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CRITICAL INTENSITY OF SWIRLING FLOWS OF A SUSPENSION AND PRODUCTIVITY IN BATCH CULTURE OF ARTHROSPIRA (SPIRULINA) PLATENSIS UNDER DIFFERENT LIGHT CONDITIONS

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The paper provides results of the experimental study of batch cultures of *Arthrospira* (*Spirulina*) platensis under conditions of critical intensity of swirling flows at different size of the working layer (the optical path). As shown, despite a 10-fold increase in the intensity of vortex mixing, the productivity of the culture decreased by 2 times, when the working layer rose by 3 times. Notably, for photosynthetic microorganisms, the key factor determining the productivity in the culture is the spatial irradiation inside cell suspension (the working layer) but with the intensity of suspension stirring taken into account. The conditions of cultivation of photosynthetic cells under which the working layer differs by 2 times, but the productivity in the culture remains the same, are experimentally demonstrated.

Keywords: vortex mixing, spirulina, photobioreactor

The productivity of photosynthetic microorganism cultures is mediated by many factors. However, when designing industrial photobioreactors (hereinafter PBRs), the key parameters are the optical path (working layer) and the intensity of cell suspension mixing. Importantly, these two parameters affect much the productivity of the cultivation system and also determine the PBR design features and its operating modes. As proven experimentally, in industrial microbiology, one of the most promising methods of the cell suspension mixing is the generation of its tornado-like vortex motion, *i. e.*, the creation of closed swirling quasi-stationary flows inside the PBR working volume [Gevorgiz et al., 2021; Mertvetsov μ др., 2002; Naumov et al., 2023a; Patent 1779690 A1 SU, 1992; Patent 2099413 C1, 1992; Patent EP 1120460 B1, 1998; Patent EP 27446382 A1, 2011]. The vortex method allows for effective mixing of the culture simultaneously providing three-dimensional circulation and meridional motion of the medium. At the same time, it is gentle enough: with no water hammer, turbulence, cavitation, increased shear stresses, and mechanical effect on cells. The mixing efficiency of the vortex formed in the suspension is the highest when radiuses of the vortex PBR and the working layer are equal [Naumov et al., 2023b]. Under such conditions, complete meridional circulation of the medium is ensured

throughout the working volume, while losses due to viscous friction against stationary walls of the reactor are small. However, unlike heterotrophic microorganisms, phototrophs must be provided with light energy in all layers of the working volume; therefore, industrial PBRs are always designed with a thin working layer [Shtol' et al., 1976]. Despite numerous publications focused on studying the effect of radiance and mixing intensity on the productivity of photosynthetic cells [Bitog et al., 2014; Wang, You, 2013; Ye et al., 2018a, b; Xu et al., 2020], there is still the question of increasing the yield in optically dense cultures by a boost in the swirling intensity and, consequently, the speed of the swirling flow.

The aim of this work is to study batch cultures of lower phototrophs under conditions of critical intensity of swirling flows at different sizes of the working layer (optical thickness of the culture).

MATERIAL AND METHODS

The research was carried out at the laboratory of advanced energy-efficient technologies at the Novosibirsk State University (Novosibirsk). The work investigated a unialgal culture of a cyanobacterium *Arthrospira (Spirulina) platensis* (Nordstedt) Gomont (strain IBSS-31) from the collection of microalgae and cyanobacteria cultures of the IBSS core facility "Collection of Hydrobionts of the World Ocean" (Sevastopol). For intensive cultivation of the cyanobacterium, the Zarrouk's medium [1966] was used, with the composition as follows (g·L⁻¹): NaHCO₃, 16.8; NaNO₃, 2.5; KH₂PO₄ × 2H₂O, 0.66; K₂SO₄, 1.0; NaCl, 1.0; Na₂EDTA, 0.08; FeSO₄ × 7H₂O, 0.01; CaCl₂, 0.04; MgSO₄ × 7H₂O, 0.2; H₃BO₃, 2.86 × 10⁻³; MnCl₂ × 4H₂O, 1.81 × 10⁻³; ZnSO₄ × 7H₂O, 0.222 × 10⁻³; CuSO₄ × 5H₂O, 0.079 × 10⁻³; MoO₃, 0.015 × 10⁻³; NH₄VO₃, 0.02296 × 10⁻³; Co(NO₃)₂ × 6H₂O, 0.04398 × 10⁻³; K₂Cr₂(SO₄)₄ × 24H₂O, 0.0960 × 10⁻³; NiSO₄ × 7H₂O, 0.04785 × 10⁻³; Na₂WO₄ × 2H₂O, 0.01794 × 10⁻³; and Ti₂(SO₄)₃, 0.0960 × 10⁻³. This nutrient medium was prepared using distilled water. To maintain a constant pH level (8.4), CO₂ was added to the culture with a pH monitor from a cylinder.

All the tests were carried out with the culture adapted to experimental conditions in two vortex-type PBRs. PBR-1 was a cylindrical container with a submerged rotating disk of radius R_1 being 145 mm. The rotating disk generated swirling flows inside the suspension, and this allowed eliminating stagnant zones in the working volume. To generate a vortex motion in PBR-2, a swirling air flow was created above the suspension by rotating a blade wheel (an activator). As a result, the vortex formed above the suspension due to air friction on the phase interface and the pressure difference between the periphery and the center of the gas-air vortex involved the cell suspension at the interface in tangential motion and generated swirling meridional circulation throughout the working volume ascending near the axis and descending at the reactor periphery [Naumov et al., 2023b]. To stabilize the phase interface in PBR-2, a free-floating flat washer was used (Fig. 1). The radius of the flat washer R_2 was 76 mm; the radius of a hole in the washer r_2 was 16 mm. The rotation speeds of the flat washer in PBR-1 and the activator in PBR-2 were constant throughout the batch cultivation and determined the culture mixing intensity.

The experiments were carried out at a constant temperature and 24-hour illumination. White CRI90 3000K LEDs were used as a light source; those created the same irradiance, $20~\text{W}\cdot\text{m}^{-2}$, on the PBR working surface in all the tests. The irradiance was calculated based on data of a Yu-116 luxmeter, the relative visibility spectrum, and spectral characteristics of the LEDs provided by the manufacturer [Gevorgiz, Malakhov, 2018]. In the experiment No. 1, in PBR-1, the working layer h_1 was 150 mm (suspension volume V_1 was 39.6 L), and the disk rotation frequency was 3.4 Hz $(\omega_1 = 204~\text{rpm})$. The maximum (critical) speed of the suspension motion in swirling flows was selected

in such a way that the interface did not oscillate or deform, *i. e.*, the working layer in all directions remained the same. Under conditions of the experiment No. 1, the value of the tangential velocity of motion v_1 was 3.10 m·s⁻¹. Conditions of the experiment No. 2 were similar to those of the experiment No. 1, but the working layer was reduced 3 times, to $h_2 = 50$ mm ($V_2 = 13.2$ L), and the disk rotation frequency was reduced 10 times, to 0.34 Hz ($ho_2 = 20.4$ rpm), with the tangential velocity $ho_2 = 20.4$ m·s⁻¹. In the experiment No. 3, in PBR-2, the working layer h_3 was 105 mm ($ho_3 = 2.7$ L), the activator rotation frequency was 1,200 rpm, and the flat washer rotation frequency was 0.88 Hz ($ho_3 = 52.8$ rpm). The maximum tangential velocity of motion $ho_3 = 2.5$ mm ($ho_3 = 2.5$ mm) ($ho_4 = 1.27$ L), and the rotation frequency of the experiment No. 3, but the working layer h_4 was 50 mm ($ho_4 = 1.27$ L), and the rotation frequency of the activator was reduced to 15 Hz (900 rpm); accordingly, the rotation frequency of the flat washer decreased to 0.7 Hz ($ho_4 = 42$ rpm). The maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity $ho_4 = 1.25$ maximum value of the tangential velocity

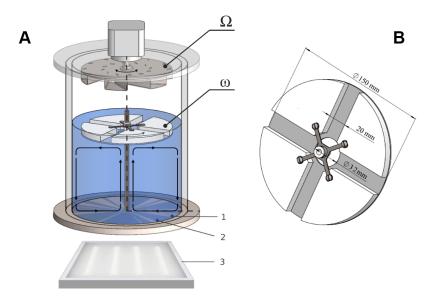


Fig. 1. Schematic diagram of gas-vortex bioreactor (A); flat washer (B). In the diagram: 1, pH sensor position; 2, point of CO_2 injection into the suspension; 3, radiation source

Throughout the experiments, the culture biomass was assessed daily by measuring the attenuation of the light flux at a wavelength of 750 nm by a layer of the cell suspension using a KFK-2 concentration photometer (T_{750} , %; a 5-mm cuvette) with a subsequent transition to optical density $D_{750} = -lg (T_{750}/100)$ and to biomass $B = D_{750} \times 0.85$ (g d. m.·L⁻¹) [Gevorgiz et al., 2005]. The standard deviation of the culture density measurements in all the experiments did not exceed 0.02 g d. m.·L⁻¹. The maximum productivity in the culture was calculated for a linear section of the cumulative curve applying the following expression:

$$B(t) = P_m \times (t - t_L) + B_L ,$$

where B is the biomass, g d. m. L^{-1} ;

 P_m is the maximum productivity, g d. m. L^{-1} ·day⁻¹;

t is time, days;

 t_L is the initial moment of the linear section of the cumulative curve, days;

 B_L is biomass at the moment t_L .

RESULTS AND DISCUSSION

In optically dense cultures, because of dark respiration and costs of maintaining the structure, a drop in productivity is always observed [Avsiyan, Lelekov, 2020; Torzillo et al., 1991]. Due to the two-stage nature of the photosynthesis process, it can be assumed as follows: an increase in the mixing intensity will reduce the time cells spend in layers with irradiance below the photosynthesis compensation point which will govern a rise in productivity. This will allow increasing the yield in industrial PBRs with a thick working layer.

Many experimental studies have shown that light conditions of cultivation are the key factor mediating the productivity in a culture [Avsiyan, Lelekov, 2020; Lelekov et al., 2020; Trenkenshu et al., 1981]. Interestingly, not only the surface irradiance is important, but also the distribution of radiation energy within the cell suspension [Novikova, 2017; Qiang et al., 1998] which much depends on the current culture density [Richmond, 2000]. For light-limited cultures, the productivity value tends to depend on irradiance linearly; thus, for *A. platensis*, linearity is observed at 5–30 W·m⁻² [Lelekov et al., 2020]. However, for intensively mixed cultures, this pattern is not always observed. For example, the results of our experiments (Figs 2, 3) show that the intensity of mixing does boost the productivity of cultures, but not linearly. Therefore, studying the effect of light conditions on productivity, it is necessary to take into account the intensity of the culture mixing as well.

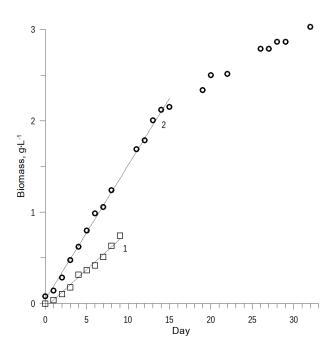


Fig. 2. Density dynamics of *Arthrospira (Spirulina) platensis* batch culture with various working layer size and vortex mixing intensity. The standard deviation of measurements does not exceed 0.02. 1, experiment No. 1: $h_1 = 150 \text{ mm}$; $\omega_1 = 204 \text{ rpm}$; $v_1 = 3.10 \text{ m} \cdot \text{s}^{-1}$; $B_1 = 0.08 \times \text{t} - 0.04$. 2, experiment No. 2: $h_2 = 50 \text{ mm}$; $\omega_2 = 20.4 \text{ rpm}$; $v_2 = 0.31 \text{ m} \cdot \text{s}^{-1}$; $B_2 = 0.15 \times \text{t} + 0.05$

The intensity of the cell suspension mixing is always limited: either by the design of the PBR or by species-specific features of cells cultured. In particular, for a plane-parallel PBR, at a certain critical intensity of air bubbling, abundant foam occurs hindering the cultivation process [Kubar et al., 2022; Shtol' et al., 1976]. For a pool-type PBR, at a certain critical speed of motion of a mechanical

stirrer, due to local pressure swings and an increase in temperature, cultured cells die [Mazzuca Sobczuk et al., 2006]. Microalgae cells are destroyed by the use of pumps [Jaouen et al., 1999], filters [Vandanjon et al., 1999], and sprinklers [García Camacho et al., 2000]. Moreover, cells are also susceptible to damage by gas bubbles and high turbulence of the culture medium [Silva et al., 1987]. Therefore, in practice, for the cultivation of photosynthetic microorganisms, the suspension is mixed not intense enough, while in large-volume PBR, the key factor affecting productivity is almost always the spatial irradiance (the working layer). According to our data, despite the maximum intensity of the suspension vortex mixing ($v_1 = 3.097 \text{ m·s}^{-1}$) in a layer h_1 of 150 mm, the productivity in the batch culture was 2 times lower (see Fig. 2) compared to that in a thinner working layer ($h_2 = 50 \text{ mm}$, $3h_2 = h_1$) and low mixing intensity ($v_2 = 0.3097 \text{ m·s}^{-1}$, $10v_2 = v_1$). Notably, with a 3-fold increase in the working layer, the productivity decreased not by 3 times, as might be expected for a light-limited culture, but by 2 times ($P_1 = 0.08 \text{ g d. m.·L}^{-1} \cdot \text{day}^{-1}$, $P_2 = 0.15 \text{ g d. m.·L}^{-1} \cdot \text{day}^{-1}$). This is due to a 10-fold rise in the intensity of the cell suspension mixing.

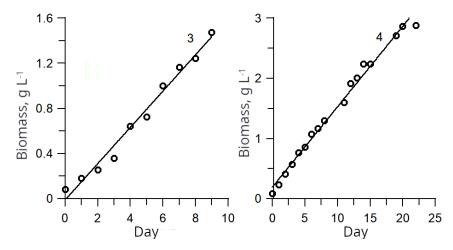


Fig. 3. Density dynamics of *Arthrospira (Spirulina) platensis* batch culture with various working layer size and vortex mixing intensity. The standard deviation of measurements does not exceed 0.02. 3, experiment No. 3: $h_3 = 105 \text{ mm}$; $\omega_3 = 52.8 \text{ rpm}$; $v_3 = 0.42 \text{ m} \cdot \text{s}^{-1}$; $B_3 = 0.08 \times t - 0.04$. 4, experiment No. 4: $h_4 = 50 \text{ mm}$; $\omega_4 = 42.8 \text{ rpm}$; $v_4 = 0.33 \text{ m} \cdot \text{s}^{-1}$; $B_4 = 0.15 \times t + 0.05$. The productivity of *A. (S.) platensis* culture (angle of inclination) is similar under different light conditions

When increasing the intensity of the culture mixing and shifting the working layer, there can be a cultivation mode established in which a 2-fold increase in the working layer will not change the culture productivity. Fig. 3 shows two cumulative curves characterized by almost the same productivity ($P_3 = 0.16 \text{ g d. m.·L}^{-1} \cdot \text{day}^{-1}$, $P_4 = 0.15 \text{ g d. m.·L}^{-1} \cdot \text{day}^{-1}$), while the working layer of the cultures differed by 2 times ($h_3 = 105 \text{ mm}$, $h_4 = 50 \text{ mm}$, $h_3 = 2h_4$). Accordingly, when culturing microalgae and cyanobacteria in industrial PBRs, in order to obtain the maximum yield with considering costs of mixing a unit volume of the suspension, it is necessary to set the maximum permissible intensity of the culture mixing and increase the working layer to a certain limit value the culture productivity does not drop at. This approach will definitely rise the yield, especially during the industrial cultivation of photosynthetic microorganisms under natural light conditions (in particular, in areas with low solar radiation flux) and during cultivation in autumn and winter [Chekushkin et al., 2022].

Conclusion. Along with light conditions, an important factor governing the productivity of microalgae and cyanobacteria culture is its mixing. The effect of this factor is especially evident when increasing the working volume and working layer of the cell suspension in industrial photobioreactors.

Increasing the intensity of the culture mixing up to certain critical values will allow obtaining the maximum yield at fixed irradiance. Currently, theoretical concepts of mixing of photosynthetic cells in culture are poorly developed; therefore, to control growth processes and find optimal solutions, it is necessary to build models of substrate-dependent growth under the effect of two factors: mixing and light supply of cells. This is a fairly complex interdisciplinary task requiring the introduction of new quantitative criteria that allow to compare different mixing methods in photobioreactors of various designs and to assess formally the intensity of cell suspension mixing in the working volume.

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КРИТИЧЕСКАЯ ИНТЕНСИВНОСТЬ ЗАКРУЧЕННЫХ ПОТОКОВ СУСПЕНЗИИ И ПРОДУКТИВНОСТЬ НАКОПИТЕЛЬНОЙ КУЛЬТУРЫ ARTHROSPIRA (SPIRULINA) PLATENSIS ПРИ РАЗЛИЧНЫХ СВЕТОВЫХ УСЛОВИЯХ

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Представлены результаты экспериментального исследования накопительных культур *Arthrospira (Spirulina) platensis* в условиях критической интенсивности закрученных потоков при различной величине рабочего слоя (оптического пути). Показано: несмотря на повышение интенсивности вихревого перемешивания в 10 раз, при увеличении рабочего слоя в 3 раза продуктивность культуры уменьшалась в 2 раза. Отмечено, что для фотосинтезирующих микроорганизмов ведущим фактором, определяющим продуктивность культуры, является пространственная облучённость внутри суспензии клеток (рабочий слой), но с учётом интенсивности перемешивания суспензии. Экспериментально продемонстрированы условия культивирования фотосинтезирующих клеток, при которых рабочий слой отличается в 2 раза, но продуктивность культуры при этом остаётся неизменной.

Keywords: vortex mixing, spirulina, photobioreactor