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Research Article

The green alga *Dictyosphaerium chlorelloides* biomass and polysaccharides production determined using cultivation in crossed gradients of temperature and light

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The green microalga *Dictyosphaerium chlorelloides* was identified as promising microorganism for biotechnological production of exopolysaccharides (EPS). In stationary phase the culture suspension solidifies to thick gel, with very high viscosity and high content of EPS which may be interesting for many biotechnological applications. To develop cultivation protocol for maximum biomass/polysaccharide production, the optimum conditions for growth and polysaccharides production were determined in this study using the crossed gradient cultivation method. Temperature and irradiance requirements of *Dictyosphaerium chlorelloides* were evaluated by statistical analyses for growth rate/biomass, extracellular (EPS) and intracellular (IPS) polysaccharides contents in crossed gradients of temperature (4–45°C) and irradiance (2–18 W/m², 9.1–82.3 μmol/(m² s)). The maximum relative growth rate was observed at temperatures around 19.2°C and relatively low irradiances in range 2.6–11 W/m² (11.9–50.3 μmol/(m² s)). The maximum IPS production was observed at temperatures around 19.2°C and irradiance around 11 W/m² (50.3 μmol/(m² s)). The maximum production of EPS was observed at temperatures around 25.7°C and similar irradiances as IPS production. Due to temperature separation of growth and EPS production, development of cultivation protocol based controlled temperature manipulation is possible.

Keywords: Biomass / Crossed gradients / Irradiance / Polysaccharides / Temperature

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1 Introduction

Many cyanobacteria and green algae cells are surrounded by slimes, sheaths, and/or capsules made up of carbohydrates, which are produced and secreted outside the cell and are termed as exopolysaccharides or extracellular polysaccharides (EPS; [1–3]). These layers can be attached to the cell surface or they can be released into environment. The producing microorganism and environmental conditions affect the composition and amount of produced polysaccharides [4]. Synthesis of the EPS represents a stress-avoidance strategy for survival in hostile conditions [5,6], and the EPS production by microalgae and cyanobacteria is controlled by different environmental variables, such as

temperature, nitrogen concentration or irradiance, and their combinations [7–9]. The microbial EPS are of diverse types and are important for biofilm formation, symbiotic interactions or resistance to stressors such as exposure to increased UV radiation, oxidative stress or water deficiency [10–12].

The EPS from microbial sources are used in many biotechnological applications, for instance, in food production as various jelly compounds, in pharmaceutical industry for capsule covering or novel anti-tumor/viral drugs, for pigments suspension for textile making, as emulsifier and detergents in cosmetics, and so on [13, 14]. The sulfated polysaccharides produced by cyanobacteria interfere in the absorption and penetration of virus in to host cells and inhibit the transcriptase activities [15]. Anti-inflammatory effects in edema tests and immunosuppressant effects in delayed hyper-sensitivity response, and on phagocytic activity [16] as well as immunostimulation in viral infection [17] were proven for the EPS of green algae. In bioremediation, the negatively charged cyanobacterial and algal EPS or biofloculants bind positively charged heavy metals

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Abbreviations: dH₂O, distilled water; EPS, extracellular polysaccharides; IPS, intracellular polysaccharides

removing them from water environment [18]. For instance, the EPS of cyanobacterium *Anabaena spiroides* were found efficient in binding of Mn(II), Cu(II), Pb(II) and Hg(II) [19]. In agriculture, the EPS can be also applied for crops improvement and for the prevention of soil erosion. The cyanobacterial and algal EPS increase the size of soil aggregates due to entrapping and binding soil particles together. Large soil aggregates are heavier, with smaller surface/volume area, so their transport by wind or water is more difficult [20]. If there is a community cyanobacteria, heterotrophic bacteria, microalgae and fungi associated with the soil particles, it is term as biological soil crusts (BSCs). The BSCs are the only microbial ecosystems contributing to nutrient cycling in the hot or cold deserts [21]. In more favorable conditions, they support the growth of other soil microorganisms, increase soil water-holding capacity and organic matter content, and improve phosphorus availability and mobility [22].

Due to various important applications of cyanobacterial and algal EPS, it is necessary to search for novel sources of these EPS and optimize the conditions for their mass production. In nature, the EPS, and of course IPS, are produced when the cells are subjected to sub- or supra-optimal conditions in general [23]. These conditions are often genus and even strain-specific, and must be determined for each biotechnologically prospective strain [23], as they may include, for instance, nutrient availability like in case of *Botryococcus braunii* [24, 25], high salinity in case of *Dunaliella* sp. [26], age of the culture [27] or high irradiance in cyanobacteria [28]. These extreme conditions often results in growth rate reduction [23], therefore, multi-stage systems for production of EPS are proposed, with respect to the strain-specific requirements [23, 29].

One of such EPS sources seems to be freshwater green microalga *Dictyosphaerium chlorelloides*, with a world-wide distribution that is widely used for studies of evolutionary ecology focused on adaptation processes in extreme conditions [30–33]. To optimize conditions for the biomass (i.e. maximum growth rate) and EPS production by *D. chlorelloides* in future biotechnological applications, the alga was grown in crossed gradients of temperature and irradiance, allowing thus rapid and effective evaluation of growth requirements as well as determination of suitable conditions for IPS and EPS production.

2 Materials and methods

2.1 Microalgal strain

The green algae *Dictyosphaerium chlorelloides* strain Kováčik 1978/11 was obtained from the Culture Collection of Autotrophic Organisms (CCALA, strain no. 330), Institute of Botany of the Academy of Sciences, Třeboň, Czech Republic, and it was originally isolated from a fish pond near Třeboň town. The stock culture was kept in twice concentrated Zehnder medium (Z8 2×; [34]) at temperature of 22°C and irradiance of 10 W/m² (45.7 μmol/(m² s)). The Z8 2× medium (final volume of 1 L) consisted of 20 mL of NaNO₃ stock solution (46.7 g/L), 20 mL Ca(NO₃)₂ stock solution (5.9 g/L), 20 mL K₂HPO₄ stock solution (3.1 g/L), 20 mL MgSO₄·7H₂O stock solution (2.5 g/L), 20 mL Na₂CO₃ stock solution (2.1 g/L),

2 mL Fe-EDTA stock solution (2.2 mL HCl (35%) + 4.5 g FeCl₃·6H₂O + 4.65 g Na₂-EDTA in 250 mL dH₂O) and 2 mL Trace metal solution (0.31 g H₃BO₃ + 0.223 g MnSO₄·4H₂O + 0.003 g Na₂WO₄·2H₂O + 0.0088 g (NH₄)₆Mo₇O₂₄·4H₂O + 0.0119 g KBr + 0.0083 g KI + 0.0287 g ZnSO₄·7H₂O + 0.0154 g Cd(NO₃)₂·4H₂O + 0.0146 g Co(NO₃)₂·6H₂O + 0.0125 g CuSO₄·5H₂O + 0.0198 g NiSO₄(NH₄)₂SO₄·6H₂O + 0.0037 g Cr(NO₃)₃·7H₂O + 0.0035 g V₂O₅(SO₄)₃·16H₂O + 0.0474 g Al₂(SO₄)₃K₂SO₄·24H₂O in 100 mL dH₂O)

2.2 Experimental setup

A unit for crossed gradients of temperature and irradiance with 20 petri dishes of 9 cm diameter was used for cultivation (LABIO Co. Ltd. Prague, Czech Republic). A thick aluminum plate (60 × 80 × 4 cm) was continuously cooled at one side and heated on the opposite side of plate length. Stable temperature was precisely controlled by pulsing energy to the cooler and heater present on opposite sides of plates, resulting in precise, stable and linear temperature gradient. The temperature was measured by a resistant Gryf 310 (Gryf, Czech Republic) thermometer equipped with bead thermistor sensor [35]. Water was placed between the plate surface and bead to improve contact. Also, wet gauze was spread under glass Petri dishes over the entire plate area to improve the heat transfer between the dishes and plate. A polyethylene sheet with proper openings was placed to accommodate the dishes over wet gauze to reduce the evaporation. The cultivation area was covered with a transparent lid. The gradient of light was upright to the gradient of temperature and was irradiated by photosynthetic active radiation (PAR) through custom made LED panel of 80 × 35 cm (4000–4500 K; 2000 lm; 120 SMD 5050 Epistar, Epistar Taiwan). The irradiance was measured by PU550 equipped with modified PAR light sensor (Metra, Czech Republic), the irradiance values measured in W m⁻² were recalculated to μmol/(m² s) according to [36]) and the generated heat was removed by fan. The decreased irradiances at the sides were partly compensated by using reflective aluminum foils. The range of temperature and irradiance in the setup was 4–45°C (later real temperatures 12–40°C) and 2–18 W/m² (9.1–82.3 μmol/(m² s)), respectively. To prevent carbon limitation, CO₂ was supplied from a CO₂ cylinder to reach final concentration of 2% (v/v) in cultivation space. Suspension of homogenized algal cells of volume of 1 mL with final absorbance at 750 nm (*A*₇₅₀) ~1.0 was inoculated in each glass Petri dish containing 19 mL of Z8 2× medium. The Petri dishes were shaken thrice a day. A total 20 petri dishes were placed on the aluminum plate with culture of *D. chlorelloides* representing the 20 different conditions of temperature and irradiance and their growth were evaluated over a period of almost 500 h.

2.3 Measurement of biomass

Growth of *D. chlorelloides* was measured in 96-well flat-bottom plates using Varioscan Flash spectrofluorophotometric plate reader (Thermo Scientific, Finland). The *A*₇₅₀ values were converted into dry weight (DW, mg/mL) using a second-order polynomial function obtained from constructed calibration

curve ($R^2 = 0.9982$)

$$\text{DW (mg/mL)} = 0.6976 \times A_{750}^3 + 0.1453 \times A_{750}^2 + 2.2185 \times A_{750} \quad (1)$$

2.4 Growth rate calculation

The relative growth rate (μ ; d^{-1}) was expressed as a slope of linear regression of dry weight vs. time during the exponential phase [37].

2.5 EPS and IPS estimation

Carbohydrates content was estimated using anthrone reagent [38]. The anthrone reagent was prepared in amber bottle by dissolving 100 mg of anthrone (Sigma, USA) in 100 mL of 75% sulfuric acid. The mixture was kept in a water bath at 85°C to dissolve the ingredient completely, and then this solution was kept in cool, dark and dry place. Homogenous suspension of cells in stationary phase of volume of 0.2 mL was sampled and was diluted 5 times with distilled water making the final volume of 1 mL. The diluted cell suspension was centrifuged in K80 centrifuge (MLW, East Germany) at 2300 g for 20 min. Cell pellet was suspended in 1 mL of distilled water and the intracellular carbohydrates content was estimated by the same way as of EPS. For EPS, supernatant was taken in another test tube and 4 mL of anthrone reagent was added in each test tube. Tubes were kept in boiling water bath for 15 min covering the mouth of each tube with aluminum foil to avoid the evaporation. The tubes were cooled to ambient temperature. Absorbance at 620 nm (A_{620}) was taken in a Systronics 105 spectrophotometer (Systronics, India) against the reference containing 1 mL of distilled water with 4 mL of anthrone reagent. From the glucose standard curve, it was estimated that A_{620} of 0.1 was equal to $33.3 \mu\text{g/mL}$ of carbohydrate.

2.6 Statistical analyses

The statistical analyses were performed using Statistica 10 (StatSoft, U.S.A.) and Canoco 5 [39] softwares. The null hypotheses stated that there was no effect of temperature, irradiance and their combination on (a) growth rate, (b) IPS production, and finally (c) EPS production. Descriptive statistics, correlation analysis, two-way ANOVA and Tukey honest significant difference test were performed using Statistica 10 software, while redundancy analysis (RDA) was performed using Canoco 5 softwares. The results were considered significant for $p < 0.05$.

3 Results

3.1 Optimum temperature and irradiance requirements for growth

The μ of *D. chlorelloides* was affected significantly by temperature ($n = 120$, $F = 294.3$, $p < 0.001$), irradiance ($n = 120$,

Figure 1. Dependence of μ of *D. chlorelloides* on temperature and irradiance. (A) 2D area plot showing optimal and marginal growth conditions. (B) The categorized dependence of mean μ on temperature. (C) The categorized dependence of mean μ on irradiance. The error bars indicate 95 % confidence intervals. The letter indicates homologous groups recognized by Tukey HSD test for $p = 0.05$.

$F = 24.33$, $p < 0.001$) and combination of temperature $\times$ irradiance ($n = 120$, $F = 32.89$, $p < 0.001$). The maximum μ was observed at temperatures around 19.2°C and relatively low irradiances in range 2.6–11 W/m^2 (11.9 – $50.3 \mu\text{mol}/(\text{m}^2 \text{s})$; Fig. 1, Table 1).

3.2 Optimum temperature and irradiance requirements for polysaccharide production

The production of intracellular polysaccharides was affected significantly by temperature ($n = 60$, $F = 5109$, $p < 0.001$),

Table 1. Comparison of μ , IPS and EPS contents (mean $\pm$ s.d., $n = 5$) at individual maxima in *D. chlorelloides*

Maximum	Temperature ($^{\circ}\text{C}$)	Irradiance (W/m^2)/($\mu\text{mol}/(\text{m}^2 \text{ s})$)	μ (d^{-1})	IPS ($\mu\text{g}/\text{mL}$)	EPS ($\mu\text{g}/\text{mL}$)
μ	19.2	7.0/32.0	0.625 ± 0.032	1998 ± 47	639 ± 122
IPS	19.2	11.0/50.3	0.534 ± 0.027	2130 ± 16	702 ± 52
EPS	25.7	15.0/69.6	0.225 ± 0.017	1504 ± 27	1075 ± 24

irradiance ($n = 60$, $F = 188.5$, $p < 0.001$) and combination of temperature $\times$ irradiance ($n = 60$, $F = 141.8$, $p < 0.001$). The maximum IPS production was observed at temperatures around 19.2°C , as maximum μ . However, the maximum IPS production occurred at the upper irradiance optimum growth limit around $11 \text{ W}/\text{m}^2$ ($50.3 \mu\text{mol}/(\text{m}^2 \text{ s})$), probably due to increased energy input compared to lower irradiances (Fig. 2, Table 1).

The production of extracellular polysaccharides was affected significantly by temperature ($n = 60$, $F = 621.5$, $p < 0.001$), irradiance ($n = 60$, $F = 54.54$, $p < 0.001$) and combination of temperature $\times$ irradiance ($n = 60$, $F = 25.19$, $p < 0.001$). The maximum EPS production was observed at temperatures around 25.7°C . The maximum EPS is shifted to higher temperatures compared to maximum μ and IPS production. The maximum EPS production occurred at similar irradiances as IPS production. Since the conditions for maximum EPS production are slightly shifted to higher temperatures and irradiances than for maximum μ , the production of EPS may be considered as response to desiccation (Fig. 3, Table 1).

The maximum IPS and EPS production was observed, when the μ was decreased (Table 1). The difference between the irradiance of maximum EPS in Fig. 3 and Table 1 is caused by the calculation algorithm, since the means of the irradiance categories in Fig. 3 are calculated from all temperatures, while the data in Table 1 are calculated for individual temperature $\times$ irradiance categories. The RDA analysis confirmed tight correlation between dry weight and IPS content and slightly different conditions for EPS (Monte Carlo permutation test for all axes, pseudo- $F = 10.0$, $p = 0.002$, Fig. 4).

Despite of significant effects of temperature and irradiance on μ , IPS and EPS contents, the correlation between the environmental parameters and μ , IPS and EPS contents were not always observed, probably due to unimodal response curve. However, the μ and IPS were negatively correlated to temperature. Contrary to temperature, no correlations were found between irradiance and EPS contents. The production of IPS was strongly positively correlated to μ , while the EPS content was independent of μ (Table 2).

4 Discussion

For the first time, the growth requirements for temperature and irradiance, and the light and temperature conditions for IPS and EPS production by *D. chlorelloides* strain Kováčik 1978/11, a prospective strain for EPS production, were determined, and a novel method for fast screening of biomass and polysaccharide production using the cultivation in the crossed gradients was applied in biotechnologically oriented research.

Figure 2. Dependence of IPS production in *D. chlorelloides* on temperature and irradiance. (A) 2D area plot showing optimal and marginal IPS production conditions. (B) The categorized dependence of mean IPS production on temperature. (C) The categorized dependence of mean IPS production on irradiance. The error bars indicate 95 % confidence intervals. The letter indicates homologous groups recognized by Tukey HSD test for $p = 0.05$.

Figure 3. Dependence of EPS production in *D. chlorelloides* on temperature and irradiance. (A) 2D area plot showing optimal and marginal growth conditions. (B) The categorized dependence of mean EPS production on temperature. (C) The categorized dependence of mean EPS production on irradiance. The error bars indicate 95 % confidence intervals. The letter indicates homologous groups recognized by Tukey HSD test for $p = 0.05$.

4.1 Growth requirements

The temperature requirements are comparable to other mesophilic microorganisms with the optimum temperature for long-term growth is around 15–20°C. Although the strain was capable to survive short-term (1 h) exposure to temperature of 40°C, long-term survival at temperatures above 30°C seems not to be possible. The temperature requirements probably reflect the adaptation/ acclimatization to temperature conditions in their natural habitat as seen in other green alga isolated from a temper-

Figure 4. RDA diagram. DW – Dry weigh, EPS – extracellular polysaccharides, IPS – intracellular polysaccharides. The first, second, third and fourth axis explained 53.54, 54.11, 96.94, and 100.00% of cumulative variation, respectively.

Table 2. r-values of correlation analysis. Marked correlations (bold) are significant at $p < 0.05$ ($n = 20$). T = temperature, I = irradiance

	EPS	IPS	μ
T	–0.2409 $p = 0.306$	–0.7980 $p < 0.001$	–0.7824 $p < 0.001$
I	0.1943 $p = 0.412$	0.0463 $p = 0.846$	–0.1262 $p = 0.596$
μ	0.3712 $p = 0.107$	0.8486 $p < 0.001$	

ate lake, *Stichococcus bacillaris* [40]. Similar conditions are used in the CCA (15–20°C, 10 W/m²), so the shift in temperature growth requirements due to acclimation *sensu* [41], i.e. response of algae to culture collection condition, and pre-cultivation (20 and 22°C, 12 and 10 W/m² / 54.8 and 45.7 μmol/(m² s), respectively) should be considered negligible.

The survival limit of ca 30°C was confirmed in *D. chlorelloides* isolated from a mountain lake in Southern Spain, however after previous exposition to elevated temperature, the alga was able to survive even at 35°C [42], indicating thus adaptation to lower temperatures. Since significant decrease of oxygen production was observed at temperatures above 21°C and complete inhibition occurred at 30°C [43], it may indicate that *D. chlorelloides* prefer temperatures between 15 and 20°C in general. However, to test this hypothesis, a large screening study with large number of strains collected worldwide is necessary.

The irradiance requirements indicate adaptation/acclimatization to relatively low irradiances. The irradiances above 11 W/m² (50.3 μmol/(m² s)) decreased growth rate, indicating thus long-term damage by continuous irradiance. Further studies are necessary to reveal limits for short-term expositions (minutes to hours) to elevated PAR and its influence on primary productivity. The saturation irradiance values from oxygen production measurements in *D. chlorelloides* range from ca 80 to 275 μmol/(m² s) (18 to 50 W/m² after recalculation according to Thimijan et Heins [36]; [43, 44]). However, the results proved tolerance

of only short-term (minutes) expositions to increased PAR of $700 \mu\text{mol}/(\text{m}^2 \text{ s})$.

Although the growth requirements of the experimental strain are similar, the slight difference may be important for development of strains-specific cultivation system. Since the best conditions for biomass production occur at temperatures from 15 to 21°C and PAR between 5 and 9 W/m^2 (22.9 and 41.1 $\mu\text{mol}/(\text{m}^2 \text{ s})$), together with broad temperature tolerance in range from 10 to almost 28°C, *D. chlorelloides* strain Kováčik 1978/11 should be suitable for mass cultivation in springs or falls in temperate zone, providing thus extended possibility of utilization of mass cultivation facilities. Such facility should be protected against excessive PAR, since it may reach up to $1000 \mu\text{mol}/(\text{m}^2 \text{ s})$ in early spring [45].

4.2 Polysaccharide production

For the first time, the conditions for IPS and EPS productivity of *D. chlorelloides* strain were determined. In green algae *sensu lato* (Chlorophyta and Streptophyta), the EPS/IPS production is common [46–50]. Synthesis and accumulation of EPS and IPS play important role in stress responses to desiccation [51, 52], freezing [53] and water stress [54]. This stress tolerance mechanism seems to be evolutionally old, since it is observed in various organisms with different degree of complexity, for instance bacteria [51] and higher plants [55]. The EPS/IPS play a key role in binding of heavy metals, and hence affecting their toxicity [56–58] in salt stress tolerance [26], soil stabilization and improvement [59–61]. Since the EPS and IPS are produced in sub- or supra-optimal conditions, μ should be decreased [23], and these optima are different from growth one [28, 62, 63]. Indeed, lower μ were observed in conditions of maximum IPS and EPS in supra-optimum temperatures and irradiances in *D. chlorelloides* in our study. In case of extreme high temperatures exceeding the growth limit, the IPS and EPS productions were reduced as well.

The maximum IPS and EPS content in the *D. chlorelloides* reached 2130 and 1075 mg/L , respectively. The maximum EPS content is higher than in *Chlamydomonas reinhardtii*. (628 mg/L [29]), and comparable to *Dunaliella salina* (944 mg/L ; [26]). Higher EPS content in range from 4 to 5.5 g/L was observed in *Botryococcus braunii*, however the production occurs at slightly higher temperatures [24]. If we consider the *D. chlorelloides* growth requirements, the lower temperature and no manipulation with medium composition to achieve similar EPS production could contribute to strain selection suitable for mass cultivation in springs or falls in temperate zone.

Despite of their importance in stress response and various biotechnological applications, less attention is paid to optima and limits for EPS/IPS production in green algae. The studies on influence of environmental variables are rare [8, 29], especially with respect to temperature and irradiance. High irradiances and continuous PAR regime increase the EPS production in cyanobacteria significantly ([28] and references therein). Since desiccation is usually connected with elevated temperatures and/or increased irradiance in nature, higher amounts of EPS and/or IPS may be synthesized and accumulated at higher temperatures as adaptation to desiccation. Indeed, the

highest contents of EPS in *D. chlorelloides* were observed in higher temperature in our study, where the maximum EPS production was observed at temperatures around 25.7°C and irradiance 11 W/m^2 (50.3 $\mu\text{mol}/(\text{m}^2 \text{ s})$). The maximum EPS is thus shifted to higher temperatures and irradiances compared to maximum biomass/ μ production.

As in the case of temperature effects, the irradiance optima for EPS production may differ from growth optima, reflecting thus influence of other abiotic and biotic factors on EPS production [28, 62, 64]. In cyanobacteria, the EPS production is enhanced by increased radiation [28]. Our study also showed the elevated EPS levels in high-light conditions in *D. chlorelloides*, indicating thus similar adaptation/acclimation processes to heat/desiccation stress.

In conclusion, the cultivation in the crossed gradients of temperature and light proved to be applicable for screening and evaluation of biomass and polysaccharide production of biotechnologically prospective microalgal strains. Although the optimum conditions for growth and polysaccharide production may differ [28, 62, 63], studies are usually performed in optimum or near-optimum growth conditions for studied strains, the real optimum for EPS production could not be revealed easily [28], and large extensive studies focused on combined effects of several environmental factors should be performed [63]. Therefore, cultivation in the crossed gradients of temperature and irradiance seems to be a good approach to this problem [35].

Due to its temperature and irradiance growth requirements and high EPS production at moderate temperatures, *D. chlorelloides* seems to be good producer of EPS with large tolerance for temperature and light intensity, suitable for large-scale cultivation, especially in spring and fall in temperate zone. Even the optimum temperature and light requirements were established for both biomass as well as IPS/EPS production in this study, yet it remains to check, and eventually optimize, the cultivation medium composition as well as other factors that may affect the carbohydrate production, such as nitrogen source and salinity of media.

The difference in growth and EPS production optima will lead to multi-step cultivation of *D. chlorelloides*. In the first step, the condition should be optimized for maximum biomass yield. In the second step, the conditions will be changed for maximum EPS production, probably by temperature manipulation. Very high density of our cultures, however, brings problem of separation of cells. Due to strong attachment of EPS to the algal-cell wall and its rigidity it will be necessary to optimize protocols for EPS separation from cells and in future, since neither centrifugation nor filtration are capable to separate cells from medium in industrial scale so far.

Practical application

The green alga *Dictyosphaerium chlorelloides* strain Kováčik 1978/11 seems to be suitable as a promising source of EPS (extracellular polysaccharides). Therefore, its growth requirements and conditions for EPS production were evaluated through the cultivation in crossed gradients of temperature and light. Since the alga preferred temperatures

below 20°C for its growth and low irradiances, it could be used for mass cultivations in spring and fall in the temperate zone. Higher temperatures needed for EPS production could result in development of cultivation protocol based on controlled temperature manipulation. The method of cultivation in the crossed gradients of temperature and irradiance can be used for fast evaluation of growth requirements and high-value compounds production of other biotechnologically interesting strains of algae and cyanobacteria.

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Nomenclature

DW [g L⁻¹] dry weight

Greek symbols

μ [d⁻¹] growth rate

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