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COMPLEX ENGINEERING APPROACH IN A PROCESS DEVELOPMENT ALGOLOGY LABORATORY

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Abstract: The process development laboratory in the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences (SAIM-BAS, Sofia) combines state-of-the-art in the fields of photobioreactors (PBRs) design, unit operation, microalgal physiology and nutrients media calculations based on the achievements of modeling and optimization frameworks. The laboratory was built in step-by-step procedures by applying knowledge database and expertise in the mentioned fields. Different concepts of PBRs design were realized and extensively studied. Microalgae of interest were purchased from one of the Czech Republic culture collections as well as from the Bulgarian Culture Collection in Plovdiv. Several new concepts of culturing microalgae were applied. Hence, the microalgal physiology of the green freshwater algal strain *Chlorella vulgaris* grown under different operational conditions involving varying light intensity, CO_2 and air loading, temperature (T), and pH was investigated. The ability of the strain to synthesize metabolites of interest was studied and the results were systematized, as well. Experiments were carried out for almost 2 years, during which changes in the PBR type, nutrient media and light intensity were carefully chosen. The responses of the microalgal cells to such changes were quantitatively analyzed. The obtained results showed that the selected strain can be used for studying the potential antimicrobial and antioxidant activities. Moreover, the published data on *C. vulgaris* and its capacity to produce bioactive metabolites were proven by our experiments and biomass of the strain was chosen to be used for food additive for broiler flocks.

INTRODUCTION

Microalgal cultures and their potential for industrial application in CO_2-fixation from flue gases for valuable metabolites production have received a lot of attention in recent years (Kroumov et al., 2016). They have been the focus of many scientists around the world mainly due to their important role in the atmospheric CO_2 remediation process. Microalgae can act both by preventing the CO_2 from going into the atmosphere through the production of biofuels, and by reducing the CO_2 levels from the air, through the photosynthesis process (Hinterholz et al., 2017). Green microalgae have been grown for commercial exploitation for several decades, with applications ranging from healthy food for human consumption, aquaculture and forage, to coloring agents, cosmetics and others (Skjånes et al., 2013). Some products of the green algae are secondary metabolites. They are derived by extraction from the biomass. The best-known examples are the carotenoids astaxanthin and β-carotene, which are used as coloring agents and for health-promoting purposes (Skjånes et al., 2013). β-Carotene is accumulated as droplets in the chloroplast stroma of *Dunaliella salina* cells, especially under stress culture conditions such as high levels of salinity, high temperatures, high light intensities, and deficiency of nitrogen sources as reported elsewhere (Kleinegris et al., 2011; Wichuk et al., 2014). The analysis of total carotenes of *Dunaliella salina* has revealed that the typical composition of the β-carotene synthesis is approximately 42% of all-trans β-carotene, 41% of 9-cis β-carotene, 10% of 15-cis β-carotene and 6% of other isomers (Ben-Amotz, 1980; Rigo Roso, 2015).

It is well known that a high yield of β-carotene by *Dunaliella salina* industrially culturing is performed under a high salinity environment in order to reduce contamination by other microorganisms.

Haematococcus pluvialis is the most favorable microalgal species for industrial-scale production of natural astaxanthin. The quantity of astaxanthin synthesized by H*aematococcus pluvialis* cells can reach up to 7% of dry biomass and 90% of its total carotenoids (Pulz and Gross, 2004; Raposo et al., 2015). *Haematococcus pluvialis* synthesizes astaxanthin as a secondary metabolite under extreme growth conditions such as nitrogen and phosphorus starvation, high solar intensities, salt stress and elevated temperature (Shah et al., 2016; Solovchenko, 2015). Astaxanthin primarily accumulates in the cytoplasm of *Haematococcus pluvialis* cells as a racemic mixture of mono- and di-esters (up to 97% of the total astaxanthin production). A minor fraction of it forms as free astaxanthin.

Scenedesmus obliquus is famous from previous studies for its high consumption of CO_2 and nutrient-deficient conditions trigger the accumulation of lipids in this microalgal species (Ho et al., 2010).

The purpose of our study was to monitor the production of biomass and biologically active substances under definite stress factors like nitrogen deficiency and high light intensity.

MATERIALS AND METHODS

Algae strain: The strain used in this study was *Chlorella vulgaris,* purchased from the Czech Republic culture collection (Fig .1).

Medium and culture conditions: The modified M-8 culture medium was used. The modifications were calculated and described previously (Kroumov et al., 2015). The composition is as follows: KNO_3 – 3 [g/L], KH_2PO_4 – 0.474 [g/L], $CaCl_2.2H_2O$ – 1.58 x10-3[g/L], $FeSO_4.7H_2O$ – 0.148 [g/L], $MgSO_4.7H_2O$ – 0.437 [g/L], $MnCl_2.4H_2O$ – 1.944 $x10^{-3}$ [g/L], $CuSO_4.5H_2O$ – 0.848 $x10^{-3}$ [g/L], $ZnSO_4.7H_2O$ – 1.19 $x10^{-3}$ [g/L]. Some experiments with different N-source were performed in order to maximize the biomass synthesis for the given working conditions.The inoculum was prepared in the incubation stage in small-scale PBRs with internal illumination (Figure 1). A culture volume (V) of 700 ml was used. Various colors of light – blue (λ= 450 - 495 nm), red (λ= 620 - 750 nm), green (λ= 495-570 nm) and white (λ= 380-750 nm, the wavelengths of the white light are combination of the wavelengths of all color lights in the visible spectrum that make up white ligh) were placed inside the PBRs (Fig. 2, 3 and 4). In the next step, the culture was transferred to the production stage in PBRs with geometrical volume (V_{geom}) of 3.3 – 3.5 L (Figure 2). The strain was cultured at room temperature between 24 and 28 °C. The internal temperature varied between 27 and 34 °C, Q_{air} was 0.05-0.2 v/v/min, whereas QCO_2 was 2-10%.

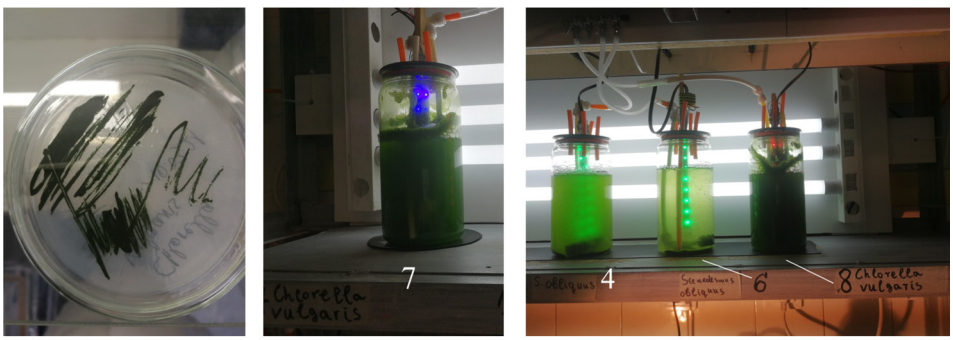

Figure 1. Culturing of Chlorella vulgaris - incubation stage, small scale PBRs with internal illumination

RESULTS AND DISCUSSION

The biomass samples were withdrawn from small PBRs with internal illumination with a variety of lights and at definite other physical factors (Fig. 1. and Fig.2, Table 1). The maximum biomass concentration achieved in the incubation stage was X=6.4 [g/L] for SC-PBR1 and X=5.5 [g/L] for SC-PBR2, respectively. These results are very promising because the geometry of the PBRs and the hydrodynamic working conditions were far from optimal in terms of fluid flow distribution and mixing. Flexibility, which the incubation stage offers for culturing microalgae, can be considered very innovative because many parallel kinetic experiments can be performed by saving research efforts, time and money.

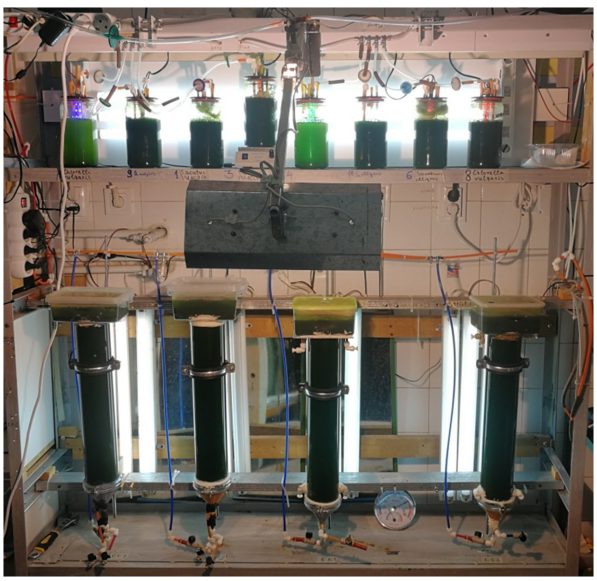

Figure 2. Air-lift and barbotage column PBRs,
inoculated with Chlorella vulgaris from cylinders with red light.

The analysis of biochemical composition (Table 1) of C. vulgaris showed the following:

•Moderate quantity of polyphenols was found in these 2 strains;

•Total carotenoids and lipids were in large amount. Variation of the production of biologically active substances was found when the same strain was grown under different colored lights inside the PBR;

•The largest amount of chlorophyll “a” and “b”, and lipids were found when cells were grown under internal blue light, then when they were grown under internal red light;

•Flavonoids were synthesized in small quantities under the given working conditions.

Table 1. Phytochemical composition of *Chlorella vulgaris* cultured in small scale PBRs under white (external) and blue or red (internal) light and influence of the operational conditions (including the light length and intensity) to the biosynthesis.

SC-PBRs	Chlorophyll a mg/g DW	Chlorophyll b mg/g DW	Total carotenoids mg/g DW	Lipids % (g/100 g DW)	PBR T
C. vulgaris, SC-PBR1	23.60	23.65	9.39	12.66	22.5 – 31.2
C. vulgaris, SC-PBR2	5.49	3.17	1.48	2.83	27.1 – 30.6

Legend: SC-PBR1 – small scale PBR, internal blue light; SC-PBR2 – small scale PBR, internal red light; Note: PBR T – the temperature inside the PBRs. The Nitrogen source of nutrients medium was supplied with KNO_3 at the beginning of cultivation. At the stationary phase the KNO_3 was exhausted which supported induction of synthesis of secondary metabolites and more particularly synthesis of total carotenoids.

CONCLUSIONS

A flexible modern engineering approach for PBR design based on computing fluid dynamics software (CFD) was used to create innovative constructions in a production stage. A multifunctional algology laboratory was created and its functioning was supported by a new approach to calculate nutrient medium recipes. Many combinations of stress factors were applied in order to find the maximum synthesis of BAC in an incubation stage. Preliminary results give us hope in the long run that the laboratory is going to play a crucial role in many process developments for the synthesis of desired metabolites. The application of different internal lights in PBRs strongly influenced the biosynthesis of certain groups of algal metabolites, thus playing the role of a key stress factor.

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Conflicts of Interest: The authors declare no conflict of interest.

Author contribution statement: A.K. planned and designed the experiments. A.B., A.K., M.M.Z., Y.I., M.K., T.K., D.Z.-D., V.B., R.G., S.R.-V., A.A. and H.N. performed the experiments. A.K., M.M.Z. and A.B. analyzed the data. A.B., A.K. and M.M.Z. wrote the manuscript.

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