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RESEARCH

Botryococcus braunii strain Showa in annular photobioreactors under artificial light

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Abstract

Botryococcus braunii strain Showa (race B) is a green microalga able to release long chain hydrocarbons, suitable for use as energy-rich compounds for biofuel applications, but also for high-value markets, like cosmetics. Its ability to grow in annular reactors up to 60 L was investigated. As expected, biomass productivities were rather low, with a maximum average of about 120 mg L^{-1} day^{-1}. Optimization of culture conditions (e.g., cell concentration, temperature, light period, light quality) is necessary to increase biomass productivity. The main weaknesses emerged were sensitivity to high light coupled to low temperatures and susceptibility to contamination by competing phototrophs. Hydrocarbon content was quite constant, reaching 26–28% of dry biomass, so hydrocarbon productivity was mainly determined by biomass productivity. The system proved to be suitable to grow *B. braunii* Showa to produce high quality inoculum, nevertheless for an economically sustainable large-scale production, even considering only high-value applications, higher biomass/component productivities would be necessary.

Keywords *Botryococcus braunii* · Chlorophyceae · Microalgae · Hydrocarbon · Artificial light · Photobioreactor

Introduction

Botryococcus braunii is a colonial green alga that is classified in different races (A, B and L) on the basis of the main hydrocarbons synthesized. In particular, in race B strains, hydrocarbons comprise C30-C37 cyclic and acyclic triterpenoids, named botryococcenes, as well as C31-C34 methylated squalenes (Metzger and Largeau 2005; Watanabe and Tanabe 2013). Recently, it has been proposed to classify the three chemical races as three different species, following comparative genomic analysis, with attribution to race B of the original name *Botryococcus braunii* (Boland et al. 2024).

Botryococccus braunii is attracting interest because of its ability to produce not only hydrocarbons, but also other biotechnologically relevant molecules, like other lipids and exopolysaccharides (Metzger and Largeau 1999). Several applications have been proposed for derivatives from this microalga, such as protein and carbohydrates (Teles Dominguez et al. 2015) for food and feed markets, carotenoids for food and health products (Ranga Rao et al. 2018), and oil and hydrophilic extracts for cosmetics (Buono et al. 2012; Atsumi 2016). In spite of the many possible uses of *B. braunii*, most of the research has so far mainly focused on its potential use for diesel and aviation fuel production (Hillen et al. 1982; Watanabe and Tanabe 2013).

Cultivation under artificial light may represent an interesting alternative to outdoor production when high value molecules are the target. Indoor cultivation permits to maintain more stable culture conditions, eliminating the daily variations in temperature and available radiation that characterize growth under natural light. It also allows year-round production, regardless of weather conditions and seasonal variability. Moreover, artificial light makes possible to vary light intensity and quality, as well as photoperiod. The above parameters may influence the synthesis of the target product besides algal growth. Finally, indoor cultivation may be expected to reduce the impact of airborne contamination. Following all these characteristics, the optimization of the target product accumulation should be easier under indoor conditions. In the case of *B. braunii* production, for example,

Mario R. Tredici is deceased in 2022.

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it is known that the type of hydrocarbon synthesized varies according to growth stage and environmental conditions (Wolf et al. 1985; Okada et al. 1995; Wang et al. 2019). Not many papers have been published on indoor *B. braunii* production with artificial light illumination at volumes larger than few liters. For example, Zhang et al. (2011) designed a 10-L airlift reactor with external lighting for mixotrophic growth of an unspecified *B. braunii* strain, Shimamura et al. (2012) grew a race B strain in 30-L cylindrical reactors with a diameter of 18 cm and external fluorescent lamps, while Khichi et al. (2018) used a 30-L flat panel illuminated by external LEDs, Indrayani et al. (2022) cultivated a race A strain in 20-L illuminated carboys, and Arbeláez Pérez et al. (2024) grew a race A strain in a 14-L cylindrical photobioreactor with four external lights.

In this work *Botryococcus braunii* strain Showa was cultivated in annular reactors (Tredici 2004), characterized by short light paths and reduction of light dispersion, thanks to the lamps lodged inside the inner cylinder, which ensure that a high fraction of the light provided reaches the cells. This design allows attaining higher volumetric productivities compared to completely filled columns and bags, used in many indoor production facilities. The use of photobioreactors under artificial light may be of interest for *B. braunii* cultivation, as light path, light intensity and their different combinations may strongly affect biomass production as well as hydrocarbon and fatty acid content in this alga (Wang et al. 2019).

This work aimed at evaluating the growth potential of *B. braunii* Showa, a race B strain, grown under artificial light in annular reactors up to 60 L volume, its composition and its potential for the production of high value products.

Materials and methods

Photobioreactor

Trials were carried out in annular reactors (ARs) commercialized by Fotosintetica & Microbiologica S.r.l., a spin-off company of the University of Florence. The AR is made of two concentric polymethylmethacrylate (PMMA) transparent cylinders of different diameter sealed at the base to form a culture chamber. The illumination system is placed inside the inner cylinder (Tredici 2004). In the case of the 5-L AR (4-L culture volume), the external diameter was 15 cm, the culture chamber width 2.6 cm and the internal illuminated surface 0.11 m^2. The illumination system consisted of four 24-W fluorescent lamps (Lumilux T5 HO, daylight 865, Osram, Germany) providing a maximum light intensity of 700 µmol photons m^{-2} s^{-1}. The 60-L AR (55-L culture volume) had an external diameter of 51 cm, a culture chamber 4.5-cm wide and an internal illuminated surface of 1.07 m^2. The illumination system consisted of twelve 39-W fluorescent tubes (Lumilux T5 H0, daylight 865, Osram, Germany) that could be turned on and off in groups of four, with a maximum light intensity providable of 460 µmol photons m^{-2} s^{-1}. No culture temperature control system was present, as the reactor was placed inside a laboratory or a greenhouse.

Strain and culture conditions

Botryococcus braunii strain Showa was cultivated in a modified Chu 13 medium (Chu 1942): KNO_3 375 mg L^{-1}, K_2HPO_4 75 mg L^{-1}, $MgSO_4.7H_2O$ 94 mg L^{-1}, $CaCl_2{\cdot}2H_2O$ 50 mg L^{-1}, $FeCl_3$ 7.5 mg L^{-1} mixed with Na_2EDTA 20 mg L^{-1}, micronutrient solution 1 mL L^{-1}. The micronutrient solution used was that from UTEX Culture Collection for Chu medium: $ZnSO_4.7H_2O$ 44 mg L^{-1}, $MnCl_2.4H_2O$ 13 mg L^{-1}, $Co(NO_3)_2.6H_2O$ 25 mg L^{-1}, $Na_2MoO_4.2H_2O$ 13 mg L^{-1}, H_3BO_3 618 mg L^{-1}, $CuSO_4.5H_2O$ 20 mg L^{-1}. Indoor growth was performed under light:dark cycles (10 h:14 h) at 700 µmol photons m^{-2} s^{-1} for the 5-L AR and at 460 µmol photons m^{-2} s^{-1} for the 60-L, except (for technical reasons) during the weekends, when the culture was kept under continuous illumination. During the adaptation phase, light intensity was increased gradually. In the greenhouse trial, as the roof was opaque to light, the contribution of natural light was negligible (< 8 µmol photons m^{-2} s^{-1}). In all the cultures, pH was kept between 7.5 and 8 by bubbling an air:CO_2 mixture (97:3, v:v), which also served for culture mixing. Harvesting of the culture was performed by filtration on a nylon cloth with a mesh size of 15 µm.

Analytical procedures

Growth of the cultures was followed by dry weight determination as reported in Guccione et al. (2014). In the co-cultivation trials, dry weight of the whole culture and of the cells passing through the nylon cloth with 15-µm mesh size was determined. Cell counts of the contaminant were performed on the filtrate and on the whole culture using a Thoma counting chamber (Paul Marienfeld GmbH & Co. KG, Germany). The average cell weight of the contaminant was then determined and the dry weight of the contaminant calculated on the basis of the cell counts performed on the whole culture. This dry weight value was subtracted from the whole culture dry weight so as to obtain *B. braunii* dry weight. This latter value was used to calculate *B. braunii* productivity.

Gross biochemical composition was determined on the biomass harvested at the end of the long-term cultivation trial, according to Lowry et al. (1951) for protein, Dubois et al. (1956) for carbohydrate and Marsh and Weinstein (1966) for total lipids. Total fatty acids and fatty acid profile were determined according to Abiusi et al. (2014).

The extracellular hydrocarbon and the carotenoid content were determined following the method by Eroglu and Melis (2010). Lyophilised biomass collected at the end of the trials was dissolved in heptane. Biomass was then vortexed with glass beads (0.4 mm in diameter), resulting in the release of extracellular hydrocarbons from *B. braunii* colonies and their solubilization in the heptane phase. Water was then added to achieve phase separation: the top heptane phase contained a clear yellowish solution (hydrocarbons + carotenoids) whereas the lower water phase contained the green biomass. Absorbance of the heptane layer was measured at 198 and 450 nm. Extracellular hydrocarbon [HC] and carotenoid [Car] content was determined using calibration curves prepared with squalene and beta-carotene, respectively, using the following equations.

$$[\mathrm{HC}] = \left\{\left[\left(\mathrm{A}_{198} - \mathrm{b}\right)/\mathrm{a}\right] \times \mathrm{MWHC}/1000\right\} \times \mathrm{V}/\mathrm{bdwt} \times 100$$

$$[\mathrm{Car}] = \left\{\left[\left(\mathrm{A}_{450} - \mathrm{b}\right)/\mathrm{a}\right] \times \mathrm{MWCar}/1000\right\} \times \mathrm{V}/\mathrm{bdwt} \times 100$$

where [HC] and [Car] are expressed in % of biomass dry weight; *A*: absorbance at 198 nm (for HC) and 450 nm (for Car); *a, b*: coefficients of the linear calibration curves ($y = ax + b$, where x is µM concentration and y absorbance) obtained with squalene for HC (a = 0.059; b = 0.062; $r^2 = 0.99$) and beta-carotene for Car ($a = 0.149$; b = 0.067; $r^2 = 0.98$); MW_{HC}: molecular weight of *B. braunii* Showa hydrocarbons assumed to be 467 g mol^{-1}, molecular weight of botryococcene; MW_{Car}: molecular weight of released carotenoids, assumed to be 537 g mol^{-1}, molecular weight of β-carotene; *V*: volume of heptane used for extraction (L); *bdwt:* amount of dry biomass extracted (mg).

This method was compared with an extraction and purification procedure: extraction was performed in hexane, purification was carried out with a flash type chromatographic column eluted with hexane, that allows the separation of hydrocarbons (R_f 0.55) and carotenoids (R_f 0). The weight of the eluted hydrocarbon fraction was determined after evaporation. The hydrocarbon content of the biomass was 26%, similar to the 25.7% estimated by the Eroglu and Melis (2010) method.

Hydrocarbon productivity was calculated based on hydrocarbon content and the biomass concentration at the start and the end of the trials. As the trials were all consecutive, the hydrocarbon content of the final biomass of one trial was the same of the initial biomass of the following trial, except for the 5-L AR trial, for which no determination of the hydrocarbon content at the start was performed, thus hydrocarbon productivity could not be calculated.

Results

Growth trials in reactors of different volumes

Initially the microalga was cultivated in a 5-L AR (Fig. 1a). The first trial was performed in a greenhouse (which roof was opaque to light, and the walls were made of semi-transparent glass) in autumn at ambient temperatures (daily minimum 15–18 °C and maximum 24–27 °C) and lasted 12 days.

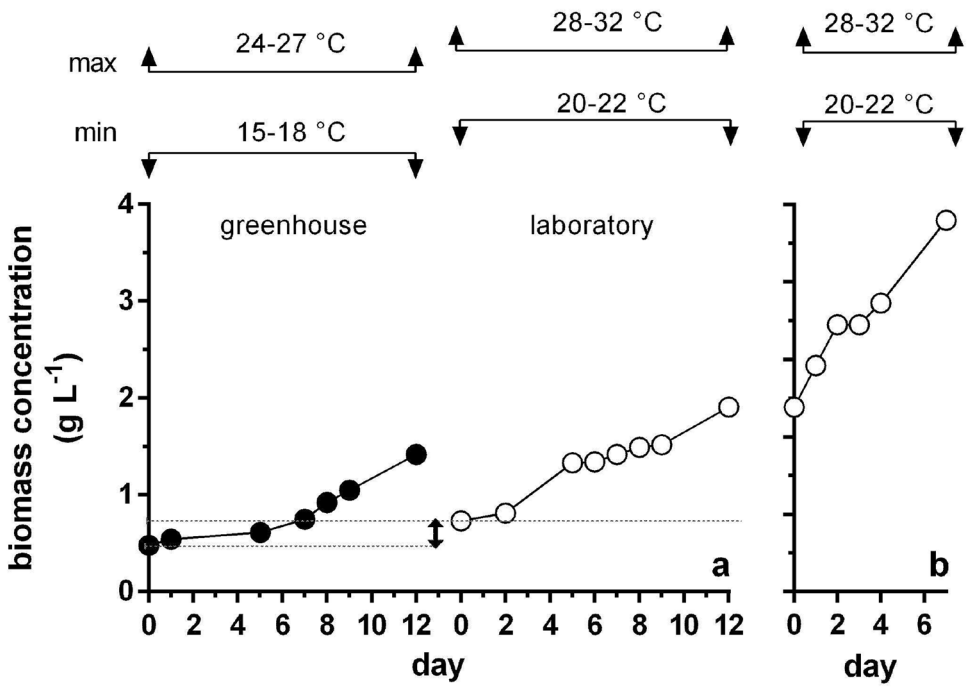

Fig. 1 Growth curves, determined as biomass dry weight concentration, of *B. braunii* Showa in 5-L annular reactors. (**a**) Comparison between growth in the greenhouse and in the laboratory. Range of minimum and maximum temperature measured during the trials are reported above the curves. Within the line chart the two horizontal dashed lines and the double arrow evidence the starting concentrations and the difference between them. (**b**) Growth during the inoculum production phase for the 60-L annular reactor

A long acclimation phase of about five days, characterized by an extremely slow growth, was followed by a period of about one week in which the culture actively grew, reaching a final concentration of about 1.5 g L^{-1}. A maximum biomass productivity of 116 mg L^{-1} day^{-1} was attained from day 5 to day 12, while the average productivity in the whole cultivation period was 78 mg L^{-1} day^{-1}. To verify whether the low temperature had a depressing effect on growth, a new culture was set up in the laboratory. In this case daily minimum and maximum temperatures were 20–22 °C and 28–32 °C, respectively. Biomass concentration reached a final value of about 2.0 g L^{-1} after twelve days. The productivity was higher compared to the greenhouse trial, reaching an average daily value of 98 mg L^{-1}, with a maximum of 173 mg L^{-1} from day 2 to day 5. The initial concentration was 0.7 g L^{-1}.

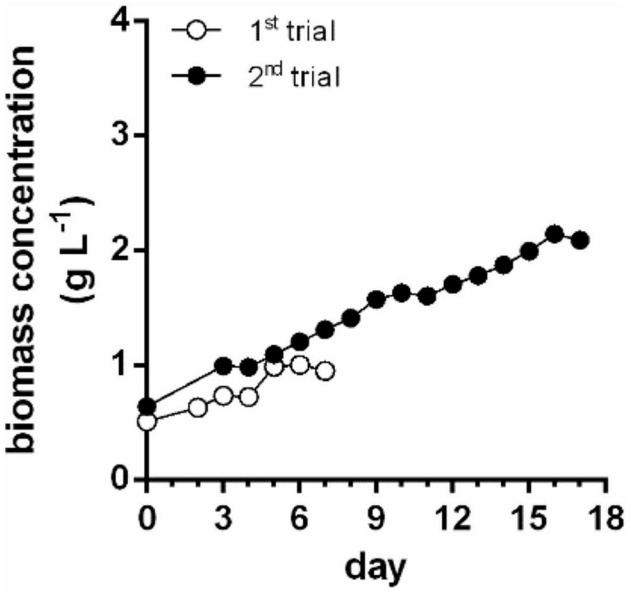

Fig. 2 Growth curves, expressed as biomass dry weight concentration, of *B. braunii* Showa cultivated in 60-L annular reactors at the final volume of 55 L

Following the 12 days of growth, the culture in the laboratory was let to grow for another week to produce the inoculum for the 60-L AR (Fig. 1b). In this growth period, a productivity as high as and 276 mg L^{-1} day^{-1} was attained. The biomass concentration at the start was about 1.9 g L^{-1}.

The culture volume in the 60-L AR was gradually increased from 15 L up to the full operative volume (55 L) (data not shown), keeping the temperature in the range 24–28 °C. This first growth trial (Fig. 2) was performed with a starting biomass concentration of 0.51 g L^{-1}. Growth ceased five days after reaching the final volume (55 L). Biomass productivity was rather low (82 mg L^{-1} day^{-1}). A second trial (Fig. 2) was performed starting from a higher concentration (0.64 g L^{-1}). The average biomass productivity was higher (96 ± 3 mg L^{-1} day^{-1}) than in the first trial.

Competition trials with contaminants

The main problem met during the cultivation of this alga in 60-L AR was contamination. The original *B. braunii* Showa culture in agar slant arrived in our laboratory, besides bacteria, included protozoa (flagellates, amoebae and ciliates) that did not graze on *B. braunii* cells and thus did not represent a direct threat for *B. braunii* growth and did not prevent attaining culture stability. These non-photosynthetic components gradually disappeared during culture scale-up. However, contamination by other photosynthetic organisms (a unicellular cyanobacterium and a green alga of the genus *Scenedesmus*) was observed following a sharp increase in pH (> 9) or in temperature (> 38 °C), accidentally occurred during cultivation. To verify whether *B. braunii* could grow under optimal conditions in co-culture with a contaminant phototroph without being outgrown, two trials were performed in which the growth dynamic of *B. braunii* and the contaminant phototrophs was evaluated (Fig. 3). The trials were performed by gradually increasing culture volume starting from 15 L.

The first trial was carried out using a culture contaminated with *Scenedesmus* as inoculum, subjected to a thorough washing treatment with sterile water on a sterile nylon cloth with a mesh of 30 μm to eliminate visible (under the

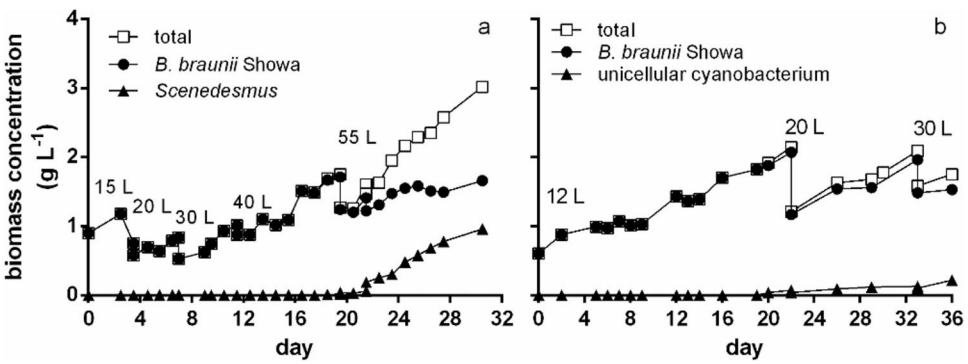

Fig. 3 Growth (expressed as biomass dry weight concentration) experiments performed in 60-L annular reactors to assess *B. braunii* Showa capacity to withstand competition of contaminant phototrophs: *Scenedesmus* (**a**) and a unicellular cyanobacterium (**b**). The numbers reported in the graph indicate the culture volume in the reactor

microscope) signs of the contaminant. Contamination started to be detectable under the microscope after two weeks of growth when the culture was at 40 L volume (Fig. 3a). For about another week *Scenedesmus* remained negligible, but a couple of days after reaching the final culture volume of 55 L, *Scenedesmus* productivity increased about ten times (from 12 to 129 mg L^{-1} day^{-1}). During the first dilution steps before reaching 55 L, the productivity of *B. braunii* was between 74 and 113 mg L^{-1} day^{-1}, while during *Scenedesmus* massive proliferation *B. braunii* average productivity decreased to 27 mg L^{-1} day^{-1}, indicating the incapability of *B. braunii* Showa to prevail over *Scenedesmus*.

Another trial was performed using the same approach with an inoculum contaminated by the unicellular cyanobacterium, washed so as to eliminate observable signs of the contaminant. The culture was kept for about 22 days at 12 L volume until the concentration reached about 2 g L^{-1}, so as to reduce the stress caused to *B. braunii* by the dilution (Fig. 3b). This strategy was decided on the basis of the observation that in smaller systems (1-L bubbled tubes) no contamination was detected and the cultures were seldom diluted and only when they had reached high concentrations (about 3 g L^{-1}). In the 60-L AR the contaminant cyanobacterium became detectable under the microscope after 20 days of cultivation. After dilution to 20 L the contaminant was still increasing, but it represented only 6% of the culture dry weight. Passing from 20 to 30 L culture volume, a higher contaminant presence was observed, and in four days it became 14% of the whole culture dry weight. A five-fold productivity increase (from 8 to 41 mg L^{-1} day^{-1}) was observed for the cyanobacterium, while productivity of *B. braunii* more than halved (from 72 to 30 mg L^{-1} day^{-1}). The trial was thus stopped. Also in this case, although contamination proceeded at a slower pace, *B. braunii* was unable to withstand competition with the contaminant.

Based on these results, a strategy to avoid outcompetition of *B. braunii* Showa in case of contamination by other phototrophs was envisaged: to filter the culture on a sterile nylon cloth (15 to 30 μm mesh size), thoroughly rinsing with sterile water to remove cells embedded in the small aggregates originated from *B. braunii* colonies, as soon as some contaminant became detectable under the microscope.

Long-term cultivation trial

A long-term (about 90 days) trial was finally performed in the 60-L AR (Fig. 4) adopting the above-mentioned strategy to control possible contaminants. During the period in which the culture was scaled to the final volume of 55 L, biomass productivity was rather low (11–53 mg L^{-1} day^{-1}) due to frequent dilutions and low morning temperatures. When the final culture volume was reached (with a starting biomass concentration of 1.1 g L^{-1}), after few days of acclimation

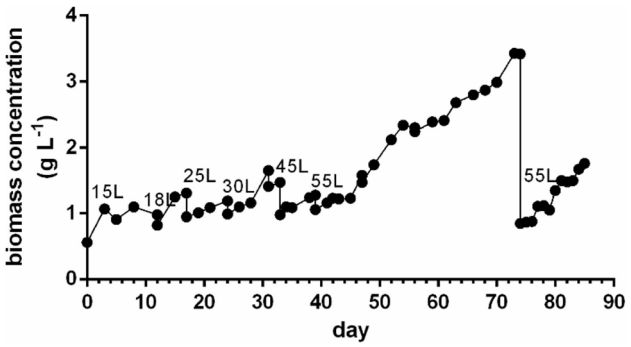

Fig. 4 Long term growth experiment performed in 60-L annular reactors with *B. braunii* Showa. Growth is expressed as biomass dry weight concentration. The numbers reported in the graph indicate the culture volume in the reactor

the culture resumed growth and a concentration of 3.4 g L^{-1} was reached, with an average biomass productivity of 78 mg L^{-1} day^{-1}. During days 65 to 74, the culture reached its maximum average productivity, 123 mg L^{-1} day^{-1}. Culture was partially harvested after 74 days and a new batch at 55 L was started from a concentration of 0.8 g L^{-1}. After five days of acclimation following the strong dilution, the culture resumed growth with the same productivity. In the whole trial, preventive filtration was performed few times.

Biomass composition

The main components of the biomasses obtained in the annular reactor at the end of the long-term cultivation trial were hydrocarbons (about 27%), protein (about 21%) and carbohydrate (about 14%), while total fatty acids amounted to less than 4% (Fig. 5). A significant part (about 24%) of the biomass was undetermined; this part includes nucleic acids, fibers, ashes. Fatty acids profile was very simple, as only three fatty acids were present in significant amount: palmitic acid (C16:0) (27% of total fatty acids), oleic acid (C18:1) and linolenic acid (C18:3 n3) (both about 35% of total fatty acids).

Extracellular hydrocarbon and carotenoid content and productivities were determined for all the experiments at the end of the trials (Table 1). In the 5-L AR under laboratory conditions, hydrocarbon content was the lowest detected in all the trials (21% of dry biomass). In the 60-L AR, hydrocarbon content was rather constant among different trials, varying from 26 to 28% of dry biomass. Hydrocarbon productivity was instead rather variable (17–30 mg L^{-1} day^{-1}), due to the much higher biomass productivity value attained in the second trial. Released carotenoid content was about 0.1% of dry biomass and productivity varied between 70 and 100 μg L^{-1} day^{-1}.

Contamination did not appear to affect hydrocarbon production, as hydrocarbon content was similar to that obtained in the non-contaminated cultures. Almost all the cells of

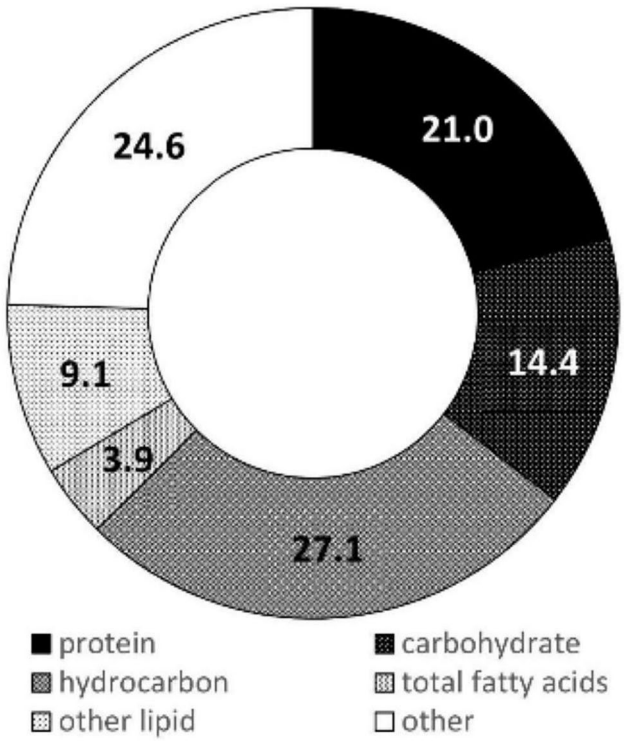

Fig. 5 Biomass composition (% of lyophilized biomass weight) of *B. braunii* Showa grown in 60-L annular reactors

both contaminants (*Scenedesmus* or the unicellular cyanobacterium) were eliminated during harvesting by filtration on a cloth with a mesh size of 30 µm, so that the analysis was carried out on a biomass essentially composed by *B. braunii* cells. Hydrocarbon content in the trial contaminated by *Scenedesmus* was 26%, whereas in that contaminated by the unicellular cyanobacterium the content decreased from 31 to 26% from start to end of the cultivation period. Hydrocarbon productivity was about 12 mg L^{-1} day^{-1} in both cases.

Discussion

Annular reactors demonstrated to be suitable photobioreactors for *B. braunii* Showa growth, also for long cultivation periods, although long acclimation phases were observed. Growth of *B. braunii* Showa in 60-L AR is not easily comparable to literature data, because of the few papers dealing with indoor cultivation of *B. braunii* at similar volume scale (≥ 10 L). Moreover, the few comparable papers concern different photobioreactor designs and different strains, often belonging to race A. Shimamura et al. (2012) obtained productivities in the range 38–133 mg L^{-1} day^{-1} with *B. braunii* strain BOT-22 (race B) cultivated in a 30-L cylindrical reactor with external illumination provided by fluorescent lamps at a light intensity of 100 µmol photons m^{-2} s^{-1} and a light path of 18 cm, conditions rather different (lower light intensity and longer light path) from those adopted in the 60-L AR in the present work. Khichi et al. (2018) grew an unspecified *B. braunii* strain in a 30-L flat panel photobioreactor with a light path of 6.8 cm illuminated from one side by LEDs. At the same light intensity that we adopted in the 60-L AR, a much higher biomass productivity (304 mg L^{-1} day^{-1}) than in our work was attained. However, it must be evidenced that Khichi et al. measured cell concentration by optical density and then converted the data into dry weight through an equation. Optical density is not a suitable growth measurement method for this alga due to its colonial habitus. The unavoidable variability in colony size can, in fact, originate variability in the relationship between optical density and dry weight and thus cause an imprecise conversion.

Indrayani et al. (2022) in 20-L illuminated carboys obtained a twofold increase in productivity (from 7 to 14 mg L^{-1} day^{-1}) when light intensity was shifted from 100 to 500 µmol photons m^{-2} s^{-1} with the A race strain *B. braunii* CCAP 807/2. In our work, the highest productivity was attained in the 5-L AR, in which a higher light intensity was provided, compared to the 60-L AR. However, besides the highest light intensity, the 5-L AR also has a shorter light path, which further increased the light availability to the cells. Moreover, in the trials performed with the 5-L AR, an increase in biomass productivity was observed when cultivation was moved from the obscured greenhouse to the laboratory. The better performance in the laboratory trial could be explained by a reduction in the stress due the combination of low cell concentration (and thus higher light availability per cell) and low temperature. In fact, the laboratory culture had a higher biomass concentration at the start, resulting in

Table 1 Extracellular hydrocarbon and carotenoid content and productivity at the end of the trials performed

Trial	Hydrocarbon content (% of dry biomass)	Hydrocarbon productivity (mg L^{-1} day^{-1})	Carotenoid content (% of dry biomass)	Carotenoid productivity (µg L^{-1} day^{-1})
5-L AR, laboratory	21	-	-	-
60-L AR, 1st trial	26	18	-	-
60-L AR, 2nd trial	28	30	0.1	100
60-L AR, long-term trial	28	17–19	0.1	70–75

a lower light availability per unit biomass (−34% compared to the greenhouse culture), and a higher minimum culture temperature (+28%). These data suggest that the combination of low culture temperature and high radiation per cell represents a depressing factor for *B. braunii* Showa growth. Similarly, with the same strain, Yoshimura et al. (2013) obtained a highly reduced growth rate when light intensity was increased at temperatures below 25 °C. van den Berg et al. (2019) obtained rather constant growth rates in cultures grown at 26 °C at 50, 150 and 450 µmol photons m^{-2} s^{-1}, a result coherent with the hypothesis that light sensitivity onsets below 25 °C. Indoor growth in a thermoregulated environment under artificial light can thus represent an interesting possibility to obtain high and stable productivities with *B. braunii* Showa. Optimization must be sought to identify the best conditions to achieve this goal and one of the aspects to be evaluated is the use of continuous illumination.

In this work, the initial biomass concentration strongly affected *B. braunii* Showa biomass productivity (0.1 g L^{-1} day^{-1} starting at 0.6–0.7 g L^{-1} and 0.3 g L^{-1} day^{-1} starting at 1.9 g L^{-1} in 5-L AR). Arbeláez Pérez et al. (2024) in a 14-L cylindrical photobioreactor reached productivities from 0.25 to 0.45 g L^{-1} day^{-1} with *B. braunii* UTEX 572 (A race), the latter value attained when the starting biomass concentration was about 2 g L^{-1}. As *B. braunii* Showa demonstrated to be sensitive to strong dilutions (either obtained by substituting part of the culture with fresh medium or by adding fresh medium to increase the final culture volume), a possible way to improve overall productivity and, thus, scalability, could be to perform a continuous culture with a standing biomass concentration of about 1.5 g L^{-1}, at which *B. braunii* Showa cultures appeared rather stable, and applying suitable dilution rates, so as to avoid the shock following strong decreases in culture density leading to long lag phases. In this way, even with a steady state productivity lower than the peak productivities observed in this work, an increase in productivity over long cultivation periods should be achieved. Moreover, under this configuration at the steady state, high light intensity might be applied, as also this parameter significantly affects productivity. This was clearly observed in the comparison between 5-L and 60-L AR results, showing a twofold increase in productivity with a 1.5 increase in light intensity and a 1.9 times decrease in light path.

Growth under fully controlled conditions, achievable in an environment with artificial light, may favour not only *B. braunii* biomass production, but also optimization of the synthesis of the molecule/s of interest by offering, besides stable climatic conditions, the possibility to play with light, in terms of either duration of the light period or light intensity and/or quality. As regards this latter point, Atobe et al. (2014) cultivated *B. braunii* Showa with LEDs of mixed colours with or without violet (peak at λ 405 nm), the exclusion of which determined a slight but significant increase in hydrocarbon production and a decrease in extracellular carotenoids and polysaccharides, while the composition of both hydrocarbons and polysaccharides was similar under the two treatments. Further studies on the effect of light quality on *B. braunii* Showa physiology are needed.

The hydrocarbon content reached in our study was similar to that observed by Eroglu and Melis (2010) with the same strain grown in 2-L flasks under semi-continuous regime (28 vs 32% of dry biomass), while extracellular carotenoids content was lower (0.13 vs 0.20% of dry biomass). Still with the same strain, Sawayama et al. (1992) obtained a 58% hydrocarbon content in 3-L flasks bubbled with an air:CO_2 mixture, while Gouveia et al. (2017) observed a 25% hydrocarbon content in 400-mL bubbled tube cultures. Yoshimura et al. (2013) obtained a stable average hydrocarbon content (34 ± 3%) under different temperatures, light intensities and CO_2 concentrations. No data are available to our knowledge for hydrocarbon content of this strain with larger volume reactors under artificial light. It has to be noted that in our work the occurrence of competing phototrophs in *B. braunii* Showa cultures did not change its ability to synthesize hydrocarbons, however, when contamination became significant it strongly reduced *B. braunii* Showa biomass productivity, thus, in the end, also hydrocarbon productivity. In our work, contamination was one of the main issues in the production of *B. braunii* Showa, in particular that derived from competing phototrophs. The contamination might have originated from the environment, or it might have been present in undetectable amounts inside the original culture, hidden within *B. braunii* colonies. Contaminants started to emerge when conditions more suitable for their growth than for *B. braunii* arose, eventually becoming dominating thanks to a higher growth rate. This dynamic appears reasonable on the basis of the results obtained in the co-cultivation experiments, and considering that the contaminants first became evident when the culture suffered technical issues in pH and/or temperature control. Thus, a strict control of culture conditions, a high-quality inoculum culture to start the process and the possibility to early detect potential contaminants (e.g., cytofluorometer or imaging techniques) might mitigate this problem, especially when large scale cultures are sought.

Besides hydrocarbon, *B. braunii* Showa grown in 60-L AR had as main components protein and carbohydrates. Gouveia et al. (2017) found about 15% total carbohydrate in *B. braunii* Showa biomass grown in flasks, a value very close to that obtained in our work.

As the use of artificial light strongly impacts on production costs (Blanken et al. 2013; Ciani et al 2021), this type of cultivation is applicable only when the target market of the produced biomass or component is that of high-value products, e.g., for medical or cosmetic applications. A brief

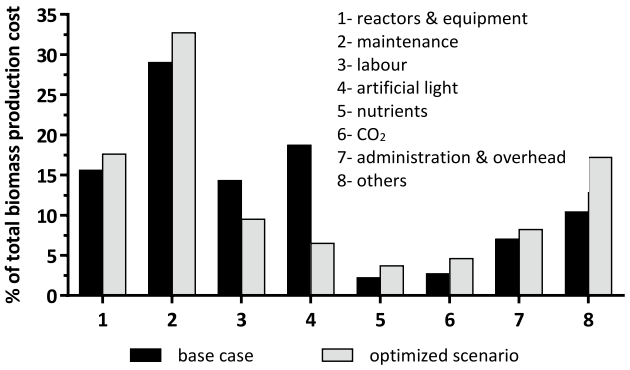

Fig. 6 Estimation of the main cost items concurring to total production cost of *Botryococcus braunii* Showa biomass produced in a hypothetical 100 m^2 indoor plant made of 60-L AC, under two different scenarios. Analysis was performed following Tredici et al. (2016), while the cost of artificial light was calculated according to Ciani et al. (2021)

summary of the production cost of *B. braunii* Showa biomass production in a hypothetical 60-L AR plant of 100 m^2 is reported in Fig. 6. In the base case, considering the highest productivity attained during this work, the total cost per kilogram would be about 490€, while in the optimized case (assuming a 50% increase in productivity, use of LEDs instead of fluorescent lights and a reduced cost of electric energy) of 290€. The main contributor to biomass cost is labour (> 29% in both scenarios), followed by the cost of electricity for artificial light (19%), the impact of which is reduced to about one third in the optimized scenario.

One of the potential markets for *B. braunii* is cosmetics, as hydrocarbons may be used to substitute mineral oil derivatives (Palavra et al. 2011). Botryococcene has shown skin moisturizing properties similar to those of squalene (Atsumi 2016) and *B. braunii* oil is already used to manufacture a hand cream commercialized by Denso Corporation (Japan) since 2014 (https://www.moina.net/, accessed February 5, 2026). Moreover, *B. braunii* carotenoids may find use as antioxidants (Ranga Rao et al. 2006), while hydrophilic extracts, rich in carbohydrates, have been shown to exert antioxidant, anti-inflammatory and enzyme-inhibiting activity, keratinocytes hydration, and stimulation of collagen synthesis (Buono et al. 2012; Lee et al. 2021). Activities of pharmacological interest have also been observed for *B. braunii* extracts, such as anti-depressant activity in mice after application of an ethanol extract (Sasaki et al. 2017) and antibacterial activity of extracts obtained with non-polar solvents (Ranga Rao et al. 2010). Nevertheless, the market price of squalene or of its hydrogenated derivative squalane for the cosmetic industry is about 30–40 € kg^{-1}, also when obtained from non-animal sources (https://datavagyanik.com/reports/squalane-hydrogenated-squalene-market-size-production-sales-average-product-price-market-share-import-vs-export/, accessed 19 February 2026; Bouriakova et al. 2020), while that of mineral oil is in the order of 12 € kg^{-1} (Making Cosmetics 2026), data that show how *B. braunii* Showa oil is currently not competitive as substitute of these products in cosmetics. In fact, considering the oil content found in this work and the cost of biomass production, the cost of oil would be in the order of at least 340 € kg^{-1}, considering oil extraction by supercritical CO_2 at about 50 € kg^{-1} (Mazzelli et al. 2022). A biorefinery approach applied to *B. braunii* biomass could allow multiple high value products to be obtained, thus improving the economic sustainability of biomass production. To reduce the biomass cost, optimization of biomass production process will be required. One possible way will be to evaluate the effect of changing light quality on biomass and/or target compound productivity. However, to make a real breakthrough in cost reduction, there should be an increase in productivity per unit occupied land. To reach competitive costs for a single component (e.g., oil) for high value applications, further optimization would be necessary to increase productivity and reduce operative costs, such as cost for energy, nutrients and CO_2.

To conclude, annular photobioreactors appear as a suitable culture system to grow *B.braunii* Showa. The strain showed, as expected from literature, rather low productivities that were particularly depressed when applying periodic culture dilutions. Hydrocarbon content was quite stable, representing from one fourth to one third of dry biomass. Contamination by other photosynthetic microorganisms was the greatest threat to the production of this microalga, that showed to be unable to compete. A simple strategy to prevent large contamination was envisaged, but it needs to be optimized for application at larger scales. Although the use of artificial light is appealing for products aiming at high value markets (e.g., cosmetic and nutraceutical), to produce *B. braunii* Showa biomass/components at economically sustainable costs, further optimization studies are necessary. The reactor appears suitable to investigate microalgal physiology and, under the conditions finally adopted in this work, to produce high-quality inoculum.

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Data availability All data supporting the findings of this study are available within the paper.

Declarations

Competing interests Natascia Biondi has no competing interests to declare that are relevant to the content of this article; the annular reactor is commercialized by Fotosintetica & Microbiologica S.r.l., in which Liliana Rodolfi has and Mario R. Tredici had a financial interest.

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