sorokiniana* (see Fig. 3 and Fig. 4).

To evaluate the effect of fluorescent and IR lamps on the composition of biomass, the total content of nitrogen and protein in microalgae cultured was determined as has been described in Section II (B. Samples preparation and determination of protein concentration). Obtained results are presented in Table I.

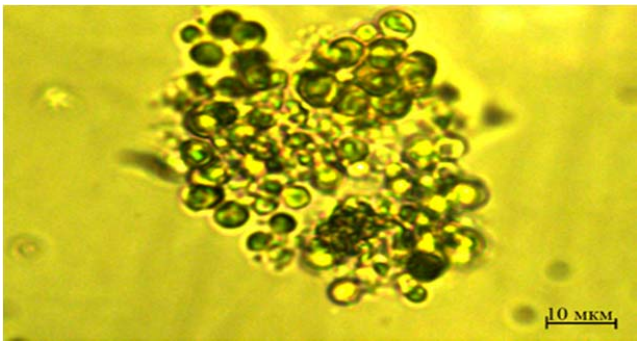

Fig. 3 Agglutinating of microalgae cells under the influence of fluorescent lamps and pH of 9.0

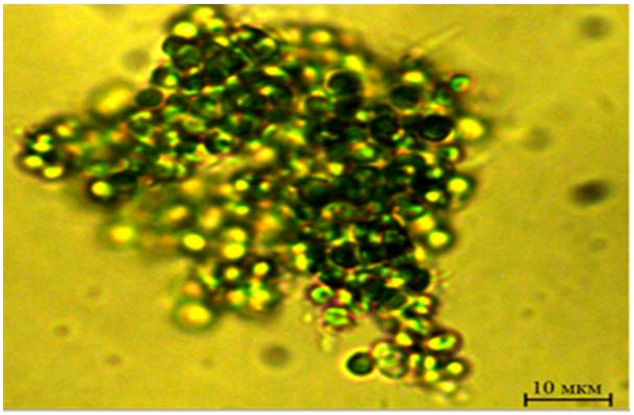

Fig. 4 Agglutinating of microalgae cells under the influence of IR lamps and pH of 9.0

As can be seen in Table I, the concentration of Kejadahl Nitrogen and soluble proteins was higher in microalgae

samples, which were cultivated under the influence of IR lamps. According to the literature, nitrogen-limited cultivation conditions are normally resulted in accumulation of saccharides and lipids in cells, along with diversion of protein synthesis [9]. Thus, under the influence of fluorescent lamps after the growth rate has reached a stationary phase on day-8 (see Fig. 2), the N-limitation has probably caused a change in biochemical composition of Chlorella sorokiniana. Under the effect of IR radiation, photo-synthetically fixed carbon was utilized for protein synthesis and continuous increase of cell concentration.

TABLE I
RESULTS OF PROTEIN DETERMINATION IN MICROALGAE *CHLORELLA SOROKINIANA*

Light source	Kejadahl Nitrogen, $mg \cdot g^{-1}$	Protein concentration, according to TKN, $mg \cdot g^{-1}$	Concentration of soluble protein, $mg \cdot g^{-1}$
Fluorescent lamp	77.27	459.76	401.31
IR lamps	81.30	483.74	426.92

All results are presented as an average value of duplicate experiments ($p < 0.05$).

It also needs to be mentioned that IR radiation has shown a positive effect on maintaining of optimal temperature in cultivation medium. In the laboratory scale cultivation conditions without additional control and adjustment, the temperature of the medium was approximately + 20-22°C and 25-28°C for fluorescent and IR lamps, respectively. Therefore, application of IR radiation to keep an optimal temperature range for *Chlorella sorokiniana* species can be considered as an advantage compared to fluorescent lamps, particularly during the cold season.

IV. CONCLUSIONS

In this study, microalgae *Chlorella sorokiniana* were cultivated in laboratory scale photobioreactors using two different light sources, namely: fluorescent and IR lamps. A comparison of the growth rates has shown that application of fluorescent lamps has resulted in higher cell concentration of microalgae, compared to IR lamps. On the other hand, the influence of IR radiation affected a biochemical composition of microalgal cell and created favorable conditions for protein synthesis. At the same time, application of IR lamps might also be beneficial when maintenance of optimal cultivation temperature is required.

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