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EXPERIMENTAL EVALUATION OF A HELICAL LABORATORY PHOTOBIOREACTOR FOR CULTIVATION OF THERMOPHILIC CYANOBACTERIA – HYDRODYNAMICS AND MASS TRANSFER STUDIES

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Dedicated to Professor Andrzej Burghardt on the occasion of his 90th birthday

The aim of the paper is to present the hydrodynamic, mass transfer and illumination characteristics of a laboratory helical-tube photobioreactor Biostat PBR-2S, commercially available and used in many laboratories in Poland and worldwide. The investigated hydrodynamics parameters were: mean liquid circulation rate, liquid velocity/residence time in the tubular part of the apparatus and mixing time, measured in the wide range of rotary speed of the circulation pump. The influence of the aeration intensity on these parameters was also checked. The volumetric oxygen and carbon dioxide transfer coefficients in the liquid phase and their dependency on the liquid circulation rate and gas inflow rate were determined. The experiments were performed in tap water and then in a real three-phase cultivation broth at the end of thermophilic cyanobacteria *T. synechococus* growth. For the final evaluation of the tested PBR there were series of test cultivations run under different conditions of illumination. The highest final concentration of the biomass of tested cyanobacteria reached the relatively high value of 4.38 g/dm^3 of the dry biomass, although the process conditions were not fully optimized. The laboratory photobioreactor PBR-2S proved to be a good tool for investigations of microalgae cultivation processes. The presented results and practical observations may help to analyze and understand the mutual influence of the specific process parameters in the described PBR, especially during autotrophic organism cultivations.

Keywords: photobioreactor, hydrodynamics, mass transfer, microalgae, thermophilic cyanobacteria

1. INTRODUCTION

Efficient microalgal species cultivation has recently received great interest since they may be applied for very different purposes, e.g.:

- for obtaining many valuable fine products such as nutraceuticals, carotenoids, polyunsaturated fatty acids and triglycerides for biofuel (Batan et al., 2016; Chisti, 2007) or bioethanol production (Su et al., 2017);
- for biosynthesis of vitamins, amino-acids and other bioactive compounds for cosmetic, pharmaceutical and food industries (Akpolat and Eristurk, 2008; Knuckey et al., 2006; Yan et al., 2016; Vaz et al., 2016);

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- for wastewater treatment and bioremediation (Fereshteh et. al, 2007; Wang and Lan, 2011);
- for binding excess CO_2 from atmosphere or waste gases (Hsueh et al., 2007; Lan and Wang, 2010; Yoshihara et al., 1996);
- as a source of energy (Kwietniewska et al., 2012).

Recently, many researchers gained interest in thermophilic and extremophilic cyanobacteria, which may be found worldwide in hot springs of water temperature even up to 98 °C (Tang et al., 2018). These species may have biotechnological potential for the production of bioactive molecules and useful temperature-tolerant enzymes, such as laccases, phosphatases and kinases. Thermophilic cyanobacteria may also have practical application as a robust photosynthetic platform for industrial CO_2 utilization (Tang et al., 2018).

Green microalgae and cyanobacteria are photosynthetic organisms with relatively simple requirements, but with high demand of light for photosynthesis. They may be cultivated in open air systems like lakes, deep channels, shallow circulating units, etc., or in closed, technically controlled installations, assuring suitable light supply for the biomass, commonly called photobioreactors (PBR). In the literature one may find many different types of PBRs described, from "classic" stirred tanks (Grobbelaar et al., 1996), bubble and air-lift columns (Guo et al., 2015; Massart et al., 2014; Wang et al., 2012), horizontal tubular bioreactors (Berenguel et al., 2004), flat panels (Sierra et al., 2008; Su et al., 2017), air-lift panels with static mixers (Bergmann and Troesch, 2016), helical, conical and torus systems (Hall et al., 2003; Pruvost et al., 2006), to different hybrid constructions (Huang et al., 2014; Huang et al., 2016; Iluz and Abu-Ghosh, 2016).

From the bioprocess engineering point of view, the suspension filling such apparatus during microalgae cultivation may be considered as a three-phase system, where gas bubbles (usually air or nitrogen, with a certain amount of carbon dioxide) constitute the gas phase, nutrient medium is the liquid phase, and living cells form the suspended solid phase. The fourth crucial "component" of a reaction mixture is light, natural or artificial, which influences complex interactions between above mentioned three phases (Vasumathi et al., 2012). Hence, a good understanding of hydrodynamic and gas transfer in PBRs is required to obtain high productivity of microalgae. It is obvious that the greatest influence on the transfer rate of gases, CO_2 and O_2, between gas and liquid phases and on intensity and amount of light reaching cells may be assured by appropriate design of the apparatus. These parameters are crucial for acceptable productivity of green biomass, especially in large-scale commercial applications (Ugwu et al., 2008).

Although closed photobioreactors generate higher capital and operational costs, they have many significant advantages over open systems: – better control over process conditions such as pH, temperature, CO_2 concentration and light, – better mixing conditions leading to higher mass transfer rates in the liquid phase, – possible higher cell concentrations, – much lower water evaporation during the process and – minimized risk of contamination in cultivations of monocultures. Artificial illumination in PBRs, although expensive, provides better control of light intensity, photoperiod and light spectra. A proper choice of these illumination parameters can result in enhanced photosynthesis and thus higher yield of biomass and valuable products. Therefore, optimization of light supply remains a critical issue in phototrophic culture biotechnology.

2. HYDRODYNAMICS AND MASS TRANSFER IN PBRS

In the literature there exists a great number of articles discussing the relationship between hydrodynamics and mass transfer in bioreactors for heterotrophic organisms, but much fewer publications on these aspects may be found for phototrophic cultures, and just a few for thermophilic cyanobacteria (Bergmann and Troesch, 2016; Eberly and Ely, 2012; Su et al., 2017).

Characteristics of hydrodynamics of the three-phase system in PBRs and gas-liquid transfer of O_2 and CO_2 include intensity of mixing, liquid velocity, gas holdup in the liquid phase in air-lift bioreactors and

the mass transfer coefficients of gases present in the system. The liquid-side volumetric mass transfer coefficient, k_La, or the overall volumetric gas transfer coefficient, K_La, are the most commonly used parameters allowing to asses gas transfer rate between different gases and liquid in two-phase (gas bubbles – liquid) or three-phase (gas bubbles-liquid-solid) suspensions in all aerobic processes and comparison between different types of bioreactors. Mixing intensity is a very important factor in all aerobic heterotrophic biological processes, as well as in photobioreactors. Good mixing conditions are required there in order to retain homogeneity of cell suspension, eliminate thermal gradients, reduce the degree of mutual light shading of cells, enable uniform nutrient distribution and improve gas-liquid exchange, which is especially important in high concentrations of biomass (Bergmann and Trosch, 2016; Janvanmardian and Palsson, 1991). In such a case the main problem is transport of CO_2 to the cells and removing oxygen in order to avoid its inhibiting local concentrations. Bosca et al. (1991) many years ago proved that the productivity of algae in the outdoor system is higher in the well-mixed culture than in the same system under the same conditions but without mixing; the same effect in the laboratory scale was reported by Sobczuk et al. (2006). In PBRs hydrodynamics determines not only the energy demand and the mixing conditions, but also directly influences cell physiology by moving – faster or slower – the phototrophic organisms through the strong light gradients and cyclic light-dark fluctuations. As reported by Grobbelaar et al. (1996), intensive mixing increased growth rate of microalgae in stirred tank photobioreactors even if mass transfer was not the limiting factor. These problems are of the greatest importance in tubular photobioreactors, which may have length of hundreds of meters and tube diameters up to 5 cm (Jacobi et al., 2010).

Mixing intensity in photobioreactors is usually expressed by the value of mixing time, i.e. time required to achieve an assumed level of mixture homogeneity (usually 95% (Chisti, 1989) or 99% (Brown et al., 2004)) after injection of a small volume of a tracer solution. In many types of PBRs there is no mechanical stirrer, but good mixing is achieved by intensive aeration and bubbling of gases in the liquid mixture (bubble or air-lift systems) or by pumping the cell suspension along tubular PBRs with sufficient rate, possibly in the turbulent range of liquid velocity (Hase et al., 2000; Lu et al., 1995). However, excessive mixing may cause cell damage because of too high shear stress. Posten (2009), pointed out that the liquid velocity greater than 1 m/s would produce microeddies, which could potentially damage the cells; the liquid velocities of 0.2–0.5 m/s were recommended. Hence, especially in tubular or column photobioreactors liquid velocity and/or circulation time are the important parameters characterizing the system.

As may be seen from the above short introduction, microalgae cultivation in closed photobioreactors and optimization of such processes receive growing attention all over the world and many authors have been reporting development of many different types of PBRs. Nevertheless, there is still demand for experimental data on hydrodynamics and mass transfer in specific apparatus.

The aim of this paper is to present the hydrodynamic and mass transfer characteristics of a laboratory helical-tube photobioreactor Biostat PBR-2S (Sartorius Stedim, Germany). The bioreactors of that type are commercially available and are used in many laboratories in Poland and worldwide (Kwietniewska et al., 2012), but there is still no comprehensive data on their performance in cultivations of phototrophic species, photosynthetic efficiency and other practical data. The authors hope that the presented results may help other investigators analyze and understand the influence of the specific process conditions in the tested PBR, especially in thermophilic cyanobacteria cultivations.

3. EXPERIMENTAL SETUP AND METHODS

3.1. Laboratory photobioreactor PBR-2S

The laboratory bench-scale photobioreactor (PBR) consists of three main elements: a culture vessel with sensors and equipment mounted in the top lid, photosynthetic module in a shape of helical glass tube with illumination unit and the basic control unit operated with a touch screen. The glass culture vessel is placed

inside the photosynthetic module and connected to it by means of silicone tubes. The liquid circulation in the bioreactor is forced by peristaltic pump. The experimental setup ready for testing is presented in Fig. 1.

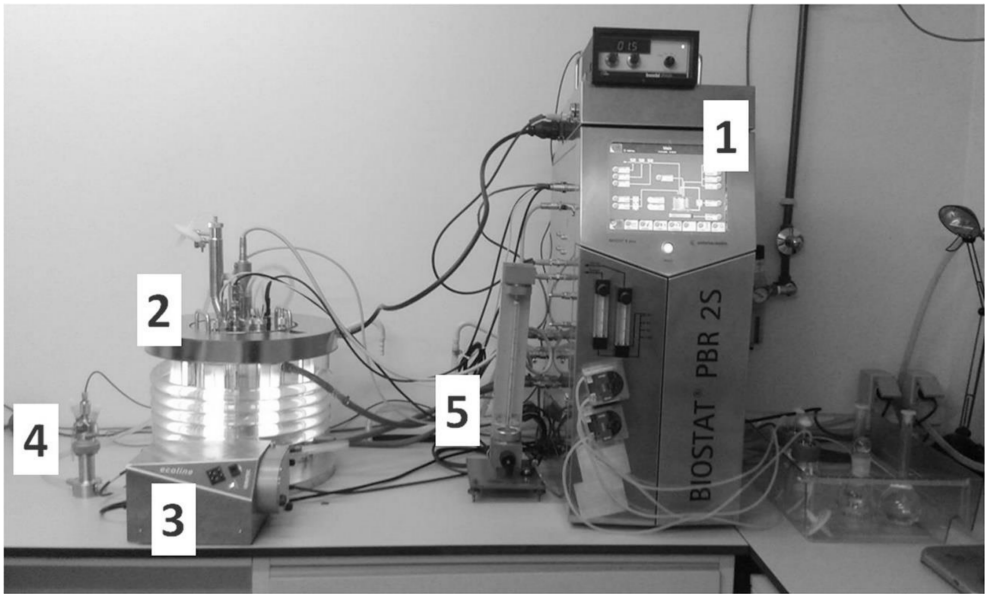

Fig. 1. The complete experimental setup ready for hydrodynamics investigations; 1 – central control unit, 2 – bioreactor with the photosynthetic module, 3 – peristaltic pump, 4 – dissolved CO_2 probe, 5 – rotameter for gases

The culture vessel of total volume 1.6 dm^3 and working volume of 0.4–1.0 dm^3 has a form of a double-jacket glass vessel with internal concave bottom and stainless steel lid with handles for easy transport. In the lid there are different ports for temperature, pO_2, pH, turbidity and – optionally – redox sensors. There is also an exhaust gas cooler, limiting evaporation of water from the bioreactor, which is important in long processes run in higher temperature, as was the case in the performed experiments. Temperature control in the apparatus is possible via a thermostat system located inside the control unit and the double jacket of the vessel. There is no stirrer mounted in the vessel, so mixing in the liquid phase is due to the medium pumping by a peristaltic pump and as an effect of bubbling of the gas in the glass vessel, which may be introduced by the porous microsparger mounted at the bottom of the vessel. In the central axis of the glass container, in the usual place for a stirrer, a triple lid port adapter with connectors for feeding the medium into the photosynthesis module, return flow from the module and for introducing medium or harvesting the product is mounted. The complete culture vessel is placed coaxially inside the cylindrical photosynthesis module. This includes a borosilicate glass pipe of diameter 20/24 mm, in spiral (helical) shape of external dimensions ca. 350 × 200 mm and volume about 1.7 dm^3 and a removable illumination unit consisting of 8 fluorescent lamps (Osram Dulux L, Osram GmbH, Germany) of cool light (T = 4000 K), each of 18 W power and giving luminous flux of 1200 lm. The photon output of the light panel may be estimated to be about 100 μmol/s on the basis of data included in the paper of Nelson and Bugbee (2014). This value is not very high, but it is only a rough estimation and the bioreactor is of a small laboratory scale. Luminous intensity may be controlled smoothly in the range of 0–100% of the maximum power via basic supply unit. A set of fluorescent lamps is mounted in the gap between the culture vessel and the helical tube, so their light may reach growing cells present in the vessel and, at the same time, flowing along the tube.

The control/supply unit enables control of many basic operational parameters (temperature, pO_2, pH, rotary speed of the circulating pump, light intensity) and, controlled by the level of dissolved oxygen and pH value, supply of up to four gases (air, N_2, O_2, CO_2) which may flow into the headspace of the vessel

(overlay line) or at its bottom through the microsparger (aeration line). The biomass concentration changes in course of the test cultivations were observed on-line by means of a turbidity sensor (Mettler Toledo, Switzerland) mounted also in the upper lid of the vessel. Moreover, there was a pCO_2 probe (Mettler Toledo, Switzerland) mounted outside the vessel in a hose leading the liquid from the culture vessel to the bottom of the photosynthetic module. Due to small dimensions of the vessel there was no other possibility of placing this probe in the apparatus.

3.2. Materials and experimental methods

During the investigations the central control unit of the PBR was connected to a computer and values of all measured parameters were collected and stored every 1 second by a data acquisition and control software MFCS/win (Sartorius, Germany), dedicated for Biostat bioreactor family.

Hydrodynamics characterization of the liquid phase

All hydrodynamic parameters were investigated in tap water. The photobioreactor was filled with 3 dm^3 of the liquid, so that the vessel contained 1 dm^3 (total working volume) and the rest circulated along the photosynthetic module of 1.74 dm^3 volume and connecting hoses. The liquid flow along the photosynthetic module was forced by a peristaltic pump. The rotary speed of the pump may be controlled by the central control unit or by the external computer software MFCS/win. The relation between the pump rotary speed and the flow rate of the liquid pumped depends on the diameter of the tubing; for the tubing used in all measurements and cultivations volumetric flow rate were determined experimentally by measuring the volume of the liquid collected in a period of at least one minute with certain rotary speed of the pump.

The liquid circulation velocity and time of mixing were determined by the tracer method using the HCl solution and a pH probe (Hamilton Easyferm Plus) as a sensor. Tracer of 10 ml volume was rapidly injected (using a syringe) as a pulse at the inlet of the liquid to the photosynthetic module and sometimes, in order to compare the data, into the vessel through the opening in the top lid. From the graph of the dynamic response of the system after impulse disturbance, the circulation time was read, as a distance between two consecutive peaks of the waning sine curve. Then, measuring the total length of the liquid path (length of one circulation loop) it is possible to calculate mean liquid velocity in a photosynthetic module. At the beginning of these experiments there was no aeration of the liquid applied, and then the influence of the volumetric flow rate of the air on the liquid circulation velocity was determined. The mixing time in the system was obtained as a total time range between the moment of a tracer injection and the complete flattening of the response curve.

Mass transfer investigations

The volumetric oxygen transfer in the liquid phase, k_La, was determined by the dynamic (gassing-in) method. As a so called "zero gas" pure nitrogen was applied, a stream of air was used for liquid aeration. Experiments were conducted firstly in tap water and then in the cultivation medium containing living cyanobacteria cells, at the end of test cultivations. Since the dynamic method was applied it was important to check dynamic parameters of the oxygen electrode. It was done outside the bioreactor by repeatedly placing the oxygen probe firstly into the flask with "zero" liquid (i.e. water with oxygen removed by pure nitrogen) and then into the flask with fully aerated liquid. For identification of time constant of the oxygen probe as well as of the k_La values from dynamic measurements, the appropriate self-written computer software was applied (Gluszcz et al., 1988).

The same procedure was used to determine volumetric CO_2 transfer coefficient, but in this case pure carbon dioxide was applied for gassing-in the liquid phase. The dynamic parameters of CO_2 probe were also identified before experiments in the bioreactor.

Test cultivations

For the final and the most reliable evaluation of usefulness of the tested PBR for specific applications, it was desirable to perform real processes with the given microorganisms in the apparatus. Hence, at the end of this study a series of test cultivations was conducted under different conditions of illumination.

In the investigated photobioreactor light intensity of the photosynthetic module may be controlled by the central control unit in the range 0–100% of the maximum power of fluorescent lamps. However, for the bioreactor evaluation it is important to know what the real luminous intensity reaching growing cells is. Hence, before starting test cultivations the relation between the relative power of the fluorescent lamps (expressed in % of maximum) and the absolute luminous intensity at the internal surface of the photosynthetic tube was found. It was obtained by means of the lux meter (Extech Instruments LT300) placed in the gap between lamps and the helical glass module. For each value of the relative power of lamps light intensity was measured in four points placed symmetrically around the helical module in the half-height of it. During these measurements all other light sources in the laboratory were switched off.

The microorganism used in test cultivations was *Synechococus sp.* PCC 6715; it is thermophilic cyanobacteria purchased from the Culture Collection of Cyanobacteria of the Pasteur Institute in Paris, France. The strain was originally isolated from Yellowstone National Park (USA) hot springs (Dyer, and Gafford, 1961). The inoculum for the bioreactor was prepared in the 0.5 dm^3 flasks containing the inorganic Blue – Green nutrient medium BG-11 optimized for cyanobacteria, according to UTEX algal media recipes (*https://utex.org/products/bg-11-medium*). The inoculated flasks were placed in the shaking cabinet Sartorius BS-1 for 10 days, in the temperature of 45 °C. Then 260 ml cell suspension was transferred into the PBR-2S bioreactor containing 2.6 dm^3 of the non-sterile basic medium BG-11 enriched with 270 ml solution of 1M $NaHCO_3$ as inorganic carbon source. When indicated in the text, an equivalent amount of distilled water was added instead of carbonate, to achieve the same starting volume of the medium. Then all the bioreactor controllers were activated with given values of the crucial operating parameters and the particular cultivation process started. Initial biomass concentration after inoculation was about 0.2 g/dm^3. As mentioned earlier, the values of all process parameters were collected and stored by the external computer software Sartorius MFCS/win remotely controlling the cultivation process via the central control unit of the bioreactor. All the cultivations were run at the same temperature of 45 °C and with the liquid volumetric flowrate in the PBR of 2.8×10^{-5} [m^3/s].

To evaluate fully the applicability of the investigated PBR for phototrophic processes, series of test cultivations were performed with different light conditions. In the first set of cultivations continuous light of 20, 30, and 40% of the maximum lamp power was applied. Then in the next cultures the control profile for the light controller was programmed and started, enabling automatic switching lamps between 0% of the light (the simulated "night" for the cells, in these experiments lasting 8 hours) and the given value of the "day" light (20, 30 or 40%, by 16 hours). The standard light controller of the central PBR-2S unit can not set control profiles, but it may be obtained using MFCS program or even easier, by means of a simple mechanical or electronic switch of the external power supplying the photosynthetic module.

In all test cultivations the main parameters for evaluation of the PBR performance were the biomass density and the growth rate of the strain. Changes of biomass density in course of the cultivations were read and stored on-line by means of a turbidity sensor Fundalux II (Sartorius, Germany), measuring the attenuation of light intensity brought about by the process medium with a single channel absorption photometer

(Optek-Danulat GmbH, Germany). At the start of each process the OD (Optical Density) meter was calibrated to "0" value just after the inoculation of the culture. Besides, biomass density was precisely measured off-line (once a day) using a double beam UV/VIS spectrophotometer T80+ (PG Instruments, Great Britain). Chosen samples were scanned in the range of 300 nm to 800 nm; for the rest the absorbance was measured only in two characteristic values of wavelength, i.e. 680 nm and 442 nm, for which the maximum absorbance of chlorophyll contained in cyanobacteria cells was reported (Lichtenthaler and Buschmann, 2005). Dry organic biomass (DOM) for each sample was determined using the earlier prepared calibration curves for the values of wavelength, according to the Polish norm PN-EN 12880:2004 and the value of Volatile Organic Solids (VOS) according the PN-EN 12879:2004 norm. Collected data enabled comparison between on-line turbidity measurements and real values of the corresponding biomass density. Biomass productivity, P, (g/dm^3/day) and final net increase of biomass concentration during the culture period was calculated as follows:

$$P = \frac{X_t - X_0}{t_x - t_0} \tag{1}$$

where $X_t$is the biomass (DOM or VOS) concentration (g/dm^3) at the end of a considered range of time, t_x is the time of the cultivation (one day or the total time of the culture) and X_0 is the initial biomass concentration at t_0, i.e. just after inoculation.

4. RESULTS AND DISCUSSION

4.1. Hydrodynamics of the liquid phase

Figure 2 shows typical dynamic response of the PBR-2S system to the impulse disturbance caused by injection of the small amount of HCl at the inlet (i.e. at the bottom end) of the helical tube. As the pH electrode was placed in the vessel there is a time delay between the moment of injection of the tracer and the first response of the pH electrode. This time interval is equal at least to the residence time of a liquid element in the tube, V_t/Q_L, where V_t is the internal volume of the tube and Q_L – volumetric flowrate of the liquid, as the photosynthetic module may be assumed to be a plug flow reactor.

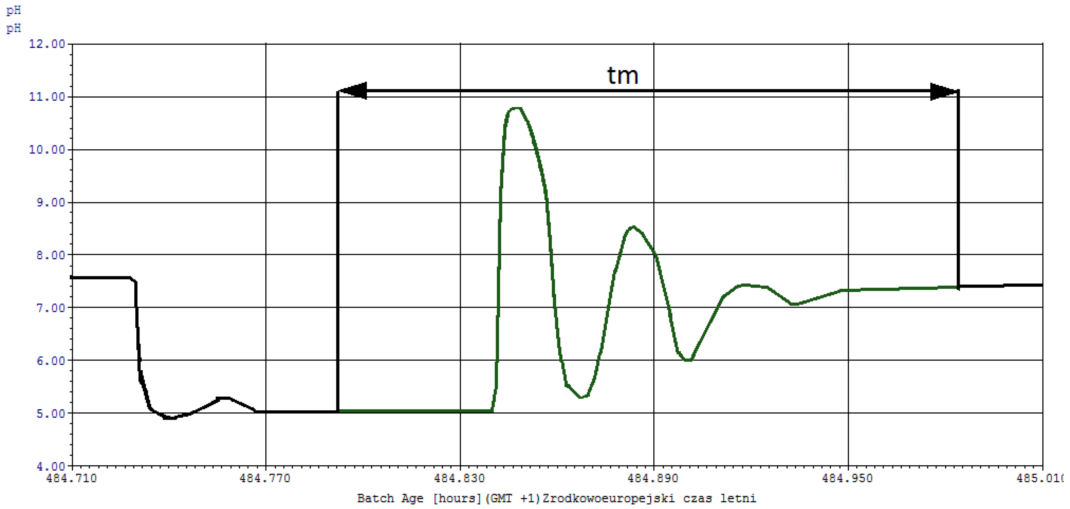

Fig. 2. Typical changes of the pH in the PBR-2S system after injection of a small amount of HCl solution as a tracer ($Q_L = 2.8 \cdot 10^{-5}$ m^3/s, rotary speed of the pump 40%, vessel not aerated)

The Reynolds number for the liquid flow in the glass tube in the entire investigated range of the pump rotary speed may change from 420 to 1760 rpm, so definitely the laminar flow prevailed there during

experiments. If it were necessary to assure turbulent flow in the tube, Q_L would have to be raised at least to the value of $5 \bullet 10^{-5}$ m^3/s, which in the investigated apparatus corresponds to the pump rotary speed of ca. 75% of the maximum. The mixing intensity in the vessel may be much higher, but only when the liquid is aerated; in this case it may be roughly assumed that the liquid phase in the vessel is well (ideally) mixed. Unfortunately, the residence time of cells in the photosynthesis tube of the PBR-2S bioreactor is almost twice higher than that in the well mixed and aerated vessel, so if mixing in the tube is poor, as is the case in laminar flow, the low mass transfer rate and prolonged mutual light shading of the significant part of the cell population obviously must have negative influence on the photosynthesis and biomass growth. In Fig. 3. the relation between circulation time (or mean total time residence of a liquid) in the apparatus and the volumetric flow rate of the medium, Q_L, is shown.

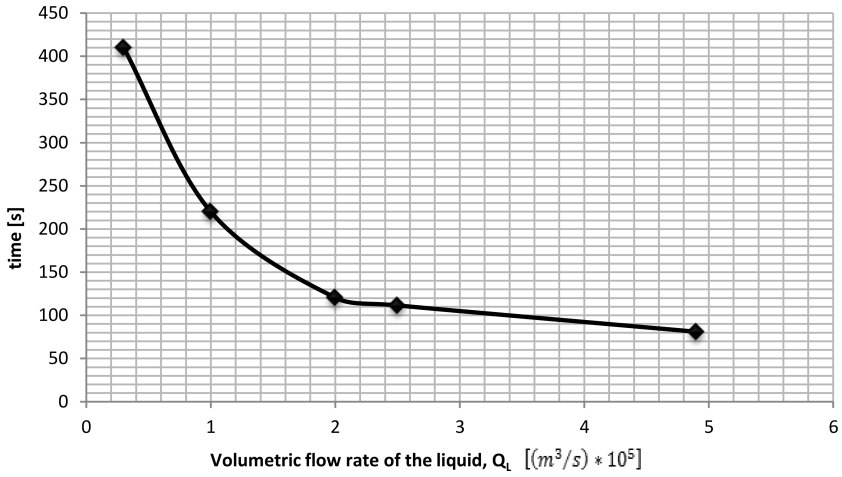

Fig. 3. Circulation time in the PBR-2S in relation to volumetric flow rate of the liquid

Analogous experiments with simultaneous aeration of the liquid in the vessel with different flowrates of the gas showed that the circulation time in the system did not depend on the aeration intensity. Moreover, air bubbles were not sucked from the vessel into the photosynthetic module. This may negatively influence the availability of CO_2 in the photosynthetic module if the gas were to be the only carbon source for cells.

Mixing time determined experimentally in the PBR-2S was relatively high, especially when the liquid in the vessel was not aerated (Fig. 4). This is caused by the specific construction of the bioreactor: the only properly aerated and, in consequence, well-mixed part of the liquid in the whole apparatus was about twice smaller than that in the photosynthetic tube of ca. 7 m length, where the laminar and almost plug

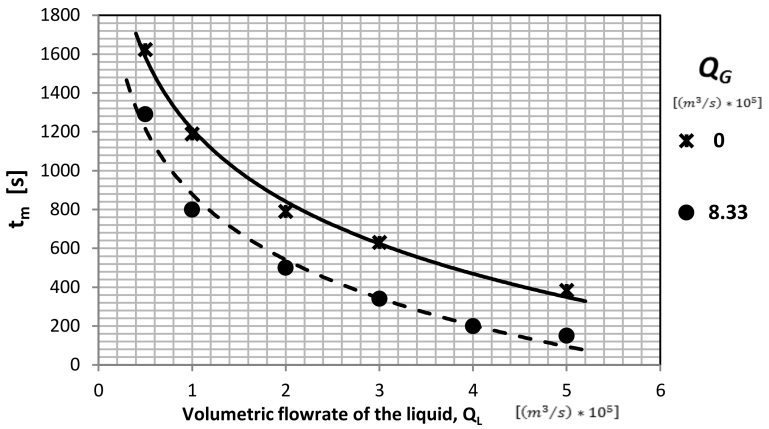

Fig. 4. Mixing time, t_m, vs. Q_L in non-aerated and intensively aerated bioreactor

flow prevails. As may be concluded from Fig. 4, even intensive aeration of the vessel does not dramatically decrease the mixing time. Hence it does not assure better mixing conditions in the photosynthetic module.

4.2. Mass transfer coefficients of O_2 and CO_2

Before starting gassing-in experiments with O_2 and CO_2 the dynamic characteristics of the probes were checked. The identification of data obtained in experiments with probes showed that both electrodes may be considered as inertial systems of the second order. Estimated values of their time constants are gathered in Table 1.

Table 1. Time constants of pO2 and pCO2 probes as inertial systems of the second order

	T_1 [s]	T_2 [s]
Oxygen probe	24.5	6.26
pCO_2 probe	84.3	0.19

Taking into account that the time constant of the gassing-out process in the case of water aeration was greater than 75 s under any conditions, and for CO_2 exceeded 100 s, the dynamics of the electrodes did not influence (delay) significantly the response of the bioreactor system to the stepwise disturbance at the gas inlet to the vessel. However, the estimated time constants of the probes (Table 1) were applied in the calculations of O_2 and CO_2 mass transfer coefficients, as the used software enabled this.

A dependency of k_La of oxygen in water on the intensity of the liquid aeration and water flow rate in the system is presented in Fig. 5. The oxygen transfer coefficient increases significantly and almost linearly with the increasing inflow rate of the air but the rising liquid circulation rate has much lower influence, especially at higher values of the latter.

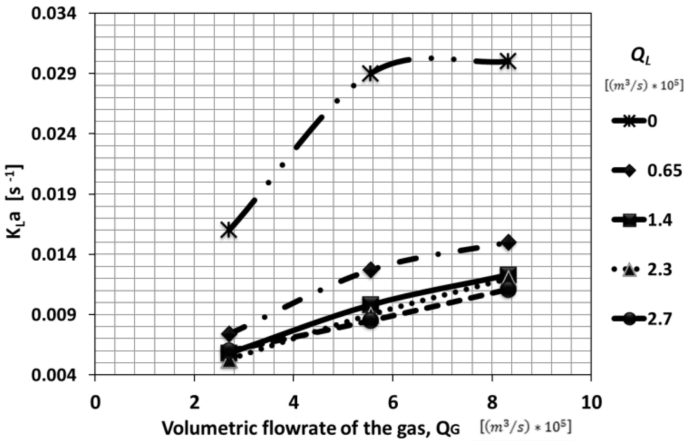

Fig. 5. Dependence of the $(k_La)_{O2}$ in water on aeration intensity and liquid circulation rate

The unusually high values of the $k_La$were found for the stopped circulation of the liquid ($Q_L = 0$). In such a case intensive aeration and oxygen absorption in water take place only in a small liquid volume in the vessel and ideal mixing conditions may be assumed. In consequence, the mixing intensity influencing the k_L coefficient and air bubbles dispersion and dissipation enhancing the interfacial area, a, are much higher than those in the liquid flowing along the tubular part of the device, which is about 7 meters long. As seen in Fig. 5. the effect is visible for Q_L below 1.0×10^{-5} m^3/s ($u_L < 3$ cm/s) and disappears for higher circulation rates.

The correlation of all oxygen transfer coefficient values determined for different aeration rates vs liquid circulation rate shows that k_La decreases slightly with increasing flow rate of water (Fig. 6), but the effect is not significant in the investigated range of Q_L. These data are consistent with the results of other authors obtained in tubular photobioreactors (Babcock et al., 2002; Razzak et al., 2016).

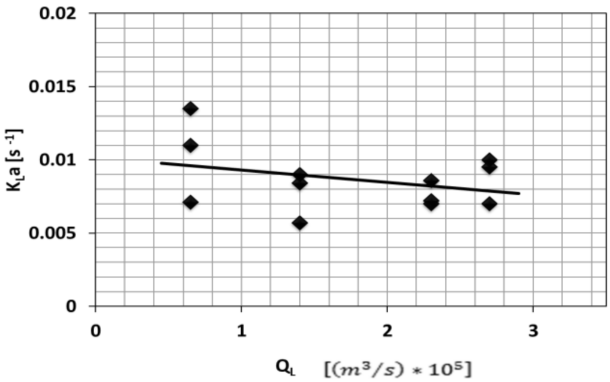

Fig. 6. Influence of liquid circulation rate on k_La of oxygen in water in PBR-2S

Carbon dioxide is more soluble in water than oxygen, but has lower molecular diffusivity; that is why the mass transfer coefficient values of CO2 which may be found in the literature are either slightly lower or equivalent to that of oxygen. Baquerisse et al. (1999), proposed a relation to calculate k_La coefficient of CO_2 in water on the basis of the k_La of oxygen:

$$(k_La)_{CO_2} = \sqrt{\frac{D_{O_2}}{D_{CO_2}}}\,(k_La)_{O_2} \tag{2}$$

but the rate and mechanism of the absorption process of CO_2 in pure water, sodium carbonate and alkaline solutions (e.g. microalgae cultivations broth) may differ significantly. Taking into account only diffusional theory to explain carbon dioxide absorption in sodium carbonate and alkaline solutions is inadequate and it is necessary to consider also the chemical reaction rates of carbon dioxide in solvents. The gas-liquid interfacial mass transfer of carbon dioxide involves at least 8 consecutive reactions and three of them are so slow that they may significantly affect the total absorption rate of the process (Kazim, 2012):

$$CO_2(\text{gas}) \leftrightarrow CO_2(\text{dissolved}) \tag{3}$$

$$CO_2(\text{dissolved}) + H_2O \leftrightarrow H_2CO_3 \tag{4}$$

$$CO_2(\text{dissolved}) + 2OH^- \leftrightarrow CO_3^- + H_2O \tag{5}$$

Due to slow reaction rates in the liquid phase, the driving force across the interface becomes small, which reduces the absorption coefficient of CO_2. The lowest value of absorption coefficient of CO_2 was observed in a dilute sodium carbonate solution as compared to water or alkaline solutions because the excessive presence of carbonates further slowed down the reaction between CO_2 and the solution. If CO_2 is absorbed in an alkaline solution, the excessive quantity of OH^- ions made the presence of HCO_3^- ions negligible, and this caused the reaction (5) to occur rapidly. Hence, higher absorption coefficient values of CO_2 have been reported in alkaline solutions than in water and carbonate solutions. The role of chemical reactions in the process of the CO_2 absorption is also the reason that the rate of gas-liquid mass transfer is temperature dependent – the higher absorption rate of CO_2 was observed at higher temperatures (Kazim, 2012). In pure water the maximum absorption rates were reported at the temperature of 70 °C.

Taking into account the above discussion, it may be assumed that it is the liquid-phase mass transfer resistance (liquid-side film) which is controlling carbon dioxide absorption in water solutions, similarly as oxygen mass transfer (Doran, 1995; Doucha et al., 2005; Kordac and Linek, 2008). Also, it is important to

note that when there is mass transfer of carbon dioxide from the gas phase to the liquid phase (water), out of all the dissolved species of carbon dioxide, that may be predicted as an effect of chemical reactions, only dissolved gas carbon dioxide itself is responsible for carbon dioxide mass transfer through the gas-liquid interface. In fact, the concentration of dissolved CO_2 is three orders of magnitude more than carbonic acid (H_2CO_3). Therefore, when the mass transfer coefficient of CO_2 is to be determined, the concentration of only dissolved carbon dioxide is taken into account (Hill, 2006; Royce and Thornhill, 1991).

In Fig. 7 the dependence of carbon dioxide overall volumetric mass transfer coefficient on the gas flow rate and liquid circulation rate in PBR-2S is given. Generally, the values of $(K_La)_{CO2}$ are slightly lower than those for oxygen absorption under the same conditions, which is consistent with the observations of other authors in different systems (Fernandes et al., 2014; Razzak et al., 2016; Ugwu et al., 2003). The values of K_La of CO_2 in water increase significantly (about 4 times) with the rising flowrate of the gas, but the liquid velocity has no visible effect. Only for the lowest water circulation rate and the highest gas flow rate a slight enhancement of K_La may be observed, which is probably caused by the same reason that in experiments with oxygen: very good mixing in a relatively small vessel. In experiments with CO_2 absorption it was not possible to stop liquid circulation in the system ($Q_L = 0$), as was the case in water aeration investigations, because the CO_2 probe is placed outside the vessel and in order to collect changing pCO_2 values the liquid flow through the probe container must be maintained all the time.

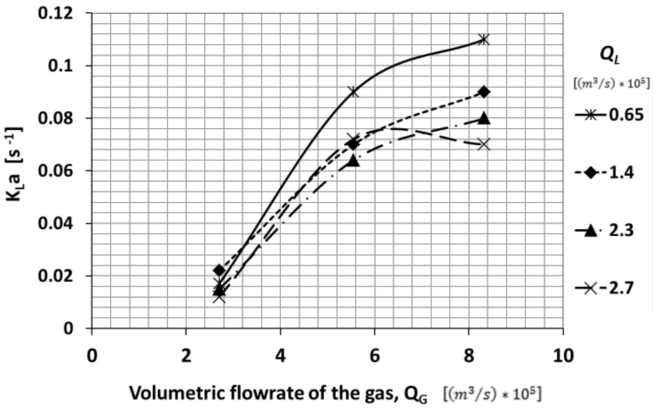

Fig. 7. Dependence of the $(K_La)_{CO2}$ in water on the gas flow rate and liquid circulation rate

At the end of the mass transfer investigations the k_La values of oxygen and carbon dioxide in culture suspensions with living cells (at the end of test cultivations) were determined. The next three figures (Figs. 8–10) allow to assess the overall effect of nutrient component and cell presence on the mass transfer rate of both gases in the cultivation medium.

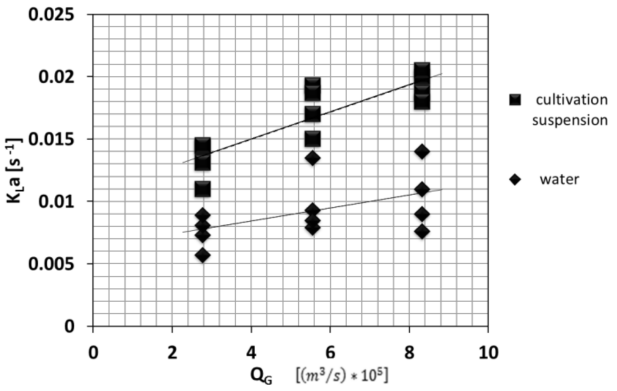

Fig. 8. Dependence of the oxygen transfer coefficient $(k_La)_{O2}$ in water and cultivation medium on the gas flow rate

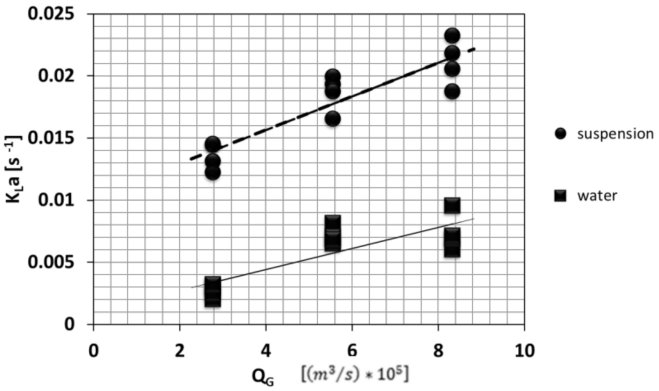

Fig. 9. Dependence of the CO_2 transfer coefficient, $(K_La)_{CO2}$, in water and cultivation medium on the gas flow rate

The effects of solids on gas-liquid mass transfer in three-phase systems and the respective physical mechanisms are not yet totally clarified. Results available in the literature, showing the influence of solid loading on liquid side mass transfer coefficient, k_La, in three-phase agitated or air-lift systems are sometimes contradictory (Mena et. al, 2011; Mills et. al, 1987; Tobajas et. al, 1999). Generally, the majority of authors reported that in bioreactors mixed by a gas stream, i.e. usually bubble columns and air-lift bioreactors, the solid loading showed complex effect on the k_La of oxygen: for low concentration of solids ($\leq 3-5$ vol.%) the mass transfer coefficient increases with the solid content and then may decrease when solid addition is higher. The main reason which may be pointed out for the enhancement of k_La in low solid content three-phase systems is that small solid concentrations of small particles do not change significantly liquid viscosity but improve surface renewal and cause micro-disturbances in the liquid film, increasing liquid-side mass transfer rate and thus k_La. The second reason may be associated with the presence of fine particles in the liquid film at gas–liquid interface which may hinder the coalescence of bubbles and in effect increase the gas–liquid interfacial area. But if the solid concentration is high enough to increase the viscosity of the suspension, surface renewal and mobility diminishe which results in reduced k_L.

Figure 10 provides a comparison of experimental mass transfer coefficients of O_2 and CO_2 in cultivation medium. Fig. 10 shows that irrespective of complex results of the presence of living microalgae cells as the third phase in the cultivation medium and different effect of the medium components (e.g. salts and/or carbonates) on solubility and mass transfer rate of oxygen and especially carbon dioxide in the solution, the overall changes of experimental k_La coefficients in the suspension for both gases are very similar. Increase of CO_2 mass transfer coefficient in the medium in comparison to water is slightly higher in the whole range of the applied volumetric flow rates of the gas than that of oxygen and the difference rises

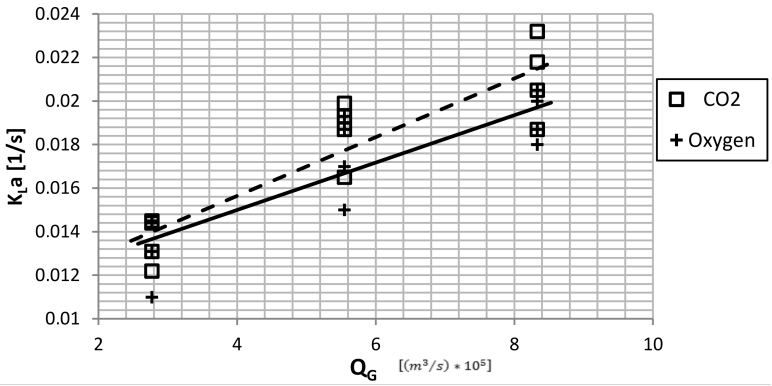

Fig. 10. Comparison of the dependence of O_2 and CO_2 mass transfer coefficients in the cultivation suspension on the gas flow rate

with Q_G enhancement. This observation confirms the earlier discussed statement that the process of CO_2 absorption in water solutions is controlled by the liquid-side film resistance, irrespective of high solubility of the gas in water. Moreover, in long tubular bioreactors the possibility of CO_2 limitation of the growth rate along the flow path is equal to the probability of O_2 inhibitory concentrations, as the mass transfer coefficients of both gases in the cultivation medium are almost the same. Due to mass transfer limitations, at the vessel (in the small liquid volume), which in the investigated PBR is the only aeration zone, a sufficient concentration of dissolved carbon dioxide (DCD) may be achieved. However, it may decrease quickly on the way along the not aerated tube; even if the DCD limitation conditions could not be reached, such fluctuations of concentration gradients usually are not advantageous for the living cells. And possible inhibiting concentration of toxic oxygen can occur after only 1 minute of residence inside a tube without gas exchange (Posten, 2009).

4.3. Test cultivations

The aim of the test cultivations was the final evaluation of the performance of the PBR-2S photobioreactor in real cultures. As light intensity and cycles are crucial factors in cultivation of phototrophic organisms, the test processes were focused on the effect of light intensity and photoperiod on biomass productivity.

The results of measurements of absolute luminous intensity at the internal surface of the photosynthetic module corresponding to the relative power of the lamps are presented in Fig. 11. For the applied fluorescent lamps and light controller this relation is almost linear above the relative power of 20% of the lamps.

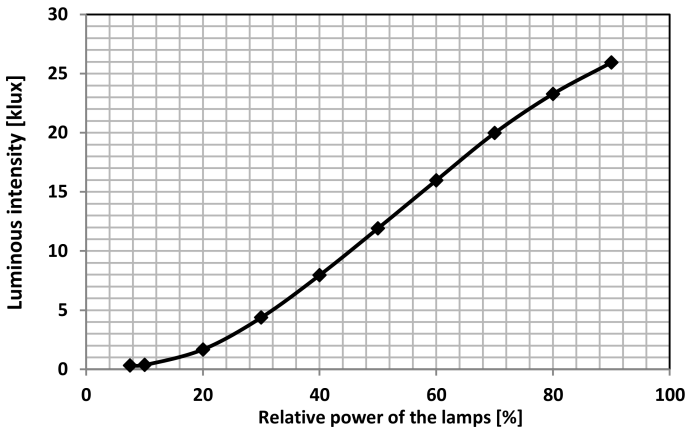

Fig. 11. The relation between the absolute luminous intensity at the internal surface of the photosynthetic module and the relative power of the lamps

Figure 12 shows results of cultivations run under different light conditions. The cyclic light/dark changes simulating the natural outdoor conditions of day and night (16 hours of a "day" and 8 hours of a "night") enabled to achieve significantly higher biomass productivity than constant light of the same intensity. The interesting observation is that the best results were achieved with the light of 40% of lamp power, in cultivations with periodical light as well as at constant illumination. Even now it is difficult to explain this effect, as 50% of lamp power give merely ca. 12.5 klux and the estimated total output of the photons is about 100–120 μmol/s. From these results it might be concluded that the higher illumination intensity, above 40–50%, caused photoinhibition of the growth of the used species. It might have been brought about by very close placement of the fluorescent lamps to the glass tube, short light path in the liquid (the diameter of the tube is only 2 cm) or so called "focusing" or "lens" effect in radial direction of the tube (Jacobi et al., 2010), but such conclusions require further investigations.

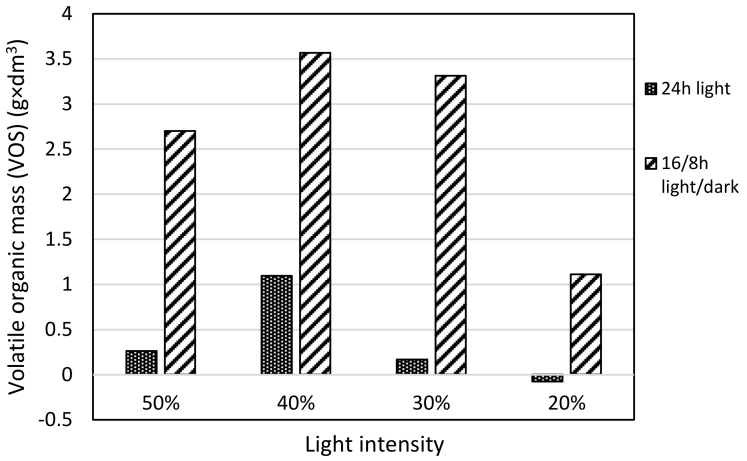

Fig. 12. The final net increase in biomass concentration under different light parameters

The highest final concentration of biomass obtained in test cultivations was 4.38 g/dm^3 of the total dry biomasss and 3.87 g/dm^3 of the VOS (Volatile Organic Solids). The net biomass increase, i.e. the final biomass concentration minus initial concentration just after inoculation, was 3.98 g/dm^3 of dry mass and 3.57 g/dm^3 of VOS and the highest productivity of biomass was 0.189 g/dm^3/day.

5. CONCLUSIONS

Taking into consideration all the obtained results it may be concluded that laboratory photobioreactor PBR-2S is a good tool for any investigations of microalgae cultivation processes. The hardware and software of the apparatus enables changes of the culture conditions in very wide ranges and may be further extended with dedicated computer software MFCS (Multi-Fermenter Control System) in a simplified Data Acquisition version (MFCS-DA) or full industrial version, capable of controlling from one to as-many-as you-wish bioreactors according to the official SCADA procedures.

The illumination parameters of the PBR-2S are sufficient for cultivation of phototrophic cells and it is possible to change freely the luminous conditions including control of the dark/light cycle in accordance to fully programmable light time and/or intensity profiles.

In test cultivations of thermophilic cyanobacteria growing at the constant temperature of 45 °C the highest final concentration of the total dry biomass reached the relatively high value of 4.38 g/dm^3 (3.87 of VOS), although the applied parameters (e.g. liquid circulation velocity, light period and intensity, age of the inoculum and others) were not earlier thoroughly optimized.

For the thermophilic strain it was not necessary to sterilize either the bioreactor or the medium and all cultivations were run in non-sterile conditions. The cleaning of the inside wall of glass tubes posed no problems even when the applied circulation rate of the medium in the photosynthetic helical module was below 3 cm/s.

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