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Cold-adapted culturing of the microalga *Monoraphidium* sp. in thin-layer raceway pond for biomass production

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ABSTRACT

Three cultivation regimes were tested in cold-adapted cultures of the green microalga *Monoraphidium* in an outdoor thin-layer raceway pond: cultivation under sunlight; its combination with continuous supplementary illumination; and nitrogen depletion using both light sources. The highest volumetric and areal productivity, 0.16 g L^{-1} d^{-1} and 3.22 g m^{-2} d^{-1}, respectively corresponding to the specific growth rate μ of 0.191 d^{-1} were achieved when sunlight was combined with supplementary illumination. The maximum total fatty acid content, 20.29 % of DW, rich in oleic acid, 54 % of total fatty acid content, was achieved under nitrogen depletion stress. An outstanding amount of lutein, 26.39 mg lutein g^{-1} DW, was detected grown under sunlight in the first trial. From the harvested and fermented biomass in the second trial 236 mL_N g^{-1}oTS of methane was generated.

1. Introduction

There has been increasing commercial interest in microalgae for a wide variety of applications including animal feed, aquaculture, biofertilizer, waste pollutant remediation, sources of nutrients and chemicals for food production or nutraceutical supplements [1–3]. The vast majority of these commercial microalgae strains belong to the group of mesophilic or slightly thermophilic microorganisms that grows optimally in moderate temperature, neither too hot nor too cold, with an optimum growth range from 20 to 45 °C. This characteristic restricts their cultivation to tropical and subtropical zones. A possible solution to extend the cultivation area and the season is therefore to look for an organism that can grow at lower temperatures. Cold habitats, such as polar or glacier regions, are potentially an encouraging source of microalgae strains for low-temperature biotechnology [4].

Light availability is one of the most crucial prerequisites of microalgae growth, especially in the short day and low irradiation seasons. Thin-layer cultivation units, such as thin-layer cascades (TLC) and thin-layer raceway ponds (TL-RWP), possess high light utilization abilities, while they have low depth (5 and 15 mm) and fast flow (0.5 and 0.2 m s^{-1}) of culture [5]. This guarantees a high ratio (>100 m^{-1} and 50–70 m^{-1}) of exposed surface to total culture volume and high turbulence of cells compared to mixed ponds or deep raceways ponds (10–30 cm depth). Thus, the light is used more effectively, compared with poorly stirred cultures. TL-RWPs and cascades differ only in circulation devices – paddle wheel or centrifugal pumps – but are equalled in light utilization efficiency. The benefit of a TL-RWP is considered mainly from the point of cell wall sensitivity to friction, in the case of the cultivation of a sensitive or a newly isolated strain, e.g. *Monoraphidium B* [6,7].

To optimize the growth regime and gain information about culture status, monitoring of physicochemical variables (pH, temperature, dissolved oxygen concentration, nutrient status, etc.) and photosynthetic activity are required. Chlorophyll fluorescence measurement and photosynthetic oxygen production have become one of the most frequently used techniques for monitoring microalgae mass cultures. The most frequently used variable, the maximum quantum yield of PSII

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(F_v/F_m) has been used to correlate photosynthesis and growth several times to improve the prediction of biomass productivity [8]. In many cases, stress is required to obtain the aimed product in a feasible yield contained by the produced biomass. Nitrogen depletion is one of the most common methodologies to improve e.g. fatty acid accumulation in microalgae cells. On the other hand, maintaining the culture under these conditions can lead to an overstressed state with its disadvantageous consequences. To avoid this phenomenon, both in-situ and ex-situ chlorophyll fluorescence measurements can rapidly show the actual activity and viability of the grown culture and the rough estimate of the accumulation state of the aimed product [9].

There are several valuable products for which the microalgae *Monoraphidium* and related genera from the Selenastraceae can be used, but the vast majority of the studies published so far focus on the production of biodiesel [10]. Microalgae can be considered a promising green source for biodiesel production as compared with other substrates due to their high production capacity for oil and can be cultivated both in the sea and freshwater [11]. Biodiesel quality depends on the profile of fatty acids which should be similar to tested features of produced biodiesel [12]. Its properties mainly affected by fatty acid composition are cetane number (dimensionless related to the ignition delay time after injection into a diesel engine), iodine value, and saponification value. The cetane number values are quite similar to those derived from edible plants (soybean, palm, canola, jatropha, and sunflower). The cetane number of fatty acid esters increases positively with higher saturation and chain length [13]. To obtain the best quality of biodiesel from microalgae, strains with high saturated (palmitic acid; C16:0 and stearic acid; C18:0) and monosaturated fatty acids (oleic acid; C18:1) are the most desirable.

Besides biofuels, microalgae have increasing importance in food supplementation, primarily as polyunsaturated fatty acids, e.g. eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [14]. However, gamma-linolenic acid (18:3; n-6), an omega-6, 18-carbon (18C-) polyunsaturated fatty acid (PUFA) found in human milk and several seed oils is typically required as a part of dietary supplements. It has gained importance for its anti-inflammatory [15] and anti-cancer [16] actions. Oleic acid is (C18:1-n9c) one of the most common monounsaturated fatty acids in daily nutrition as well.

Microalgae can be the source of several carotenoids, e.g. lutein which, together with zeaxanthin, has gathered increasing attention. Currently, lutein is obtained from the petals of marigold after an extraction process [17] which is a labour-intensive land-demanding process. The only other possible sources with a sufficient content to be considered for lutein production are certain strains of microalgae [18,19].

After the extraction of valuable products from microalgae, there is a large quantity of biomass left over. By following the biorefinery strategy, the production prices of valuable products can be further decreased by finding additional uses for process residues. For example, the anaerobic fermentation of microalgae biomass into biogas containing methane can be a feasible scenario [3,20]. *Auxenochlorella protothecoides* was firstly grown phototrophically and then heterotrophically up to 120 g L^{-1} [21]. The high-density biomass was used for lipid extraction and then, the residues were further anaerobically fermented with the methane yields of 0.4 L g^{-1} volatile solids. As a result, biodiesel production with lipid-extracted algal residues to generate methane could increase the overall process energy output by up to 40 %.

Cold-adapted microalgae possess the unrevealed potential to expand the borders of microalgae biotechnology as concerns climate and location. In this work, the cold-adapted microalgae *Monoraphidium B* in TL-RWP was studied under low irradiation and temperature conditions in outdoor pilot-scale cultivation units. We attempt to come round the boundaries of a light-limited and low-temperature environment and describe its acclimation to harsh cultivation conditions by monitoring physicochemical variables and chlorophyll fluorescence techniques. Additionally, the utilization of the produced biomass was further investigated for fatty acid and carotenoid content and the rest was fermented for biogas production.

2. Materials and methods

2.1. Strain selection

In this study, the unicellular green microalga (Supplementary Fig. 1) was selected for its ability to adapt to the cold climate and low irradiation environment. *Monoraphidium* (Selenastraceae, Chlorophyceae) is a genus of coccoid green microalgae that grows in many freshwater habitats such as lakes, bogs, or small ponds at temperatures below 20 °C. The strain *Monoraphidium* sp. B (further as *Monoraphidium B*), was isolated in 2009 from the surface layer of the lakes Omega 1 on the James Ross Island (64°10′S, 57°45′W), north-eastern Antarctic Peninsula by plating on an agar plate with BG11 medium and colony selection [22] and it is deposited in the collection of Prof. Josef Elster (the Institute of Botany, Třeboň, Czech Republic). Both molecular (18S rDNA, ITS2 rDNA) and morphological data were used for the identification of the strain; the sequences are available in the NCBI Nucleotide Sequence Database under accession numbers KX671910 and KX671912.

The temperature requirements for the growth of the strain were specified in the laboratory between 6 and 20 °C which was very similar to their original habitat. The capability of the strain to grow under low irradiation intensities and low temperatures is essential for outdoor cultivation from late autumn to early spring as it is typical for the moderate climate zone in Třeboň, Czech Republic.

2.2. Indoor cultivation

Monoraphidium B was stored on agar plates containing BG-11 medium placed at 18 °C under average photon flux density (PFD) of 50 μmol photons m^{-2} s^{-1} before inoculation to a 50 mL tissue culture flask. The volume was gradually increased up to 2 × 500 mL volume which was performed in an 800 mL tissue culture flask in BG11 medium. The culture was kept on a horizontal shaker at 18 °C and illuminated continuously with an average PFD of 80 μmol photons m^{-2} s^{-1}. The mixture of air with 1.5 % CO_2 (v/v) was continuously purged into the cooled incubator. The seed culture was transferred into a double-jacketed glass column photobioreactor (PBR; an internal diameter of 190 mm and a total height of 400 mm) with internal LED illumination and scaled up stepwise to 10 L. The measured average PFD on the outer surface of the PBR was set to 30 μmol photons m^{-2} s^{-1} until the culture reached its maximum density. The temperature was set to 20 °C and the culture was mixed with the stream of filtered air (+1.5 % CO_2) using a stainless steel loop with apertures (i.d. 0.5 mm, 10 mm apart) placed at the vessel bottom maintaining oxygen stripping.

2.3. Outdoor cultivation

Once the stationary growth phase in the laboratory PBR was reached, the culture was transferred into the outdoor thin-layer raceway pond (TL-RWP) which was placed in a polycarbonate double-layer greenhouse to protect cultures from cross-contamination and unfavourable outdoor conditions (Supplementary Fig. 2) [5]. TL-RWP was characterized by the microalgae grown in a thin layer (the culture depth of 15 mm) and hence, this unit is well suitable for light utilization during cultivation. TL-RWP was operated continuously with a volume of 100–120 L. The loss of water was compensated every morning before samples were taken for measurements. The automatic addition of CO_2 maintained the pH value between 7.5 and 8.0 (pH-stat). The temperature in the greenhouse was regulated by two heaters and cooling fans on both ends of the greenhouse maintaining the required temperature range between 6 and 24 °C.

Three trials were performed subsequently. They differed in the illumination intensity and cultivation medium. After each trial, a part of the culture was harvested and biomass density was set back to the initial

level (0.4–0.6 g L^{-1}) by adding tap water. Before the start of the first trial, *Monoraphidium B* was pre-cultivated in TL-RWP for one week to adapt to the outdoor environment. In the first trial, the culture was grown under sunlight in full BG11 media. In the second trial, sunlight was continuously (24 h d^{-1}) supplemented with low-intensity artificial illumination (~30 μmol photons m^{-2} s^{-1}) and the culture was grown in a full BG11 medium. In the third trial, sunlight was continuously (24 h d^{-1}) supplemented with low-intensity artificial illumination and the BG11 medium was depleted from nitrate. Nitrate depletion was conducted by supplementation with tap water after the second trial. The samples for various measurements were taken daily at 09:00 h. The evaporation was compensated every morning before sampling (09:00 h) by the addition of tap water to keep the culture volume constant.

2.4. Location and weather conditions

The outdoor trials were carried out in the Centre Algatech (48°59′16.6″N, 14°39.9″E) in November and December 2020. Both the ambient and culture temperatures were monitored and recorded online (Supplementary Figs. 3a, b, c and 4a, b, c). The daily lowest culture temperatures ranged between 5.9 °C and 13.4 °C, while the midday maximum temperatures were between 10.9 °C and 23.3 °C; which fits into the proper cultivation range [22]. The average temperatures of the first and second trials were 13.4 °C and 13.1 °C, while during the last trial it was 10.9 °C. Photon flux density inside the greenhouses was recorded by a weather station (IP Warioweather, model ME 13). Artificial illumination was provided by a light panel placed horizontally above the TL-RWP, containing high-frequency cool fluorescent tubes (36 W/830 Lumilux, Osram, Germany), and four LED panels mounted below the roof of the greenhouse. The artificial PFD increment was measured manually (+about 30 μmol photons m^{-2} s^{-1}) and added to the values recorded by the weather station during the second and third trials. The average PFD of the first trial was 19.5 μmol photons m^{-2} s^{-1}, while in the second and third trials it was 53.1 μmol photons m^{-2} s^{-1} (23.1 μmol photons m^{-2} s^{-1} + 30 μmol photons m^{-2} s^{-1}) and 46.1 μmol photons m^{-2} s^{-1} (16.1 μmol photons m^{-2} s^{-1} + 30 μmol photons m^{-2} s^{-1}) (Supplementary Fig. 3f).

2.4.1. Online outdoor photosynthesis measurement

In-situ photosynthetic activity of *Monoraphidium B* culture was measured by a portable pulse-amplitude modulation fluorimeter (Junior-PAM, H. Walz, Germany). The fluorimeter fiber optics and irradiance microsensor were merged into the middle of the photic zone. The submerged depth of the sensors was adjusted every morning after the adjustment of the water level. The actual photochemical yield Y_{II}, non-photochemical quenching NPQ, electron transport rate rETR and photosynthetically active radiation PAR estimated in the photic zone were recorded simultaneously. Relative electron transport rate rETR was calculated as irradiance intensity multiplied by Y_{II} in the photic zone:

$$\mathrm{rETR} = Y_{II} \times E_{PAR}$$

2.5. Analytical measurements

2.5.1. Dry weight determination

The biomass content was measured in triplicate once a day in the morning (at 9:00 h after the culture compensation for evaporation). The biomass density was measured as dry weight (DW) by filtering 5 mL of culture samples on pre-weighed glass microfiber filters (GC-50). The filters with the cells were washed twice with deionized water, dried in an oven at 105 °C for 8 h, then transferred to a desiccator to equilibrate to laboratory temperature and weighed (precision of ±0.01 mg) [9]. The specific growth rate was calculated as follows:

$$\mu = (ln\ X_2 - ln\ X_1)/\Delta t\ [d^{-1}]$$

2.5.2. Analysis of fatty acids

The collected microalgae samples for fatty acid analysis were stored at −20 °C. The amount corresponding to 5–10 mg of lyophilized biomass was transferred to a breaking vial, and about 0.4 mL of zirconium/silicon beads (⌀ 0.1 mm), 1 mL mixture of 3 M hydrochloric acid in methanol, and 50 μg of internal standard (C15:0) were added. Then, the microalgae cells were disintegrated using 5 consecutive 30 s cycles, using a bead beater (Mini-Beadbeater-16, BioSpec Products, USA). After the disintegration, the samples were cooled down on the ice. Then, the content of the breaking vial was transferred into a screw-top test tube and the breaking vial was washed twice with 1 mL of methanol. The sample was sonicated for 15 min (Kraintek 6, Czech Republic) and then, the reaction mixture was heated at 90 °C for 1.5 h in a thermoblock, then cooled to laboratory temperature and 2 mL of hexane and 2 mL of 1 M NaCl were added. The mixture was shortly vortexed for 10 s and centrifuged at 900 ×*g* at 4 °C for 10 min (Eppendorf centrifuge 5804 R). The upper organic phase was separated and analysed.

The separation of methyl esters of individual fatty acids (FAMEs) was performed on Thermo Trace 1300 gas chromatography system with the TR-FAME column (60 m × 0.32 mm, df 0.25 μm) when helium was used as a carrier gas at a pressure of 200 kPa. The temperature regime was as follows: the starting value of 140 °C was increased to 240 °C at the rate of 4.5 °C per min and then it was maintained at 240 °C for 10 min. The injector was controlled at 260 °C and the detector at 250 °C. The retention times of FAMEs were compared to known standards from menhaden fish oil (Supelco® 37 Component FAME Mix; PUFA No.3 Supelco). The amounts of individual fatty acids were estimated using internal standards with a known amount of glycerol tripentadecanoate (C15:0), and calculated by multiplying the integrated peak areas by the correction factors of the FID response.

2.5.3. Nutrient analysis

The sample (5 mL) of microalgae culture was centrifuged at 2300*g* for 10 min and the supernatant was stored at −20 °C before the analysis. The sample was diluted ten times with deionized water. The analysis was performed on an ion chromatography system (ICS-90, Dionex fitted with an AS22-Fast 4 × 150 mm, Dionex IonPac™ column). A solution of known $NaNO_3$ concentrations was used as a standard to construct the calibration curve. Nutrient concentrations were calculated in mM.

In the first two trials, *Monoraphidium B* was cultivated in a replete BG11 medium. Between trials, biomass density was decreased by harvesting culture and replacing the missing volume with tap water which finally caused the rapid decrease in nitrate concentration. To avoid the depletion in the second trial, concentrated nitrate was added. In the third trial, nitrogen depletion was induced again (Supplementary Fig. 5).

2.5.4. Pigment analysis

Chlorophyll a, b and total carotenoid concentrations were determined spectrophotometrically in methanol extracts. Samples of 0.5 mL were collected in 2-mL Eppendorf tubes and centrifuged at 13,000 rpm for 3 min (centrifuge Minispin, Eppendorf). The pellet was re-suspended in 0.5 mL of 100 % methanol, 0.1 mL of sea sand was added, and the tubes were put into a laboratory ultrasound bath (Kraintek 6) for 2 min, then cooled down on the ice and centrifuged at 10,000 rpm for 1 min. The extraction was repeated until the pellet was colourless. The absorbance of the combined supernatants from all extraction steps was measured at 470, 665.2, 652.4, and 770 nm using a high-resolution spectrophotometer (UV 2600 UV-VIS, Shimadzu, Japan, slit width of 0.5 nm) and the concentrations of Chl a, b and total carotenoids were calculated as described previously [23].

2.5.5. Lutein analysis

2.5.5.1. HPLC-DAD analysis of extracts and fractions. The

Monoraphidium B extracts and HPCCC fractions were analysed using an Agilent HPLC system (Agilent 1100 Series, Germany) with a diode array detector (DAD). The chromatographic separation of the target compound was carried out on a reversed-phase column (Luna® C8 column, 100 × 4.6 mm, 3 μm) at 30 °C. The mobile phase – the mixture of water (A) and methanol (B) – was pumped at a flow rate of 0.8 mL min^{-1}. The gradient elution was programmed using the following pattern: 0–20 min, 20 %–0 % A; 20–25 min, 0 % A; 25–27 min, 0 %–20 % A; 27–30 min, 20 % A [18,24]. The HPLC analysis of samples was monitored at 440 nm. For lutein quantification in extracts, a commercial standard of lutein (Extrasynthese, France) was used to construct a calibration curve of five concentration points in the concentration range from 0.03 to 128 μg mL^{-1}. The regression equation was calculated as y = 49.837x + 16.026 (R^2 = 0.9999), where x expresses the lutein concentration (μg mL^{-1}) and y is the HPLC peak area.

2.5.5.2. HPCCC isolation of lutein. To carry out the separation of lutein from the extract of *Monoraphidium B*, a high-performance countercurrent chromatography (HPCCC) instrument was used (Spectrum model, Dynamic Extractions Ltd., Slough, UK) equipped with a 134 mL capacity column (PTFE bore tubing = 3.2 mm). The rotation of the HPCCC column was fixed and monitored by a speed regulator installed in the equipment. The temperature in the column was kept at 30 °C using a H50/H150 Smart Water Chiller (LabTech Srl, Sorisole Bergamo, Italy). The mobile and stationary phases were pumped through the HPCCC column using a Q-Grad pump (LabAlliance, State College, PA, USA). Finally, the separation process was controlled at 440 nm using a Sapphire UV-VIS spectrophotometer (ECOM s.r.o., Prague, Czech Republic). The trace line of the chromatogram was recorded using an EZChrom SI software platform (Agilent Technologies, Pleasanton, CA, USA).

Lutein isolation was preceded by the production of an extract of *Monoraphidium B* dried biomass, which was obtained through ultrasound-assisted extraction (UAE) of 15 g of dried biomass for 30 min with 0.9 L of the lower phase from the biphasic solvent system utilized for the HPCCC separation of lutein [18]. The solvents from the resulting liquid extract were removed using a rotary evaporator under reduced pressure at 38 °C leading to 4.51 g of dried extract. The HPCCC separation of lutein from *Monoraphidium B* dried extract was carried out using a biphasic solvent system composed of the mixture of *n*-heptane, ethanol, and water using the ratio, 5:4:1.5, v/v/v [18]. The preparation of the biphasic solvent system was done by mixing the individual solvents in a separatory funnel. The resulting mixture was intensively stirred and then allowed to stand until the formation of two clear immiscible liquid phases. The lower phase was used as the mobile phase, while the upper phase was the stationary phase. To start the separation process, the HPCCC column was firstly filled with the stationary phase by pumping two column volumes. After that, the rotation of the HPCCC column was started at a speed of 1600 rpm at a controlled temperature of 30 °C. After the total filling of the column, the mobile phase was pumped through it until reaching the hydrodynamic equilibrium between the two immiscible phases within the column. The hydrodynamic equilibrium is reached when the mobile phase emerges from the column without the loss of stationary phases. Under this steady condition, the chromatographic system is ready for sample injection. A quantity of *Monoraphidium B* extract dissolved in a volume of mobile phase was the sample to be injected. The resulting fractions of the separation process were manually collected and analysed using HPLC-DAD.

The retention of the stationary phase (Sf) within the HPCCC column was calculated using the following equation:

$$\mathrm{Sf}(\%) = \frac{\mathrm{Vs}}{\mathrm{Vc}} \times 100$$

where Vc is the HPCCC column volume and Vs is the stationary phase volume in the column when hydrodynamic equilibrium has been reached [25].

To predict the retention time of the target compound t_R in the HPCCC separation process, the following equation was used:

$$t_R = \frac{V_M + (K \times V_S)}{F}$$

where V_M is the mobile phase volume when the hydrodynamic equilibrium is reached, K is the partition coefficient of the target compound, V_S is the stationary phase volume when the hydrodynamic equilibrium has been reached, and F is the mobile phase flow rate [25].

2.5.5.3. Confirmation of the chemical identity of the purified target compound. The chemical identification of the isolated lutein from *Monoraphidium B* extract was determined by mass spectrometry using a Dionex UltiMate 3000 HPLC system (Thermo Scientific, Sunnyvale, CA, USA) connected to high-resolution mass spectrometry (HRMS) detector with atmospheric chemical ionization (APCI) source (Impact HD mass spectrometer, Bruker, Billerica, MA, USA) (HPLC-APCI-HRMS). The confirmation of the isolated target compound was performed by comparing the commercial standard of lutein and literature data. The conditions for the chromatographic separation are indicated in a previous section (Section 2.5.5.1). To favour the ionization of the target molecule, formic acid (0.1 %) solution was added to solvents A and B. The mass spectrometry operating parameters are described as follows: 4.2 kV of the spray needle voltage; 250 °C of drying temperature, and nitrogen used as the nebulizing (3 bar) and drying gas (12 L min^{-1}). The scanning range was 50–2000 *m/z* operating in the positive ion mode and with a scan rate of 2 Hz. The lutein fragmentation was generated with collision energy set at 35 eV using nitrogen as the collision gas.

2.5.6. Anaerobic digestion experiment

Biochemical methane potential (BMP) measurements were used to determine the potential and biodegradability of *Monoraphidium B* biomass. Experiments were carried out in 160 mL reactor vessels (Wheaton glass serum bottle, Z114014 Aldrich) containing a 60 mL liquid phase at mesophilic temperature (37 ± 0.5 °C). All fermentations were done in triplicates according to the VDI 4630 protocol (Vereins Deutscher Ingenieure 4630, 2006). The inoculum sludge originated from an operating biogas plant (Zöldforrás Ltd., Hungary) fed with maize silage (68 % VS) and pig manure slurry (15 % VS) maintained in semi-CSTR digesters (VS: volatile solids). The inoculum was filtered (>1 mm particles filtered out) and used as a negative control in the BMP test and the "base" inoculum. Maize silage, which is a common substrate in biogas plants (as a first-generation substrate) served as a positive control.

The total solids (TS) content was quantified by drying the microalgae biomass at 105 °C overnight and weighing the residue. The CH_4 content was determined with an Agilent 6890 N GC (Agilent Technologies) equipped with an HP Molsieve 5 Å (30 m × 0.53 mm × 25 μm) column and a TCD detector. The temperature of the injector was 150 °C and split mode 0.2:1 was applied. The temperature of TCD detector was set to 150 °C. The column temperature was maintained at 60 °C. The carrier gas was Linde HQ argon 5.0 with the flow rate set at 16.8 mL min^{-1}.

3. Results and discussion

3.1. Growth

The *Monoraphidium B* culture was grown in an inorganic BG-11 medium in the outdoor TL-RWP at three cultivation regimes. They differed in illumination and medium quality. In the first trial, the culture was grown in ambient irradiance and full BG11 medium. In the second trial, the sunlight was supplemented with low-intensity artificial illumination (~30 μmol photons m^{-2} s^{-1}) and the culture was grown in the BG11 medium. In the third trial, the sunlight was supplemented with low-intensity artificial illumination and nitrate was step wisely depleted

in the medium by dilution of the culture with tap water and the consumption of the biomass during the growth.

In the first trial, the biomass concentration of the culture increased from 0.44 g L^{-1} to 0.98 g L^{-1} (Fig. 1a) and from 8.75 g m^{-2} to 19.68 g m^{-2} (Fig. 1b), which provided a daily 0.08 g L^{-1} d^{-1} and 1.56 g m^{-2} d^{-1} average biomass productivity increase (Fig. 1c). The average specific growth rate during this trial was $\mu = 0.12$ d^{-1}. Microalgae growth in TL-RWP has been already tested in the summertime with pure *Chlorella vulgaris* and mixed *C. vulgaris* and *Scenedesmus acutus* cultures in BG11 medium and in municipal wastewater where additional illumination was not utilized [6,7]. The average specific growth rate of *C. vulgaris* cultured in TL-RWP was $\mu = 0.14$ d^{-1}, while the average specific growth rate of *C. vulgaris* and *S. acutus* cultures grown in wastewater centrate was $\mu = 0.15$ d^{-1}. The growth of *Monoraphidium* sp. A was studied in a thin-layer cascade unit [26]. Its initial biomass density was 0.5 g L^{-1}, while its final concentration was 13.6 g L^{-1}. The average PFD was higher (32 μmol photons m^{-2} s^{-1}) compared to our first trial (19.5 μmol photons m^{-2} s^{-1}) (Supplementary Fig. 3f). As a result of the higher average irradiance, the specific growth rate was $\mu = 0.15$ d^{-1}; it is important to note that $\mu = 0.12$ d^{-1} for *Monoraphidium B* was achieved in the winter period with low irradiance and temperature.

In the second trial, the sunlight was supplemented with low intensity (~30 μmol photons m^{-2} s^{-1}) continuous artificial illumination to prove that a similar specific growth rate can be reached with the addition of only very weak light [7]. When extra illumination was provided the biomass concentration increased from 0.4 g L^{-1} to 1.53 g L^{-1} (Fig. 1a) and from 8 g m^{-2} to 30.54 g m^{-2} (Fig. 1b) in 7 days. The volumetric and areal biomass productivity were 0.16 g L^{-1} d^{-1}, 3.22 g m^{-2} d^{-1} (Fig. 1c), while the average specific growth rate was 65 % higher ($\mu = 0.12$ d^{-1} vs. $\mu = 0.19$ d^{-1}) compared to the first trial. The maximum PFD of the days ranged between 150 and 350 μmol photons m^{-2} s^{-1}, moreover, the length of the daylight period was much shorter than in summer. The culture of *C. vulgaris* MACC-1 strain grown in a thin-layer cascade in summer showed $\mu = 0.19$ d^{-1}, and $\mu = 0.14$ d^{-1} when it was grown in TL-RWP [7]. However, in another trial the growth rate for *Chlorella* sp. R-117 (production strain used for large-scale cultivation) was 0.32 d^{-1} in an outdoor thin-layer cascade unit used exclusively for this strain. Overall, in the winter season, the daily average PFD is rather low to compete with summer production rates, but with the supplement of very low-intensity artificial illumination, the growth rate can be significantly improved to approach significant productivity. Another explanation for increased productivity could be the diminishing of the biomass loss during the night period caused by cell respiration, which can reach even 30 % of the dry matter generated during the light period [27]. The low PFD could be enough to balance the anabolic and catabolic processes in the cells and prevent the culture from biomass loss during the night period, which overall resulted in higher biomass productivity. As LED technology is rapidly developing and its cost is continuously decreasing, the application of artificial illumination may be feasible for biomass production. Investment costs can be minimized in thin layer systems, as they require only low-intensity illumination for significant production improvement [28].

Nutrient depletion is a common way to induce stress in microalgae as described in our previous experiments [1]. Several studies have also described that nitrogen depletion can induce fatty acid accumulation. Starting the cultivation with a low amount of nitrogen source is many times beneficial, as a higher amount of fatty acid is attainable when the culture is not suddenly exposed to nutrient limitation stress [29]. In our third trial, we aimed to induce a higher fatty acid accumulation rate by nitrate depletion. Initial nitrate concentration was set to 0.64 mM, while the total deprivation was reached after one week by the consumption of all the remaining nitrate. As a result, the diluted culture grew from 0.62 g L^{-1} to 1.78 g L^{-1} and from 12.36 g m^{-2} to 35.57 g m^{-2} in 10 days. Despite the supplementary illumination, its average specific growth rate (0.12 g L^{-1} d^{-1}, 2.32 g m^{-2} d^{-1} and $\mu = 0.11$ d^{-1}) did not reach the level of the second trial (0.16 g L^{-1} d^{-1}, 3.22 g m^{-2} d^{-1} and $\mu = 0.19$ d^{-1})

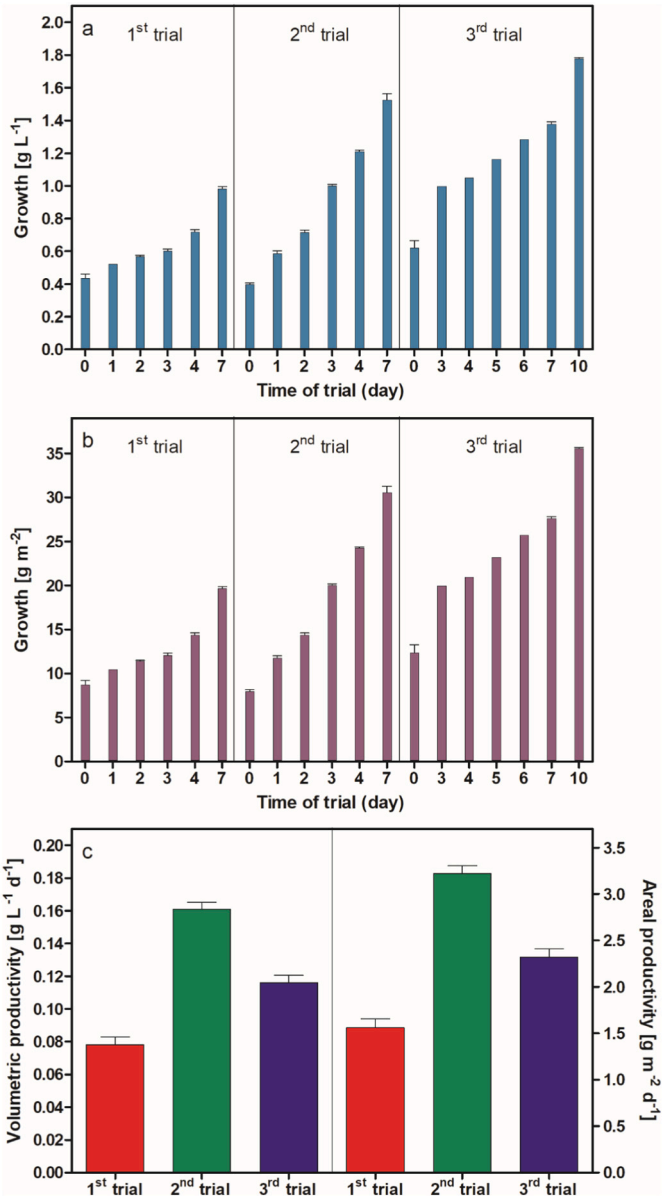

Fig. 1. Changes in the biomass density and productivity in the *Monoraphidium B* cultures grown in TL-RWP in three subsequent trials: a) volumetric dry matter; b) surface dry matter; c) volumetric (left side) and surface (right side) growth rates. The first trial was conducted under sunlight, the second trial was run with additional artificial illumination and the third trial was carried out under sunlight with artificial illumination and nitrogen depletion. Error bars represent analytical standard deviation as the samples were determined in triplicate.

(Table 1). It was only comparable with the first trial (0.08 g L^{-1} d^{-1}, 1.56 g m^{-2} d^{-1} and $\mu = 0.12$ d^{-1}). We suppose that lower average temperature and irradiance partly contributed to the declined growth figures. The average weekly temperature and PFD were lower during the third trial compared to the second one (10.9 °C vs. 13.1 °C and 46.1 μmol photons m^{-2} s^{-1} vs. 53.1 μmol photons m^{-2} s^{-1}, resp.). Overall, the planned simultaneous biomass production and lipid accumulation (from 8.9 % to 20.3 % TFA of DW) was achieved, thus significantly increasing the fatty acid production.

3.2. Correlation between rETR and average specific growth rate

The photosynthetic activity of the culture was monitored ex-situ and

Table 1
Main operating conditions and results of the three trials.

	Trial 1	Trial 2	Trial 3
Cultivation conditions	Sunlight	Sunlight + artificial illumination (30 μmol photons m^{-2} s^{-1})	Sunlight + artificial illumination (30 μmol photons m^{-2} s^{-1}) Nitrogen depletion
Average temperature	13.4 °C	13.1 °C	10.9 °C
Average PFDs of the trials	19.5 μmol photons m^{-2} s^{-1}	53.1 μmol photons m^{-2} s^{-1} (23.1 μmol + 30 μmol)	46.1 μmol photons m^{-2} s^{-1} (16.1 μmol + 30 μmol)
Volumetric biomass productivity	0.08 g L^{-1} d^{-1}	0.16 g L^{-1} d^{-1}	0.12 g L^{-1} d^{-1}
Areal biomass productivity	1.56 g m^{-2} d^{-1}	3.22 g m^{-2} d^{-1}	2.32 g m^{-2} d^{-1}
Average specific growth rate	$\mu = 0.12$ d^{-1}	$\mu = 0.19$ d^{-1}	$\mu = 0.11$ d^{-1}

in-situ by three techniques: saturation pulse analysis of fluorescence quenching to record rapid light-response curves (RLC), fast fluorescence induction kinetics (OJIP), and photosynthetic oxygen evolution and respiration (POE/R) to construct steady-state light-response curves (SS-LRC). The methods and results of the ex-situ measurements are described in detail in the Supplementary materials. In-situ measurements are discussed in this section.

The relative electron transport rate through PSII (rETR = $Y_{II} \times E_{PAR}$) was monitored online in-situ by the Junior-PAM fluorimeter. The average rETR and the average specific growth rate values (Fig. 2a) showed a good correlation. As much the average specific growth rate increased ($\mu_{1st\ trial} = 0.06\ d^{-1}$ vs. $\mu_{2nd\ trial} = 0.18$) the more the average rETR values multiplied (1.67 vs. 6.54). A similar phenomenon was observed when the third trial was compared to the second one. Despite the additional illumination, the average specific growth rate in the third trial was significantly lower than the second one ($\mu_{2nd\ trial} = 0.18\ d^{-1}$ vs. $\mu_{3rd\ trial} = 0.11\ d^{-1}$) (Fig. 2a). Similarly, the average rETR was 6.54 in the second trial, while it was 3.34 in the third trial. The average specific growth rate/rETR ratios of the different trials resulted in very similar quotients: 0.04, 0.031 and 0.032. Their average was calculated as 0.034 (Fig. 2b). We assume that these averaged quotient variables might be a tool to balance the fluctuations of the varying daily biomass production rates. The biomass average specific growth rate strongly depends on the yield of the daily average irradiance (Fig. 2a and Supplementary Fig. 3e and f), but the utilization of light energy by the microalgae cells can be influenced by other factors, e.g. excess irradiation, suboptimal temperature, or the lack of required nutrients which in the end alter the planned daily biomass production rate [30]. Cultures, especially in open outdoor cultivation units, are exposed to variable and often barely predictable weather conditions [31]. The variables Y_{II} and rETR measured continuously in-situ (if sensors are properly arranged in the photic zone) provide rapid information about the culture health and about how effectively light energy is used for photosynthesis. In practical application, the calculated variables can be used to reach a planned daily biomass production rate if the averaged daily rETR was calculated after the end of the daily photosynthetically active radiation. If the calculated average rETR did not reach the desired value, we can suppose that the daily production rate will not reach the planned level. Desired daily production rate can be improved by artificial illumination. The necessary illumination intensity, and thus the minimum necessary energy investment can be set based on the actual rETR value which can be set according to the averaged daily rETR value calculated from the quotient. After keeping the rETR value in the calculated range during the rest of the day the desired daily average rETR can be reached and thus the planned daily average specific growth rate might be achieved as well. This methodology has the potential to balance the impact of variable weather conditions on growth and minimize the necessary extra financial and energetic investments for low irradiation season cultivation. However, this is a theory based on our observations and further trials are needed to prove its applicability.

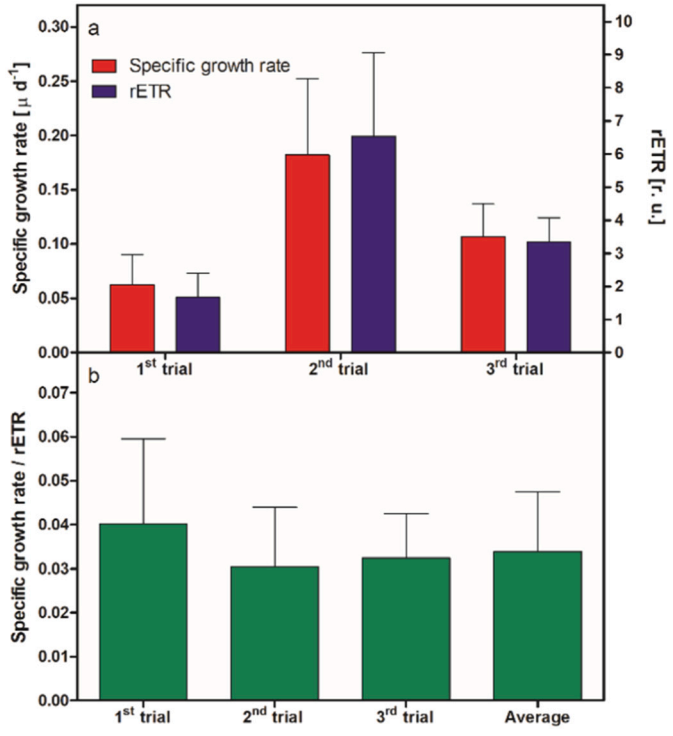

Fig. 2. Correlation of the average specific growth rate and the relative electron transport rate through PSII in the three trials: a) average specific growth rates (left axis, red columns) and the average relative electron transport rate through PSII (rETR) (right axis, blue column); b) the ratios of average specific growth rates and rETR. The first trial was performed with natural illumination; the second trial was run with additional artificial illumination and the third trial was conducted with artificial illumination under nitrogen depletion. Error bars represent analytical standard deviation as the samples were determined in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Fatty acid profile

During the three trials, two saturated fatty acids (SFAs) (palmitic acid: C16:0 and stearic acid: C18:0), two monounsaturated (MUFAs) (palmitoleic acid: C16:1n-7 and oleic acid: C18:1n-9c) and three polyunsaturated fatty acids (PUFAs) (linoleic acid: C18:2n-6c, γ-linolenic acid: C18:3n-6 and stearidonic acid: C18:4n-3) were detected (Fig. 3 c and Table 2). The quantity and quality of the fatty acid content were mainly defined by the status (stressed or unstressed) of the culture. Regarding the cultivation records, the main stressing factor was the actual concentration of nitrate. In the first and second trials, when the nitrate concentration was sufficient, the total fatty acid yield was relatively low and the profile was balanced (Fig. 3a and b). The fatty acid content varied between 6.0 % and 7.6 % of DW. Approximately 50 % of the total fatty acids (TFAs), 35 mg g^{-1} were PUFAs, while the other half of the fatty acids were MUFAs or SFAs. In the second trial, the nitrate concentration decreased to a critical level of 1.02 mM L^{-1}, which induced the accumulation of excess fatty acid in the cells as a reaction to nitrogen depletion (Supplementary Fig. 5 and Fig. 3c). The relative fatty acid yield rose from 7.4 % up to 10.0 % of DW (from 74 to 100 mg g^{-1}). However, whereas the nitrate yield was supplemented on day 3 during the second trial and the concentration reached up to 8.1 mM, the fatty acid accumulation reversed and declined to 6.9 % of DW (69 mg g^{-1}). In the third trial, the quantity of the fatty acids increased following the same scenario. The nitrate concentration was decreased to 0.64 mM by

harvesting the biomass and diluting the culture with tap water. In ten days, the remaining nitrate was also consumed. The total fatty acid content of the dry weight increased from 8.9 % up to 20.3 % of DW (from 89 to 203 mg g^{-1}) in ten days (Fig. 3a).

Regarding the quality of the fatty acid profile, oleic acid (C18:1n-9c) became dominant together with palmitic acid (C16:0) in the second and the third trials as well (Fig. 4b). In the second trial, the oleic acid level increased from 9 % of TFA up to 23 % of TFA (from 6.7 mg g^{-1} to 23.1 mg g^{-1}), then after the nutrient supplementation, it declined to 12 % of TFA (8.1 mg g^{-1}) by the seventh day. In the third trial, oleic acid increased from 16.0 mg g^{-1} to 111.3 mg g^{-1}, while palmitic acid increased from 18.9 mg g^{-1} to 33.4 mg g^{-1}.

Microalgae are generally recommended as food supplements for improving the daily fatty acid profile intake. The most favoured fatty acid components are two PUFAs: eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Although these two PUFAs were not identified in the biomass, the fatty acid profile of

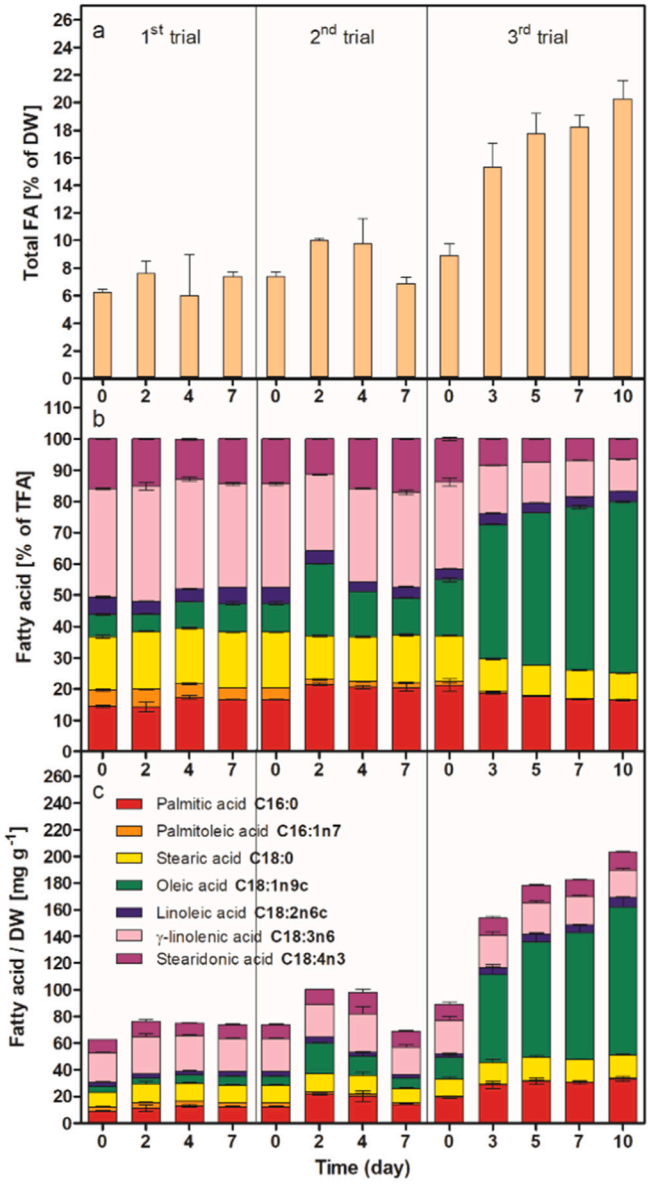

Fig. 3. Changes in the fatty acid profile during the three trials: a) total fatty acid content of the dry biomass; b) individual fatty acid content; c) the amount of specific fatty acids in 1 g dried biomass. Error bars represent analytical standard deviation as the samples were determined in triplicate.

Table 2
Summary of fatty acid profiles of the three trials.

	Trial 1	Trial 2	Trial 3
Cultivation conditions	Sunlight	Sunlight + artificial illumination (30 μmol photons m^{-2} s^{-1})	Sunlight + artificial illumination (30 μmol photons m^{-2} s^{-1}) Nitrogen depletion
Fatty acid content on the last day of the trials	7.4 % of DW 74 mg g^{-1}	6.9 % of DW 69 mg g^{-1}	20.3 % of DW 203 mg g^{-1}
SFA	34.5 % of TFA	35.9 % of TFA	25.1 % of TFA
MUFA	12.9 % of TFA	13.2 % of TFA	54.8 % of TFA
PUFA	52.6 % of TFA	50.9 % of TFA	20.1 % of TFA
Palmitic acid: C16:0	16.5 % of TFA	20.5 % of TFA	16.4 % of TFA
Palmitoleic acid: C16:1n-7	3.8 % of TFA	2.1 % of TFA	0.2 % of TFA
Stearic acid: C18:0	17.9 % of TFA	15.4 % of TFA	8.5 % of TFA
Oleic acid: C18:1n-9c	9.1 % of TFA	11.7 % of TFA	54.8 % of TFA
Linoleic acid: C18:2n-6c	5 % of TFA	3.6 % of TFA	3.4 % of TFA
γ-Linolenic acid: C18:3n-6	33.2 % of TFA	30.2 % of TFA	10.2 % of TFA
Stearidonic acid: C18:4n-3	14.4 % of TFA	17.1 % of TFA	6.5 % of TFA

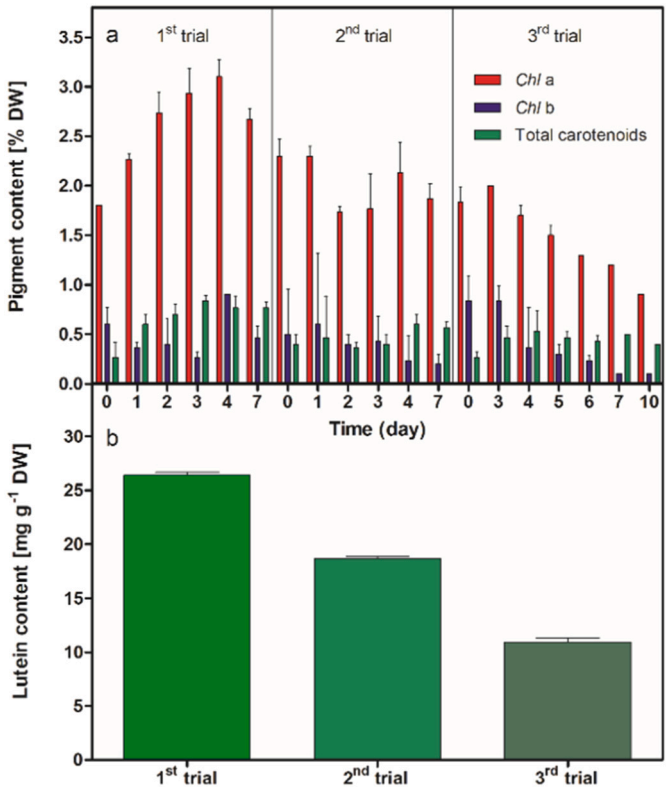

Fig. 4. a) Changes of Chl a (red column), Chl b (blue column) and total carotenoids (green columns) during the three trials. b) Lutein content in dried biomass of *Monoraphidium B* cultivated in three different conditions. 1st trial was conducted in natural illumination. The 2nd trial was run with additional artificial illumination. 3rd trial was cultivated with additional artificial illumination and nitrogen depletion. Error bars represent analytical standard deviation as the samples were determined in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Monoraphidium B was notable. The SFAs in general have different effects on the concentration of plasma lipoprotein cholesterol fractions. For example, lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids increase LDL cholesterol whereas stearic (C18:0) has no effect [32]. In *Monoraphidium B*, the palmitic acid content varied around 15 % of TFA while stearic acid was about 17 % of TFA (Fig. 3b). The maximum recommended SFAs energy intake percentage is 10 %, which is significantly lower in the investigated *Monoraphidium* biomass; thus the health risk of this amount is negligible [33]. On the other hand, the yields of MUFAs and PUFAs, which may cause health benefits, were high, approximately 68 % of TFA (Fig. 3b). Palmitoleic acid (16:1n-7c) represents the smaller portion of MUFAs, about 3–4 % of TFA. It has numerous health benefits such as increased cell membrane fluidity, reduced inflammation, protection of the cardiovascular system, and inhibition of oncogenesis [34]. The other MUFA was the omega-9 oleic acid (18:1n-9c) being 6–9 % of the TFA. Its appearance and health benefits are explicitly known from olive oil. Diets enriched in oleic acid affect fat balance, body mass as well as energy expenditure [35]. As the result of nitrogen depletion in the third trial, the oleic acid content (55 % of TFA) and thus the ratio of MUFAs and PUFAs increased to 75 % of TFA making the biomass more valuable for nutraceutical purposes. Omega-6 fatty acids, γ-linolenic acid and linoleic acid represented 35 % and 4 % of the TFA, resp. in the first and second trials (Fig. 3b). Linolenic acid is an essential fatty acid as it cannot be synthesized by humans. It has an important role in arachidonic acid synthesis [36]. γ-Linolenic acid is produced by the human body from linoleic acid catalysed by Δ6-desaturase. Omega-3 fatty acids were found as stearidonic acid in the biomass; about 14 % of TFA was assayed (Fig. 3b). The preventive and therapeutic relevance of dietary consumption of stearidonic acid relies mainly on its efficient conversion to EPA and DHA in the human body stearidonic acid is considered as 'pro-eicosapentaenoic acid' [37]. The recommended ratio of omega-6/omega-3 suggested in the diet is 4:1 or 2:1 [38]. In *Monoraphidium B* the ratio of omega-6/omega-3/omega-9 fatty acids was close to the ideal, it was 4.3:1.5:1. Regarding the beneficial fatty acid portfolio, the fatty acid profile makes this microalga a suitable food supplement to the everyday diet.

Microalgae, besides palm or rape, are generally recognized as a promising stock for biodiesel production. Biodiesel properties are determined by fatty acid composition, such as carbon chain length and unsaturation extent. Quantitative differences were observed in the fatty acid compositions of the three trials: SFAs were 34.5 %, 35.9 % and 25.1 % of total fatty acids, MUFAs were 12.9 %, 13.2 % and 54.8 % of TFA and PUFAs were 52.6 %, 50.9 % and 20.1 % of TFA in the first, second and third trials, respectively. For this reason, the final fatty acid compositions in the three trials were investigated and compared with the results of other studies. The longer the fatty acid carbon chains and the more saturated the molecules, the higher the cetane number [39]. Low cetane numbers have been associated with more unsaturated components. The degree of unsaturation (DU) of the oil is a prominent parameter in determining the cetane number and iodine value of the final biodiesel product. Oils that have unsaturation degrees higher than 137 do not meet the European Standard for cetane number. In former studies, the DU values of different sources showed high variation: *Monoraphidium* sp. FXY-10 (51.5, heterotrophic) and (136.0, autotrophic), palm (64.2), rape (121.9) and sunflower (152.2) [39,40]. In the present study, the DU values were 118.1, 103.2 and 94.2 in trials one, two and three, respectively. *Monoraphidium B* can be recognized as a good oil producer for biodiesel based on the DU values and the fatty acid profile. The highest fatty acid content of the biomass (20.3 % of DW) was reached when fatty acid accumulation and culture growth were still in the increasing phase. Therefore, the effect of the longer cultivation period on lipid productivity and composition needs to be investigated for clarifying the potential lipid productivity.

3.4. Pigment profile

Different environmental variables such as temperature, irradiance, photoperiods, pH, nutrient limitation, nitrogen supplements, salinity, pesticide and heavy metal presence can affect the production of microalgae pigments [41]. Here, we presume that the main affecting factors were the amount of PFD and nitrogen depletion. The pH, carbon dioxide supply and temperature values were monitored and adjusted automatically in an optimal range. Maximum PFDs of the days were remarkably different between the first and second trials and the average PFDs were supplemented by artificial illumination in the second and third trials. This resulted in, similarly to other studies, substantial differences in pigment yields [42]. The pigment content in the biomass was higher in low average PFD and lower during high average PFD (Fig. 4a). In the first trial, the maximum Chl a and total carotenoid contents were 3.1 % and 0.83 % of DW, resp. while in the second trial they were 2.3 % and 0.6 % of DW. If we consider the average values of all three trials, Chl a and total carotenoid contents were 2.58 % and 0.66 % of DW, respectively, while in the second trial they were present in the amount of 2.02 % and 0.47 % of DW. Nitrogen availability was limited twice during the trials (Supplementary Fig. 5). Thus, it must be mentioned that in the second trial pigment values were lowered because of nitrogen depletion on days 0 to 2. Similarly to other reports, after adding the nitrate supplementation, the pigment content was restored [43]. In the third trial, the intention was to stress the culture by nitrogen depletion. This led to a decrease in the overall pigment amount in the biomass (Fig. 4a). Both chlorophyll and carotenoid contents were affected.

A high amount of lutein was detected by HPLC analysis. Its content in the last days of the three cultivation trials accounted for 26.4, 18.7 and 10.9 mg g^{-1} of DW. Currently, marigold flowers (e.g., *Tagetes erecta*, and *T. patula*) are the only lutein commercial source, which shows lutein contents within the range between 0.83 and 7.95 mg g^{-1} and between 0.60 and 12.31 mg g^{-1} for dried powders of *T. erecta* [44], and *T. patula* [45], respectively. However, its production is limited by seasons, climate, location and high labour costs [46]. Thus, microalgae biomass represents an alternative source of lutein showing advantages such as high productivity and low demand for water compared to marigold plants. For instance, the lutein content in several microalgae species obtained through mutagenesis and metabolic engineering strategies and grown under directed cultivation conditions was reported to be in the range of 4.5 and 13.81 mg g^{-1} [47]. In the present study, *Monoraphidium B* biomass cultivated during the first trial showed a lutein content of 26.39 mg g^{-1}, which was comparable to that of marigold flowers; hence, it may be a potential candidate for lutein commercial source. In the present study, 15 g of *Monoraphidium B* biomass from the first trial was selected for the preparation of biomass extract with 0.9 L of lower phase under ultrasound-assisted extraction for 30 min. The amount of 4.5 g of dried extract was obtained, which was subsequently used for the isolation of lutein by HPCCC. A detailed description of the high-throughput isolation of lutein from *Monoraphidium B* biomass using HPCCC and the analysis of the isolated compound can be found (Supplementary materials together with Supplementary Figs. 10, 11, 12 and 13).

3.5. Biochemical methane potential

Anaerobic fermentation was performed to reveal the potential of the microalgae biomass to be used as a substrate for biogas production. The experiment focused on two scenarios. The dried biomass of non-stressed and stressed cultures from the last days of the second and third trials was fermented which lasted for 31 days. A remarkable amount of methane was detected in the produced biogas (Fig. 5). In the second trial, 236 ± 4.6 mL_N g^{-1}oTS methane was produced from 1 g dried biomass. However, a lower amount, 213 ± 2.9 mL_N g^{-1}oTS of methane was produced from the dried biomass harvested at the end of the third trial. In comparison, methane production in the previous studies varied between 215 mL_N g^{-1}oTS (*Chlorella luteoviridis*) and 331 mL_N g^{-1}oTS (*Navicula*

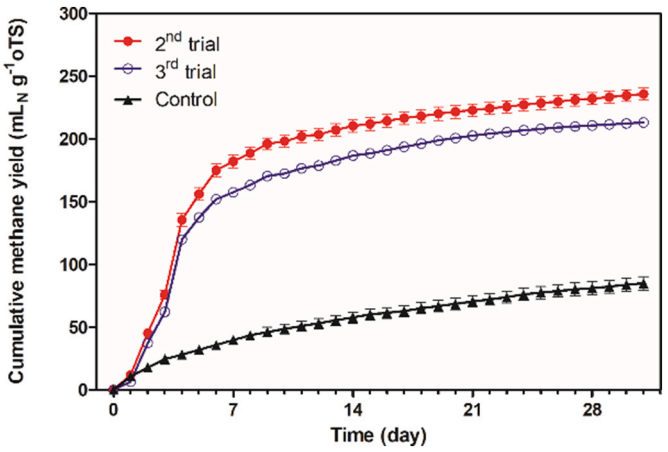

Fig. 5. Methane yields (mL$_N$ g^{-1}oTS) from the fermentation of *Monoraphidium B* dried biomass, harvested at the end of the trials. The samples taken from the non-stressed culture of the 2nd trial (red closed circle) and the culture stressed by nutrient depletion (blue open circle) from the 3rd trial were collected. Error bars represent analytical standard deviation as the samples were determined in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

salinicola) [20,48,49]. Regarding these studies, *Monoraphidium* belongs to the lower methane producers. In screening trials of several *Monoraphidium* strains, the highest methane evolution was obtained in *Monoraphidium neglectum* – of about 280 mL$_N$ g^{-1}oTS [48]. However, the results shown in this study indicate that *Monoraphidium B* biomass cannot be exclusively targeted for biogas production. Additionally, residues of *Monoraphidium B* might be used as a good feedstock for fermentation after lutein or fatty acid extraction.

4. Conclusion

The productivity of *Monoraphidium B* grown in ambient irradiance was significantly improved by the addition of artificial illumination. The culture productivity was mainly slowed down by short days (limitation of photosynthetic activity by insufficient irradiance) and to a lesser extent by temperature. A good correlation was revealed between relative electron transport rates measured in-situ and the average specific growth rates which suggest the potential for production improvement. The biomass analysis showed fatty acid profiles valuable for nutraceuticals and biodiesel, while pigment content revealed a promising amount of lutein. Considering the biorefinery principle, biochemical methane production of the harvested *Monoraphidium* biomass was also tested and found sufficient. All in all, *Monoraphidium B* possess the potential for mass cultivation in moderate climate zone during cooler periods out of the usual cultivation season.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102926.

CRediT authorship contribution statement

Gergely Ernő Lakatos: Conceptualization, Funding acquisition, Methodology, Investigation, Data curation, Visualization, Writing – original draft. **Karolína Ranglová:** Methodology, Investigation, Data curation, Writing – original draft. **Daniela Bárcenas-Pérez:** Methodology, Investigation. **Tomáš Grivalský:** Investigation, Data curation. **João Câmara Manoel:** Investigation. **Mykola Mylenko:** Methodology, Investigation, Data curation, Writing – original draft. **José Cheel:** Visualization, Writing – original draft. **József Nyári:** Investigation. **Roland Wirth:** Methodology. **Kornél L. Kovács:** Funding acquisition, Supervision. **Jiří Kopecký:** Funding acquisition, Supervision. **Linda Nedbalová:** Writing – original draft. **Jiří Masojídek:** Funding acquisition, Methodology, Investigation, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

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