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Cost-effective and sustainable microalgae cultivation: A low-cost artificially integrated LED photobioreactor ensuring high-quality algal biomass production from industrial CO_2 flue gas in a high latitude country

José Ignacio Gayo-Peláez [a,b], Darren L. Oatley-Radcliffe [a,b,*], Alla Silkina [b], Andrew R. Barron [c,d]

[a] Department of Chemical Engineering, Faculty of Science and Engineering, Swansea University, Fabian Way, Swansea SA1 8EN, UK
[b] Algal Research Group, Bioscience Department, Faculty of Science and Engineering, Swansea University, Singleton Park, Swansea SA2 8PP, UK
[c] Algae Products International, Swansea Enterprise Park, Swansea, Wales, SA7 9LA, UK
[d] MiDAS Green Innovations, Swansea, SA1 8RD, UK

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ABSTRACT

The equipment required for large-scale production of quality microalgal biomass is costly to set-up. To address this challenge a novel low-cost internally illuminated reactor 'the Cube' has been developed, which is suitable for deployment in high latitude countries with low natural light conditions. This innovative concept combines the use of low-cost materials, LED lighting, effective temperature and pH control technology to produce high quality algal biomass from industrial CO_2 effluent at low initial capital cost. This study evaluated the ability of the cube by growing *Arthrospira platensis* (Spirulina). The reactor achieved biomass concentrations of 1.37 g L^{-1} and 80.5 mg L^{-1} day^{-1} productivity in a 1 m^3vol that occupies 1 m^2, with consistent productivity spanning 78 days, surpassing some of the existing most cost-effective microalgae cultivation system designs currently available. Aerial productivity was demonstrated as 92.1 kg $year^{-1}$ m^{-2} compared to 4.2 kg $year^{-1}$ m^{-2} for a comparable raceway. Protein composition was 54.1% and phycocyanin content was 78.27 mg g^{-1} of biomass. An economic appraisal gave capital cost as £12,776.60 per m^3 reactor and potential profits from pigment and protein production lead to a payback period of only 1.7 years. This novel reactor demonstrates sustainable profits from carbon capture and reuse.

1. Introduction

The field of microalgae biotechnology and algal cultivation is growing exponentially, mainly due to countless applications, processes, and new products finding their way to new markets; such as food, food supplements, animal feed, proteins (and amino acids), lipids (such as omega 3 or fatty acids), enzymes, biomass, polymers, vitamins, toxins, pigments, bioremediation, and green energy (Rempel et al., 2021; Torres-Tiji et al., 2020; Yousuf, 2020). Additionally, microalgae can make a significant contribution to circular bioremediation for wastewater treatment and net zero, with applications in carbon capture, reuse, and storage (Schneider et al., 2024). The choice of microalgal species for any scalable system is very important. Every year the global population is increasing by about 82 million people, leading to increased pollution and enhanced global warming (Ganivet, 2019). To support this population expansion, an increase in food production systems is highly desirable. Global hunger currently affects 1 in 9 people (Torres-Tiji et al., 2020) and global food demand is estimated to increase by 50–60 % by 2050 (Falcon et al., 2022). A solution to combat some of these problems and satisfy future food demand in a sustainable way is the production of alternative proteins from microalgae. (Torres-Tiji et al., 2020). Some microalgae species contain up to 65–70 % (w/w) of protein and among them is *Arthrospira platensis* (commonly known as Spirulina). This organism is already industrialised and contains high protein content (typically 51–58 % w/w) (Wang et al., 2021b). Current global production is estimated as 12,000 tons per year of dried Spirulina (median price of 20–30 USD/kg) and is the most commercialised microalgae on the planet (Costa et al., 2019; Rumin et al., 2020). Spirulina is one of the few species authorised by alternative food regulation for human consumption due to Novel Food Status issued by the

* Corresponding author.
E-mail address: d.l.oatley@swansea.ac.uk (D.L. Oatley-Radcliffe).

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European Food Safety Authority (EFSA) in Europe and the Generally Recognized as Safe (GRAS) status given by the Food and Drug Administration (FDA) in America (Lupatini et al., 2016; Prüser et al., 2021; Vigani et al., 2015).

Spirulina is a blue-green microalgae, specifically a cyanobacteria, that can grow in different salinities, high alkalinity (pH 8.5–11.0), and high temperature (above 30 degrees Celsius) (Lupatini et al., 2016; Ragaza et al., 2020). This makes Spirulina a great candidate as a source of high-quality protein (Lupatini et al., 2016) as large-scale cultivation is relatively easy. In addition to high protein levels, Spirulina is also well known for nutritional value in producing human and animal food, as well as a source of therapeutic molecules (Ragaza et al., 2020). Spirulina biomass is rich in essential amino acids, vitamins, pigments, and other valuable molecules. Especially relevant are pigments, as they have a high value, both economically and therapeutically. In the case of Spirulina, phycocyanin (PC), a phycobiliprotein, is the most common pigment to be commercialised, with a global market value of 9.6 % and an estimated market value of USD 279.6 million by 2030 (Athiyappan et al., 2024). Phycocyanin is a natural blue pigment that can be used in the nutraceutical, pharmaceutical, food, animal, nanotechnological, and medical fields with anti-inflammatory, antioxidant, anticancer, antiviral, neuroprotective, cardioprotective, and immune-stimulating properties (Ashaolu et al., 2021).

Photoautotrophic algal growth is the most common cultivation technology and is characterised by light (usually natural sunlight) as a source of energy for photosynthetic biomass production. Nutrients such as carbon dioxide (CO_2), nitrogen (NO_x), and phosphorus (PO_x) are also required for successful growth (Abreu et al., 2022; Sutherland and Ralph, 2021). Photoautotrophic cultivation is the most widely applied technology because of favourable economic and environmental advantages, sunlight is after all free energy. However, to achieve the best algal biomass production efficiency, the reactor design needs to deliver the photosynthetic and operational requirements of microalgae. The resulting system must be easy to operate at large-scale cultivation volumes and low cost to be economically viable (Carvalho et al., 2006). This is essential as an expensive technology will be limited in use and hinders global expansion. Many reactor technologies used today are simply too expensive or too difficult operate and scale-up, limiting the growth of the microalgae sector and potential biomass applications (Novoveská et al., 2023).

Microalgal cultivation systems are classified into two main classes: open and closed systems. Open systems are mainly characterised by the fact that the microalgae culture is in close contact with the environment, without any barrier, and therefore the cultivation is limited to the local environmental conditions and designs have been adapted to improve microalgal growth. The most common open systems are ponds or raceways. This is mainly because of their relative low construction costs and ease of management. They have many disadvantages such as low capacity to control parameters, low cell densities, high probability of contamination, and are restricted to only a few microalgal species (Carvalho et al., 2006; Paul et al., 2021; Prado et al., 2023). These disadvantages are resolved with closed systems. Closed systems are characterised by their hermeticity with the surrounding environment, cultivation conditions are independent of the location and can be manipulated according to the desired operation. Closed systems are most appropriate for locations where climatic interaction will harm the algal growth rate (temperature, rainfall, light, dust) and when algal culture is required without contamination (Prado et al., 2023). Among the most used systems are the so-called photobioreactors (PBRs), which have numerous variants and are effectively repurposed fermenters. The main disadvantage of these systems is high capital cost and difficulty in scale-up of some system designs (Carvalho et al., 2006; Paul et al., 2021).

The main problem with all cultivation systems is that they need to be cost-effective for the intended purpose. The capital expenditures associated with closed photobioreactors, and the labour costs are substantial (Pawar, 2016) when compared to the open systems. Consequently, a potential avenue for advancement would involve reducing initial capital expenditures, while maintaining the production rate of biomass with comparable quality. Designing a system that can produce quality microalgal products, while maintaining consistent productivity throughout the entire year poses the greatest challenge. Current open systems in high latitude countries often fail to deliver due to variable climate conditions. Consequently, the construction of alternative designs becomes essential to effectively address and overcome these challenges.

The aim of this work is to design, construct, and validate a novel immersed LED-based PBR for cost-effective algal cultivation, ensuring a low initial investment while maintaining the same quality of Spirulina biomass production currently delivered at industrial scale. The design should be capable of producing high-quality microalgae, rich in protein and derived high-value products to supply microalgal applications and provide simple eco-friendly technology to produce tomorrow's food.

2. Material and methods

2.1. Experimental design

The cyanobacteria Spirulina (*Arthrospira platensis* CCMP1295) was cultivated for 78 days to demonstrate the effective use of novel low cost PBR design using a modified 1000 L Industrial Bulk Container (IBC) tank. For this process, the new design was constructed and validated with the growth of cyanobacteria with a liquid volume of 900 L and deployed at an industrial location. During the time of the experiment, validation of the new PBR was made by repeatable microalgal cultivation during 3 complete cycles. In addition, measurements of biotic and abiotic parameters for the algal culture were made and the resulting biomass was characterised for biochemical composition.

2.2. Strains, medium and pre-cultivation conditions (lab conditions)

The microalgae *Arthrospira platensis* (CCMP1295) was originally obtained from Bigelow [The Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA), USA] and was conditioned as indoor and outdoor cultures in a dedicated laboratory facility at Vale Europe Ltd. (Clydach nickel Refinery, Glais Road, Clydach, Swansea SA6 5QR). For this purpose, autoclaved reverse osmosis (RO) water (20 min at 121°C, Classic Prestige Medical, UK) was used to prepare small scale cultivation in flasks (ranging from 250 mL to 1 L) together with 0.2 g L^{-1} SP commercial medium (Cell-hi SP™, Varicon Aqua, UK), 5 g L^{-1} of sodium bicarbonate (Fischer Scientific, UK), and 5 g L^{-1} of salt (sodium chloride, Fischer Scientific, UK).

The cultures were grown in 250 mL and 1 L flasks indoors with approximately 300 μmol m^2 s^{-1} light irradiance from cool white fluorescent light tubes (T5HO, SunBlaster, UK) perpendicularly located to the culture, with a light:dark cycle of 18:6 h, light irradiance was recorded using a light meter (Walz Model ULM-500). The temperature in the laboratory was constant at 20 ±3°C, however, the temperature of the culture was maintained at 30 ±3°C with the use of heat mats (Brewing Mate, UK). Mixing of the culture was achieved by bubbling air into the solution 24/7 from an air compressor (ACO-9810 HAILEA, China).

After scaling up the culture from 250 mL to 1 L, the algae were inoculated into 25 L carboys, previously chemically sterilised by the use of sodium hypochlorite (0.5 mL L^{-1} – 24 h) and neutralization by sodium thiosulphate (0.2 g L^{-1}), both chemicals obtained from Fischer Scientific UK. The conditions of the cultivation were as previously described.

2.3. PBR design

The cube PBR design was based on the structure of an IBC tank [1000 L – 1200 mm (L) x 1000 mm (W) x 1160 mm (H)]. The lid was cut from

the top of the tank and a metal box frame structure was introduced (placed) inside of the cube reactor (Fig. 1, A). This structure is custom made from 10 mm square metal frame, welded to generate the required construction as depicted in Fig. 1 A. The approximate dimensions of the frame are 1 m x 0.65 m x 1 m square with the support strut elevated by 10 cm from the base and lowered by 10 cm at the top. This frame supports the light panels in place such that eight light panels are spaced 12 cm apart. The aeration system consists of a main feed tube (1inch PVC) that runs down the edge of the vessel and splits at the bottom the tank and then forms two branches that run along the perimeter/edge of the

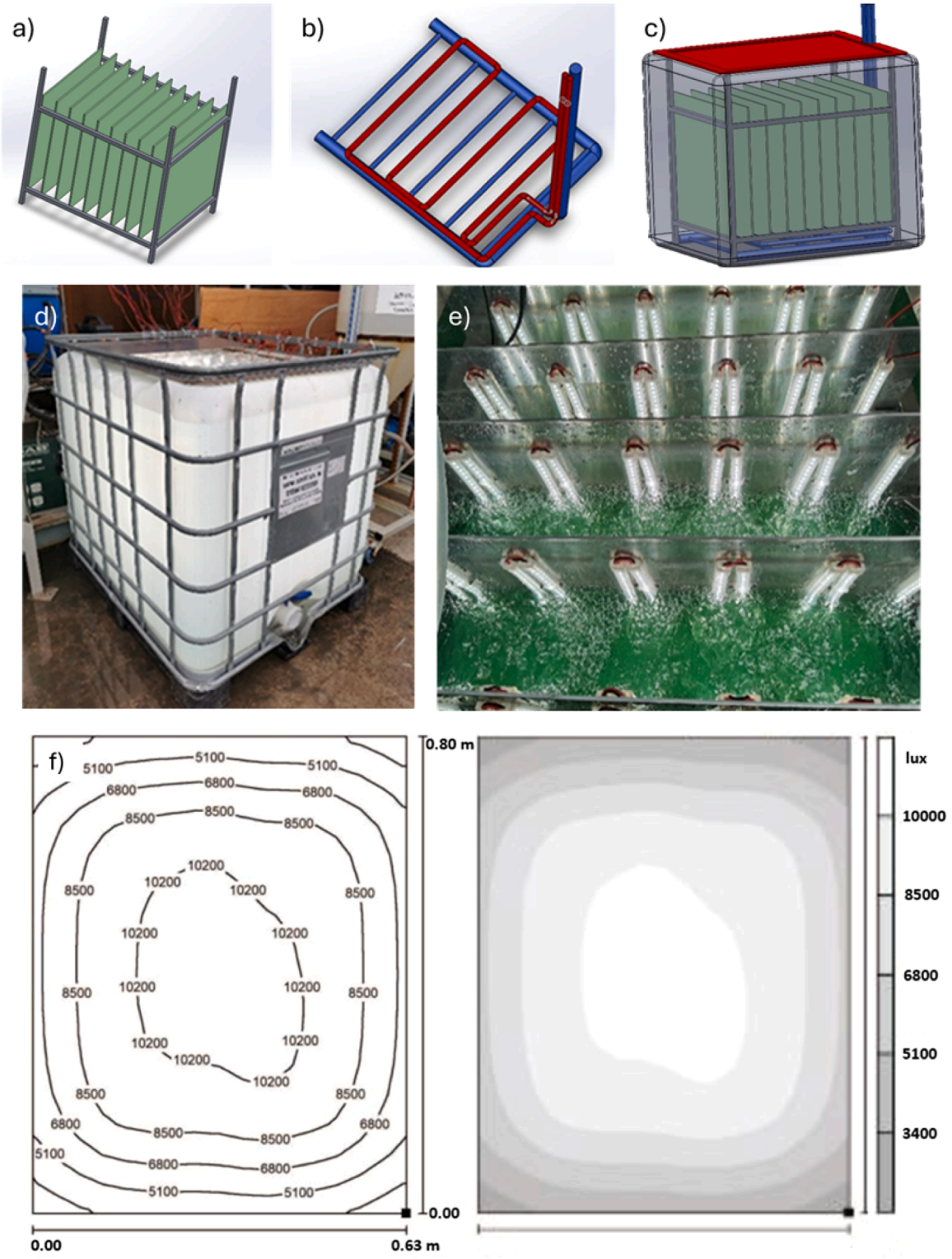

Fig. 1. The Cube PBR reactor designed for this study. a) a schematic representation of internal steel box frame with aluminium panels inserted; b) the PVC aeration pipework (blue) and the steel heat transfer pipe (red) inserted to the bottom of the tank; c) the assembled tank with all components inside and the lid at the top; d) a photograph of the IBC tank used from the outside; e) a photograph looking inside the reactor illustrating the panels, aeration, and LED light configuration; f) light intensity distribution modelling indicating surface profile (lux) of the panel.

vessel in a square U shape. Between the two legs of these main pipes are five 0.5 inch PVC tubes inserted in parallel. Each of the five tubes are 20 cm apart with the first tube placed between panel 1 and panel 2 of the lighting system. Thus, the first aeration tube is placed in the centre of the first two light panels, and the rest of the aeration tubes are then located between the subsequent light panels such that every other channel between the panels is aerated. This creates a series of airlifts around each panel. Each aeration tube has a series of 2 mm holes drilled into them and the whole structure is connected via the feed line to a 200 L min^{-1} (0.15 bar) aeration pump (ETD 200A, Charles Austin Pumps LTD) (Fig. 1b, blue pipework). The air flowing into the reactor is supplemented by 2 %CO_2 from the nickel refinery. This CO_2 emanates from steam reforming of methane and is purified by pressure swing adsorption to greater than 99 %. The residuals in this stream will be carbon monoxide (CO) and trace amounts of hydrogen (H_2). The heating-cooling system follows a serpentine construction with four parallel pipes traversing the base of the tank. Each parallel tube is set 20 cm apart from the next and the tubes are offset to the aeration tubes, see Fig. 1b red pipework. Each of the heat transfer tubes are 0.5inch stainless steel (SS316L), within which heat transfer fluid is pumped from a recirculating heater/chiller system (F250, Julabo, UK) according to a set temperature. The light system consists of aluminium panels with dimensions of 800 mm (H) x 630 mm (W) x 3 mm (T) where 11 LED strips of lights (Epistar chips, CRI 90) are attached to each side of the panel. The lighting system consisted of a 700 mm length LED Strip of 12 V, 14.5 W and a Cool White Neutral White CCT 4000 K providing 4000 μmol m^2 s^{-1}. The lights were placed in a zig-zag arrangement (see Fig. 1e) and simulation of the configuration suggested that the Emax was 10,761 lx, Emin was 2370 lx, and the Eave was 7999 lx, see Fig. 1f. Light irradiance was recorded using a light meter (Walz Model ULM-500) (Fig. 1, c). Each panel was placed inside of the tank with a distance between them of 120 mm, a total of 8 internal LED panels were used in this design, in essence splitting the tank into 10 compartments. Note that the LED plates are located centrally in the vertical direction such that the liquid phase flows around the plate, similar to the operation of an airlift. However, the working volume of the PBR in this study was limited to 900 L to ensure that the gassed liquid did not overflow the vessel. This was not the case and future work could increase operational liquid volume to 1000 L. Once all of the internal components were inside the tank, a metal lid was placed over the resulting hole such that the tank was sealed and effectively closed to the environment, indicated in red Fig. 1c. Algae is harvested from the tank by use of the base valve, i.e. simply open the valve and algal solution can be withdrawn into a bucket. Replenishment of the reactor fluids was achieved by temporarily removing the lid and pouring in. Temperature and pH in the tank are monitored using a Hanna marine water monitor (HI-981,520, Hanna Instruments, UK) with a HI-1286 pH Electrode probe and an EC temperature probe. On completion of the tank set-up, the lid was modified to include an insulation layer and placed back on the top of the vessel. This was done to avoid both heat and moisture loss through the top of the vessel.

This new reactor offers several advantages, namely:

- Extremely low-cost construction.
- Durable materials that avoid corrosion in saline environments.
- Integrated lighting that avoids diurnal cycle and climatic conditions.
- Direct contact LED lighting that avoids light attenuation.
- The light path is 5 cm which avoids light shading.
- The aeration system introduces CO_2 and creates appropriate mixing.
- The temperature control system maintains culture conditions.
- Simplistic design means simple operation and directly scalable.

2.4. Culture conditions in the Cube PBR tank

The industrial location of the Cube PBR was inside a non-heated polytunnel greenhouse at the Vale Europe site. Inoculation of the Cube was with a total volume of 150 L (6 carboys of 25 L) and represented 16.7 % of the total 900 L operational volume. The initial Spirulina culture concentration in the Cube was 0.102 g L^{-1} dry weight. The set conditions in the bioreactor were similar to those explained at the laboratory scale. Temperature was kept at 30 ± 3°C by manual adjustment of the heater/chiller according to the exterior temperature and heat rise from the LEDs. The water for the cultivation was pre-treated by Reverse Osmosis (RO) and mixed to form 5 g L^{-1} of bicarbonate and 5 g L^{-1} of salt. The microalgae were consistently fed with 0.2 g L^{-1} of SP medium to maintain a concentration average of 205.87 mg L^{-1} of total nitrogen and 25.6 mg L^{-1} of phosphate, checked daily with the Total Nitrogen kit (LCK338, Hach, Germany) and phosphates with the MQuant® Phosphate test (HC985964, Germany). Aeration (200 L min^{-1} - 0.15 Bar) was provided 24/7 by an air pump (ETD 200A, Charles Austin Pumps Ltd., UK), this is the equivalent of 0.22 vvm. LED lights (SIRIM, Malaysia) were used in a light: dark cycle of 24:0. The pH was not adjusted but monitored throughout the experiment. All parameters were constantly monitored and recorded (at least 3 times a day). The cultivation was maintained for a total period of 78 days with three growth cycles, i.e. the algae was harvested 3 times (days 25, 48 and 78) to check algal biomass composition at different stages of the cultivation process. Each harvest removed 800 L of culture which was replaced with fresh cultivation media and prepared water. During the third growth cycle, nutrient replenishment was intentionally no applied, and the culture was maintained until the onset of the decline phase to evaluate biomass composition under nutrient-stress conditions.

2.5. Methods of analysis (biotic parameters)

2.5.1. Dry weight (DW)

The concentration of algae as dry weight was measured 5 times a week, according to the Sorokin protocol (Sorokin, 1973). Whatman filters (47 mm diameter and 0.22 μm GF/C) were weighed using an SLS SR-250AZ precision balance (LAB PRO, UK) prior to filtration. Oven dried filters were obtained by placing in an oven (Genlab DC125, UK) at 80°C for at least 4 hours or until constant weight. Culture samples of 20 mL were then filtered in a Buchner funnel using a vacuum pump (RS-1, VEVOR, UK). After filtration, the wet filters containing the algal cake were placed back in the oven at 80°C for at least 12 hours. After this time the filters were weighed again and the difference in weight between the initial dry filter and filter with cake was calculated according to the following formula:

$$DW = (Y - X)\frac{1000}{V} \quad (1)$$

where: DW is the dry weight of the sample (g L^{-1}), X = weight of clean dried filter (g); Y = weight of filter after biomass recovery and drying (g); V = culture sample volume (mL).

2.5.2. Optical density measurements (OD)

Optical Density was used as a proxy for the biomass concentration of the cultures. Absorbance at 750 nm wavelength was recorded using a spectrophotometer (DR3900, Hach, Germany).

2.5.3. Growth rate (μ)

The culture specific growth rate (μ) was calculated by the following equation:

$$\mu = \frac{1}{t} Ln\left(\frac{X}{X_0}\right) \quad (2)$$

where μ is the specific growth rate ($days^{-1}$), t is the time difference for the sample taken (days), Ln is the natural logarithm, X and X_0 are the measured dry weight (g L^{-1}) or absorbance at 750 nm at the initial time (X_0) and the final time (X).

2.5.4. Duplication time (DT)

The duplication time (DT) or doubling time is the time (days) needed for the culture to double in concentration and is calculated as:

$$DT = \frac{1}{\mu} Ln(2) \quad (3)$$

where Ln is the natural logarithm and μ is the specific growth rate obtained from Eqn. (2).

2.5.5. Biomass productivity

Biomass productivity (P) is the difference between the DW of the sample and that of the previous day. Results are given in mg L^{-1} day^{-1}.

$$P = \frac{X - X_0}{t} \times 1000 \quad (4)$$

where X and X_0 are the measured dry weight (g L^{-1}) at the initial time (X_0) and the final time (X), t is the time (days).

2.6. Biomass composition analysis

All analysis was performed in triplicate with the average reported. Moreover, biomass from the seed carboy cultures, from an in situ tubular reactor with natural lighting, and commercially obtained biomass (from Terrafertil and Naturya wholesales companies) were analysed for comparative purposes.

2.6.1. Pigments

Phycocyanin (PC), was extracted using a modified version of the method developed by Coward et al. (2016). Culture samples were taken in triplicate at different experimental times (days 0–3, 7, 15, 22) of the 3 experimental cycles and on day 25 of the final cycle. A sample of 1 mg dry biomass was weighed (KERN ABS 80–4 N, Germany) for the analysis. The sample was mixed with 3 mL of 0.1 mol L^{-1} phosphate buffer (pH = 6) in a 15 mL falcon tube. The sample was then placed in a -20°C freezer for a minimum of 2 h until frozen. The sample was then thawed at room temperature and sonicated in an ultrasonic bath (XUBA3, Grant, UK) with ice for 10 min. Finally, the samples were vortexed for 5 mins and placed back into the -20°C freezer. This process was repeated 5 times. Following extraction by cell disruption in freeze-thaw cycles, the sample was centrifuged at 4700 rpm for 10 mins at 4°C and the supernatant was used to determine PC concentration using a spectrophotometer (UV-2600, Shimadzu, UK), with the measurements at 592, 618 and 645 nm.

The concentration PC was determined using the equations of Beer and Eshel (1985):

$$PC\left(mgmL^{-1}\right) = [(OD_{618nm} - OD_{645nm}) - (OD_{592nm} - OD_{645nm}) \times 0.51] \times 0.15 \quad (5)$$

where OD is the optical density of the pigment at the given wavelength. The value obtained from Eq. 5 was then expressed in mg g^{-1} by normalisation to the original dry mass of algae used in the analysis.

2.6.2. FTIR spectroscopy measurements

The bulk biochemical composition of the algal biomass was assessed by Fourier Transform Infra-Red (FTIR) spectroscopy using the method developed by Mayers et al. (2013). Measurements of FTIR attenuated total reflectance (ATR) spectra were made using a PerkinElmer Model Spectrum Two (Perkin Elmer, UK), equipped with a diamond crystal ATR reflectance cell and a DTGS detector scanning over the wavenumber range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1}. The prepared algal biomass (approximately 5 mg) was placed into the surface of the crystal and then pressed onto the crystal head. Each sample was measured in triplicate using 12 scans for each run. The background correction for the scans was made using ambient air. Scans were recorded using the spectroscopic software Spectrum (version 10. Perkin Elmer, UK). As well as bulk materials, the output from the scans was capable of identification of carboxylic acid functional groups, primary amines (amide 1) and secondary amines (amide 2).

2.7. Energy measurements, CAPEX, and OPEX

All stages of the microalgae cultivation from laboratory to the CUBE were evaluated to provide an overall understanding of the energy requirements to run the new reactor. Capital investment (CAPEX) was determined by simple addition of the costs for each component of the new reactor. In addition, the capital investment for the downstream process was taken from actual equipment purchases, the details of which other than costs are not relevant to this paper. Operational expenditure (OPEX) was made by consideration of the electricity consumption for the process. For this purpose, a standard energy meter (MECHEER, UK) and an industrial (230v 16A) kWh MID-approved power meter (Tough Leads Ltd., UK) were used. The energy meter was connected for at least 7 days to the equipment to determine the daily consumption of electricity and therefore to extrapolate the monthly and annual consumption rate. Note that the CUBE was placed inside a controlled environment, so annual temperature fluctuations will not impact the energy usage. Energy running costs were calculated according to the energy measurements and local energy prices.

3. Results and discussion

The following results were obtained and demonstrate that the design of the new Cube PBR was validated by successful algal growth and biomass quality.

3.1. The abiotic parameters

3.1.1. Temperature and pH

The temperature and pH recorded during the cultivation period is illustrated in Fig. 2.

Maintenance of the temperature helped to avoid stress of algal cells and helps to grow the culture in optimal conditions. As shown in Fig. 2b, temperature was maintained and measured consistently during entire experiment. Following initial start-up, the data demonstrated a few minor perturbations where the outside temperature fluctuated due to local weather conditions (minimum 12°C and a maximum of 37.1°C), however, the temperature inside the tank remained relatively constant during the whole cultivation period at 30 ±3°C. Two large decreases in temperature are noted at day 25 and 48. These decreases in temperature were detected following harvest, this was caused by the lower temperature of the fresh media replacing 800 L taken from the tank. In future operations, pre-conditioning of the fresh media could remove this issue. Obviously, manual control of the temperature is not perfect, inclusion of an automated temperature control loop would be beneficial and could keep the culture to ± 0.5°C, with an obvious cost associated. However, the two major perturbations recovered in less than 48 h and demonstrate that the heating/cooling system was adequate to maintain homeostasis during the operation. The pH was monitored at 10.2 ± 0.52. The pH was not adjusted throughout the experiment as the culture was maintained under constant aeration, so this parameter represents a direct correlation with observed growth due to photosynthetic activity. Perturbations are observed on harvest days when the culture media was replaced with fresh media, seen by a slight lowering of the culture pH on days 25 and 48. Research by Shi et al. (2016) demonstrated Spirulina platensis growth while varying different parameters and found that the highest growth occurred at 30°C and pH 9.5–10.0, the same parameters used in this study.

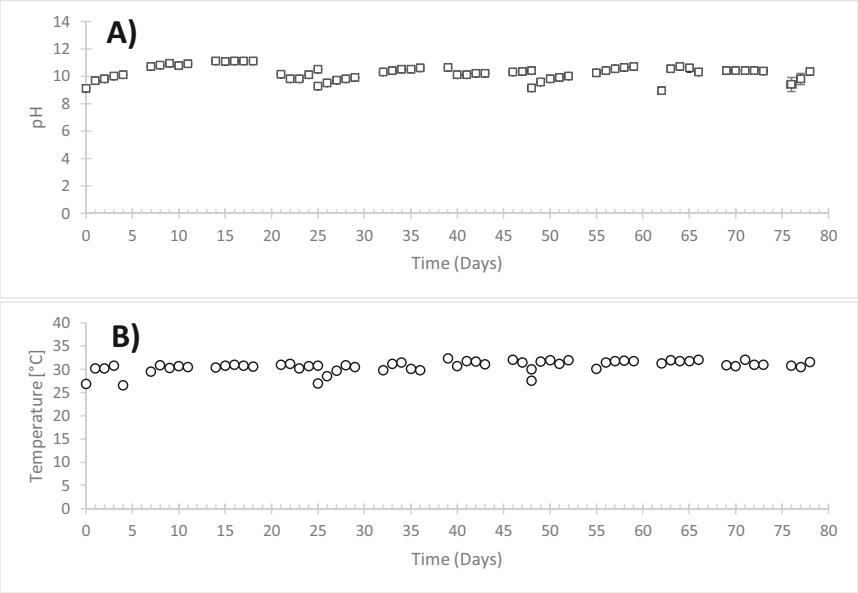

Fig. 2. Overview of the abiotic parameters during the 78-day experimental trial. (A) pH variation with time and (B) temperature variation during the cultivation process.

3.2. Biotic parameters

3.2.1. Algal growth rate

As shown in Fig. 3, both the optical density measurements and dry weight analysis show good correlation across the experimental period. When directly compared, an equation linking the two parameters is obtained as DW = 0.7392OD (R^2 = 0.992). For cycle 1, the biomass increased from the inoculation concentration of 0.1 g L^{-1} (0.05 OD) to a maximum of 1.37 g L^{-1} (1.6 OD) for first 24 days. The growth at the beginning of this period is clearly exponential (linear increase in biomass) and then alters trajectory at day 11. The growth in this second stage is still relatively linear and indicates that the culture is limited, this is most likely light limitation caused by the culture density increasing and the organism is self-shading. The maximum specific growth rate was calculated from the first few days of growth as 0.384 $days^{-1}$, which represents a doubling time of 1.81 days and productivity of 72.3 mg L^{-1} day^{-1}. Interestingly, the growth rate calculated from the second stage is 0.020 $days^{-1}$, which is significantly lower than the initial growth rate. The culture was then harvested on day 25. Growth cycle 2 followed a similar growth pattern to that of cycle 1, although in this case the culture achieved a maximum concentration of 0.9 g L^{-1} (1.3 OD). The maximum specific growth rate was calculated as 0.543 $days^{-1}$. Two of the lighting panels failed during the experiment at this time and the lower cell density was attributed to the lower light intensity now existing in the reactor. Similar to cycle 1, the growth appears to change trajectory at day 35 (day 10 of this cycle). Again, this could be attributed to the culture density increasing and causing light limitation, with the difference in absolute concentration resulting from the lower light intensity. This material was harvested on day 48. During growth cycle 3, similar growth behaviour was initially observed, and the culture was maintained until the biomass entered the decline (death) phase. In growth cycle 3, nutrient replenishment was not applied, and cultivation was

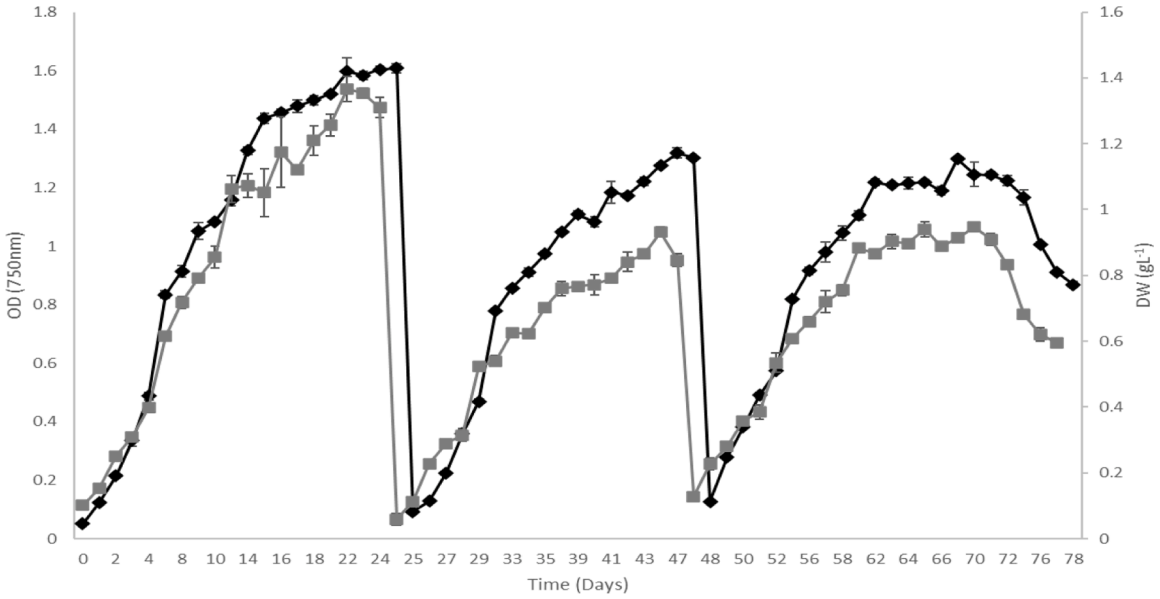

Fig. 3. *Arthrospira platensis* CCMP1295 growth as measured by optical density (OD,) at 750 nm and dry weight (DW, g L^{-1},) over the cultivation period of 78 days.

continued until the culture entered the decline phase, with the exponential, stationary, and death phases clearly distinguishable. The exponential phase was observed between days 48 and 59, the stationary phase between days 59 and 71, and the death phase between days 71 and 77, with final harvest on day 78. Maintaining the culture until the death phase was conducted to analyse the resulting biomass and determine if the cellular content and metabolites generated were different from that of the exponential growth phase. During each growth cycle there was no observed adaptation phase (lag phase), which shows that the reactor parameters were constant and in optimal conditions for growth. This avoided crashes, stress, and contamination issues affecting algal culture growth crush. The growth parameters associated with the three production cycles were calculated and are available in Table 1.

The first two harvests (day 25 and 48) produced 1794 g (1048 g and 746 g respectively) of Spirulina biomass, and the third (if harvested at its maximum peak - day 71) provided 755 g, giving a total of 2549 g or 2.55 kg. This is the equivalent of 0.0359 kg (algae) day^{-1} and 0.040 kg (algae) day^{-1} m^{-3} (reactor). This equates to an annual production rate from the Cube of 14.56 kg (algae) $year^{-1}$ m^{-3} (reactor). This could have been improved by harvesting more conservatively; reducing the harvest volume and increasing harvest frequency to maintain the species in the higher exponential growth phase for longer.

In the study by Shi et al. (2016) there was a focus on variation of light wavelengths and how this affected growth. They discovered that the best light to grow Spirulina was red with a maximum dry matter content of 1.346 g L^{-1} and a combination of red/blue light could enhance this growth to 1.518 g L^{-1}. The study used small scale (<1 L) at laboratory conditions compared to this study (~1000 L) at the industrial setting and achieved only 0.859 g L^{-1} when using white fluorescent lights in the control group. In this study, the growth was 1.37 g L^{-1} using white LEDs, similar to using the red light and slightly lower than the combination of lights.

When Spirulina is grown in raceways, Wang et al. (2021a) reported maximum growth of 66 mg L^{-1} day^{-1} in a 20-m^2 raceway pond and a maximum of 36 mg $L^{-1}day^{-1}$ in a 605-m^2 raceway pond (193.6 m^3). The cube PBR was able to achieve an average of 75.72 mg $L^{-1}day^{-1}$ in 1 m^3, therefore achieving 14.7 % more productivity than the best scenario (20 m^2 raceway) and 110.3 % more than the large-scale raceway (605 m^2). The cube offers a much higher aerial productivity than raceways or even closed PBRs as 4 CUBE reactors may be stacked, i.e. 4 m^3 per 1.2 m^{-2} = 3.33 m^3 m^{-2}; this means that the aerial productivity per m^2 could be 252.4 mg $L^{-1}day^{-1}$ (up to a maximum of 268.3 mg $L^{-1}day^{-1}$). The 605 m^2 raceway has a working volume of 193,600 L, therefore, this gives an aerial productivity of 4.2 kg $year^{-1}$ m^{-2} compared to an average of 23.0 kg $year^{-1}$ m^{-2} in the cube, being 92.1 kg $year^{-1}$ m^{-2} when 4 cubes are stacked. This is an increase of 87.9 kg $year^{-1}$ m^{-2} and a percentage increase in aerial productivity of ~2000 %, which is significant.

In closed systems, Stunda-Zujeva et al., (2023) reported a maximum specific growth rate in Arthrospira strains of 2.34 ± 0.07 day^{-1} using continuous lighting (cold white light) at 33 to 35°C in a small-scale PBR (0.5 L PET bubble columns). The growth rate achieved is much higher than in this study (0.419 day^{-1}), the operational volume was very low, and the inoculum was much more concentrated (2.6 g L^{-1}) compared to this study (0.102 g L^{-1}), which allowed the algae to adapt and grow more quickly from onset. Higher yields are expected when the inoculum concentration is higher; however, Stunda-Zujeva et al., (2023) also reported that most studies in the literature show a specific growth rate below 0.4 day^{-1}, which is in line with this study. In contrast to the CUBE PBR, these yields are obtained at low volume and are dependent on many factors, which are usually obtained under optimal conditions. However, the duration of these optimal conditions may be limited to space-time and may not be representative throughout the year, especially in high-latitude countries with low daylight hours. The CUBE design has achieved 1.37 g L^{-1} in a semi-continuous production run with no dependence on external factors. Therefore, the productivity and yields achieved can be considered constant throughout the year.

Table 1
A summary of the growth parameters for Spirulina using the CUBE reactor.

Parameters	Cycle 1	Cycle 2	Cycle 3	Average	Units
Growth rate (Maximum)	0.384	0.543	0.329	0.419	**d^{-1}**
Duplication time (Minimum)	1.81	1.28	2.11	1.73	**days**
Biomass productivity (Maximum)	72.3	80.5	74.3	75.72	**mg L^{-1} day^{-1}**
Biomass concentration (Maximum)	1.37	0.93	0.94	1.08	***g* L^{-1}**

An internally illuminated LED reactor was designed in the study by Ahangar et al. (2023), but the cultivated microalgae was *Chlorella sorokiniana* at a volume of 10 L. In this case, the authors reported a maximum production of 2.52 g L^{-1} in the best-case scenario with a similar inoculum concentration as used in this study (0.06 g L^{-1}). The design used mirrors in the walls of the reactor to reflect light and provide uniform light distribution for microalgal growth. This is an improvement that could be easily adapted to the CUBE design. They also reported concentrations of 1.61 g L^{-1} when the light was internal but without mirrors (similar to the Cube) and 1.95 g L^{-1} when the PBR was illuminated externally. Although the volume is low and the microalgae species is different, the production rate and concentrations reported are very similar to those obtained in this study. In addition, the Cube has demonstrated robustness in maintaining key parameters, such as pH and temperature, at optimal culture levels for the species used. This has prevented stress on spirulina and consequently avoided any visual appearance of biofilm. Furthermore, maintaining constant conditions is reflected in the absence of a lag phase following inoculation. In contrast and with a similar design to the CUBE, Erbland et al. (2020) experienced a lag phase of ~126 h with *Tetraselmis chuii*.

3.3. Biomass composition

3.3.1. FTIR

FITR measurements provide qualitative compositional analysis of the microalgal biomass composition for the different compounds of interest.

In the case of proteins, as can be seen in Fig. 4, the most abundant group of compounds composition generally increases throughout the exponential phase and decreases as the stationary phase approaches, reflecting the growth of the biomass. Although the protein levels remained relatively consistent across the entirety of cycle 3. The maximum percentage due to optimum conditions in the medium was recorded during the first cycle where a percentage of 54.1 % of protein was achieved. The first cycle is the most representative in terms of protein composition, the second and the third cycles are more homogeneous, most likely due to adaptation and light limitations in the Cube PBR. In the case of lipids, Spirulina, like most cyanobacteria, is a microalgae that is not associated with high lipid accumulation in normal cultivation conditions (Yalcin, 2020). Therefore, the variation observed across the growth cycles is much smaller than in the case of protein. In this case, optimal conditions were always achieved in the reactor and lipids were not accumulated. For carbohydrates, as expected and reported (Rosero-Chasoy et al., 2022), the composition is higher at the onset of growth, but in this case then remains reasonably consistent across the three cycles.

A comparison between the Spirulina biomass composition from the culture produced in this study and the various alternative Spirulina biomass indicates some differences, see Fig. 5. The measured protein level in the Cube reactor product is 46 % (average over the 3 cycles) and significantly higher compared to the samples of commercial Spirulina biomass (34 %), this can be due to the freshness of the produced biomass as the other two samples show similar protein levels, with the carboy at 44 % and photoautotrophic tubular PBR 42 %. The other cellular components are quite similar across all the samples, with both commercial

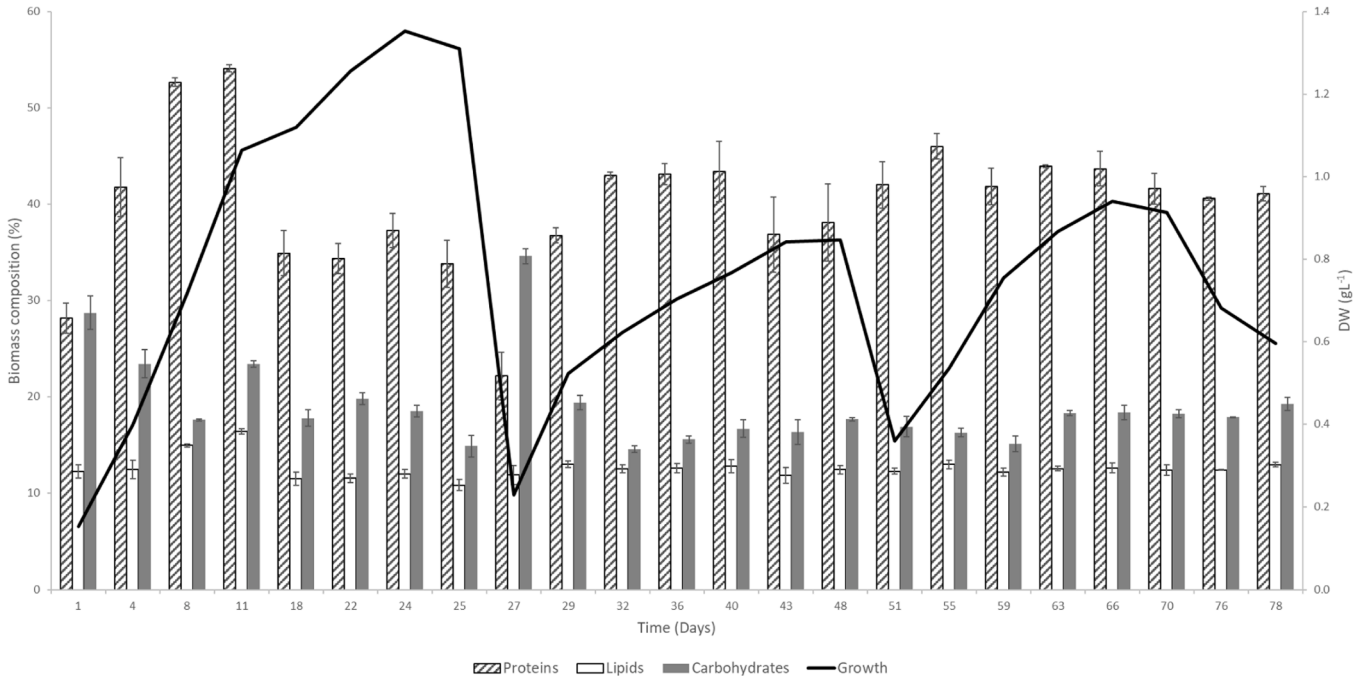

Fig. 4. Spirulina composition from FTIR measurements for the three CUBE cycles. () Proteins, () lipids, () carbohydrates, and () growth (DW, g L^{-1}).

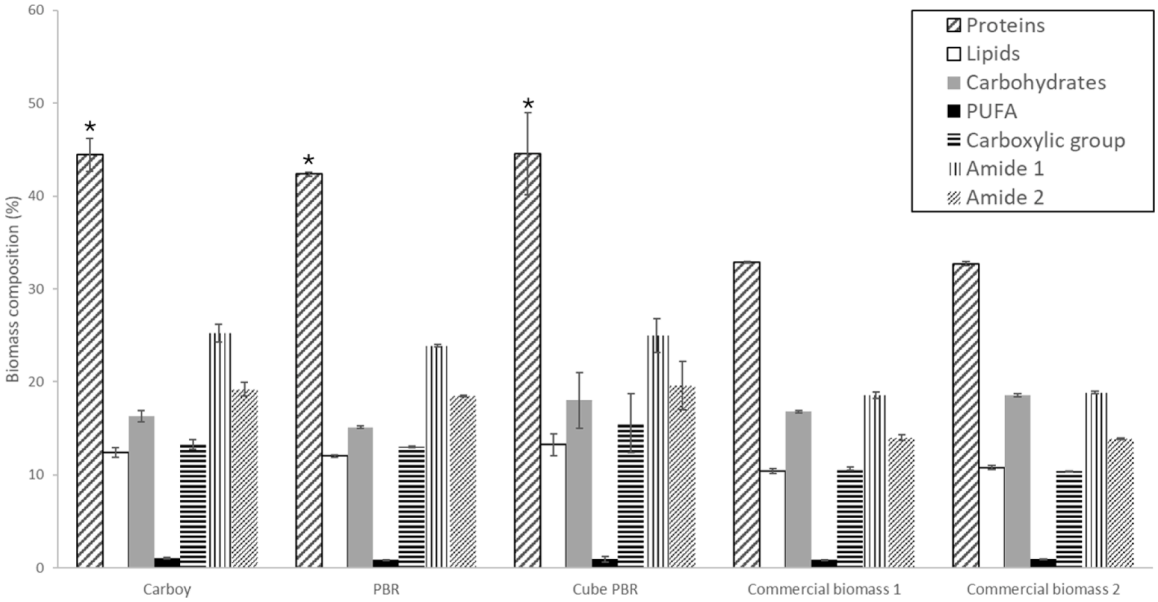

Fig. 5. Comparison of the biomass composition obtained from the Cube PBR (average from each of the three cycles) against fresh carboy and PBR cultivation, as well as two purchased commercial spirulina samples. () proteins, ()lipids, () carbohydrates, () polyunsaturated fatty acids (PUFA), () carboxylic group, () amide 1 and () amide 2.

samples being slightly lower than that of the fresh biomass. A study with similar cultivation parameters and using Spirulina sp. was conducted by de Jesus et al. (2018) in a 240 L raceway at two locations in Brazil. The first site achieved a maximum of 1.6 g L^{-1} from a 0.5 g L^{-1} seed culture and a productivity 54 mg L^{-1} day^{-1} (this study 1.37 g L^{-1}, 80.5 mg L^{-1} day^{-1}). The maximum protein composition was reported as 62.97 ± 1.45 % compared to this study as 54.1 ± 0.35 %. The second site achieved only 0.9 g L^{-1} culture density, 9 mg L^{-1} day^{-1} productivity, and 53.6 % protein. This suggests that while the productivity is higher in the cube PBR, there is still potential to improve the protein content further. Protein concentration is important as this provides higher value to the biomass. The reported maximum level of 54.1 % protein in this study is typical of that observed in many studies, with ranges from 46 to 75 % in special circumstances reported (Al Hinai et al., 2019; Lupatini et al., 2016; Avila-Leon et al., 2012). Ramírez-Rodrigues et al. (2021) report a concentration of 47 % protein content and describe this result as a promising sustainable source of protein for highly nutritional food product prototypes. This protein concentration makes spirulina one of the most value sources of protein and shows excellent performance as a functional ingredient due to higher digestibility (up to 78 %) when compared to other microalgal sources (Quintieri et al., 2023). The results from this study demonstrate that the biomass was not significantly different to that of commercially purchased biomass (although higher in protein content) and the algal culture produced in the cube is of high

quality. This has the potential for commercialisation and may be used for various food, cosmetic, and nutraceutical applications.

3.3.2. Phycobiliproteins content - Phycocyanin

As shown in Fig. 6, the PC increases according to the microalgae growth over time, reaching maximum concentration before harvesting. The maximum concentration achieved was at day 22 and 70, representing 7.83 % and 7.34 % or 78.27 mg g^{-1} and 73.41 mg g^{-1} of biomass respectively. Particularly relevant is the fact that the concentration of phycocyanin increases with the growth of the microalgae from day 0 to the first peak at day 22. After each harvest the concentration slightly decreases, but not as much as at the initial point of cultivation, reaching the highest peaks at the maximum growth points in the three cultivation cycles before harvest. Also of note is the fact that while the culture density was lower in cycle 2 and 3, the PC concentration remained at similar levels.

When comparing the phycocyanin content of culture produced in the Cube PBR (Fig. 7) with the content produced using other cultivation equipment or commercial Spirulina biomass, there are clear differences. The phycocyanin content level in the cube reactor is 58.6 % and 38.12 % higher than in both the commercial biomass samples, and 35.47 % higher when compared to the other large-scale system (Tubular PBR). To put this in context, de Jesus et al. (2018) achieved a PC content of 311 ±0.001 mg g^{-1} and 73.74 ±1.96 mg g^{-1} for their site 1 and site 2 respectively. The increased PC content at site 1 was attributed to higher growth temperature, which was ~30°C on average. PC content is associated with growth temperature but also with specific light wavelengths available for capture and photosynthesis. This may explain how similar growth parameters using two different light sources (sunlight and LEDs) could allow the phycocyanin content to vary. This indicates that there is the potential to use LEDs with more specific wavelengths to enhance the productivity of phycocyanin. A study growing Arthrospira platensis (Rizzo et al., 2015) using a white 50–150 µmol m^{-2} s^{-1} fluorescent lamp reported PC content of 7–10 % of dry biomass. This a similar result as obtained in this study, although the light intensity was lower. A study (Yim et al., 2016) with internal LED lighting looked at the effects of different wavelengths on the growth of *Arthrospira platensis* and phycocyanin content. A maximum growth rate and maximum biomass density of 0.39 $days^{-1}$ and 0.10 g L^{-1} day^{-1} was achieved respectively, at 1000 µmol m^{-2} s^{-1} using red LEDs and 0.36 $days^{-1}$ and 0.08 g L^{-1} day^{-1} for white LEDs. In terms of phycocyanin content, a maximum of 0.2 mg mL^{-1} in 2000 µmol m^{-2} s^{-1} was achieved when using green, red or white LEDs. Lima et al., (2018) reported a biomass productivity of 148 mg L^{-1} day^{-1} and maximum phycocyanin concentration of 0.168 mg mL^{-1} using LEDs that had a 70 % red and 30 % blue composition and a light intensity of 100 µE m^{-2} s^{-1}. In this study, 0.04 mg mL^{-1} was achieved in a significantly higher volume (900 L compared to 250 mL) and with no specific wavelengths to induce phycocyanin accumulation. In addition, the reported literature studies used an alternative phycocyanin extraction methodology, which may also account for some of the difference observed.

3.4. Energy requirements, CAPEX, and OPEX

The major construction costs for the CUBE PBR are outlined in Table 2.

As can be seen in Table 2, the most expensive item in the reactor construction was the lighting system. LED lights are currently not widespread for underwater applications and those used were proprietary technology and relatively expensive. Given time, this cost is expected to significantly reduce (Olajiga et al., 2024). The second most expensive item was the heater/chiller, which in this case was only used for temperature control of the reactor. However, if the Cube is housed within a true climate-controlled environment, i.e. a warehouse or greenhouse where the temperature is set to that of the culture, then this equipment may not be required, or the expected duty may be significantly reduced. The total for producing the CUBE in this case was £12, 776.60 and is the equivalent of 1 m^{-3} reactor operational volume. If the system was housed in a climate-controlled environment, then this overall cost could be reduced to below £10,000. This cost may decrease further as Cube technology, such as immersive lighting, becomes more affordable in the future. Similarly, in the case of a large-scale installation, the CUBE reactors may be stacked vertically and horizontally in close proximity. This configuration would lend itself to large scale automation and a central heating/chilling system, both of which are not accounted for here. In addition to the reactor, in order to produce algae as a product there will be additional equipment required. The algae will need to be harvested from the reactor, dewatered to remove excess fluid, and then dried. A filtration rig suitable for harvesting/dewatering process is included. Dry biomass is not a proposed option for this analysis, so no equipment such as a spray dryer has been included. Further downstream processing to produced dried pigments will also require a freezer (included for freeze-thaw cycles and storage of product), a filtration platform for pigment isolation and purification, and a freeze dryer. Appropriate costs for this equipment have also been included. For the non-reactor items, the costs included are real world in the sense that they reflect actual equipment purchases, however, item specific details have not been included as the equipment was not physically used in this study. This results in a total CAPEX expenditure of £75,898.10 for the as built installation.

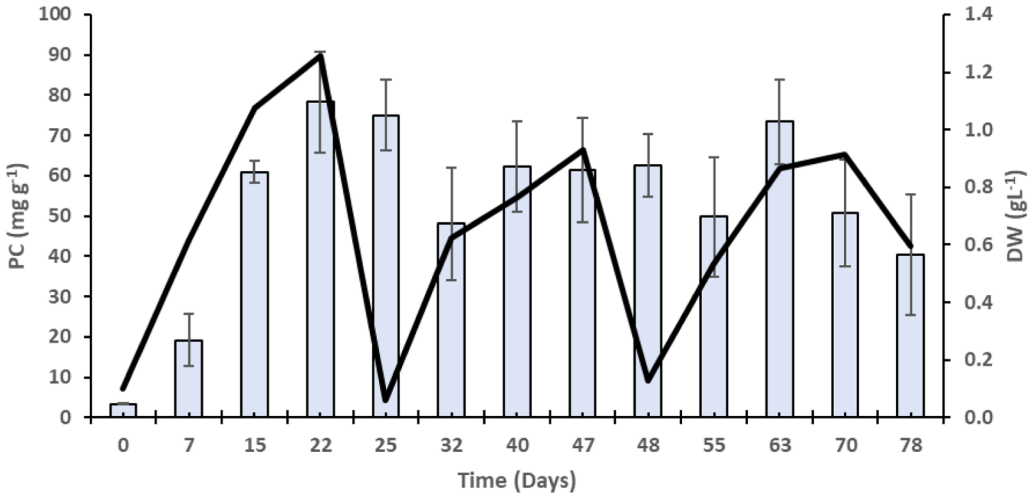

Fig. 6. Variation of Phycocyanin (PC,) concentration (mg g^{-1} dry biomass) across the experimental period overlaid with growth () (DW, g L^{-1}).

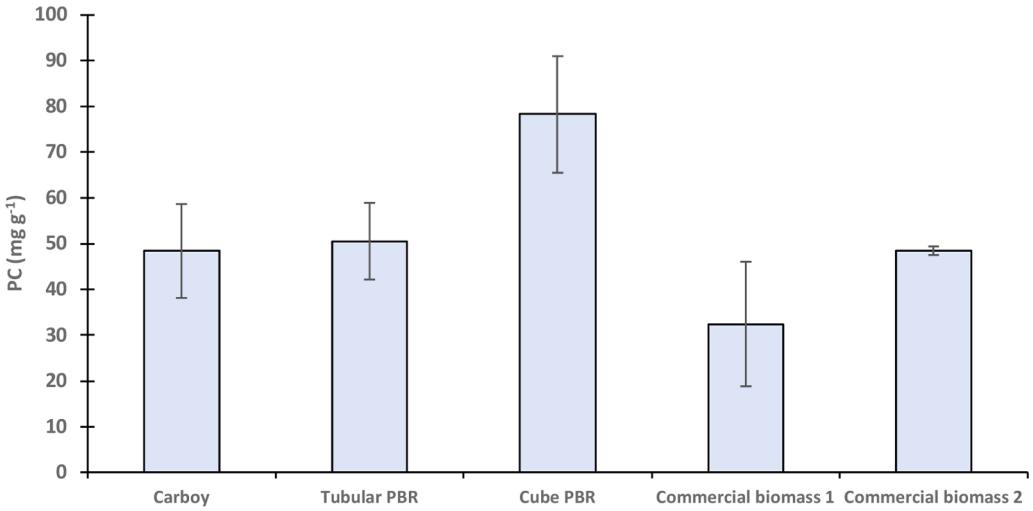

Fig. 7. Comparison of Phycocyanin content (PC) in mg g^{-1} from the Cube PBR against alternative Spirulina sources.

Table 2
Major capital expenditure (CAPEX) costs for the Cube PBR.

Capital Expenditure - CAPEX			
	Price (£)	Quantity	Total (£)
LED Lighting panels	1092.80	8	8742.40
Air pump	300.00	1	300.00
Probes and monitoring	313.81	1	313.81
Heater/Chiller system*	2956.00	1	2956.00
Tank internals and lid	250.00	1	250.00
IBC Tank	214.39	1	214.39
Reactor Total			**12,776.60**
Harvesting rig			37,450.00
Freezer			500.00
Pigment isolation			11,575.00
Drying			13,596.50
Total			**75,898.10**

* Chiller used was over specified, a more appropriate model price included here.

The electrical consumption of the different items in the CUBE PBR are outlined in Table 3. When analysing the energy consumed for the cultivation, the lights and the heater/chiller are, as expected, the most energy-consuming parts of the microalgae cultivation process, with daily consumptions of 32.64 and 5.808 kWh, respectively. In total the equipment consumes 14,708 kWh annually. With an annual production rate of 14.56 kg (algae) $year^{-1}$, this equates to 1010 kWh kg^{-1} algae. Similarly, this figure represents three full growth cycles. However, for an industrial production system the harvesting would be optimised for highest productivity, which would maintain the culture in exponential growth, i.e. more frequent harvesting at lower culture densities. This would have an impact on the energy cost per kg of algae.

According to the Department for Energy Security and Net Zero (2023), the cost (£) per kWh for industrial small band Industries with an annual consumption between 20 and 499 kWh in the UK is £0.2729. Labour, nutrient consumption, and consumables has been factored in on an annual basis. The labour charge is based on an activity mapping for the system estimating the required hours needed on a weekly basis for general operations. The expected annual hours required by a full-time equivalent (FTE) employee is 1040 hours. A standard FTE working years in the UK is 2000 hours and a typical salary is £35,000 per annum full cost. Therefore, the cost to the business for labour is £18,200 per annum. The nutrient consumption is directly linked to the algae production rate and was 5.2 kg of nutrient for the 3-cycle run. Scaled to one year of operation this gives nutrient consumption as 24.3 kg and the price per kg is £27. Thus, the annual nutrient consumption cost is £657. Other consumables such as gloves, disposable sampling pots, filter papers, and cuvettes for analysis have been estimated from the consumption during the 3-cycle operation and scaled to one year of operation as £1000. A furether cost of £2500 has been estimated for maintenance and repair. Therefore, the total running costs associated with the equipment are £26,370.82 annually. This generates an algae cost of £1811 kg^{-1}.

Given this data, several scenarios can be generated to assess the economics of this new PBR design and subsequent downstream processes. Note that in these scenarios only the OPEX costs given in Table 3 are used Table 4.

A cultivation period of 11 months for production was considered which allocated a month for planned downtime (maintenance and cleaning of the equipment). Similarly, as the process is not reliant on

Table 3
Operational Expenditure (OPEX) costs of running the equipment according to energy use and prices (Department for Energy Security and Net Zero, 2023).

	Operational Expenditure - OPEX							
	kWh	Amount	kWh/Day	kWh/Week	kWh/Month	kWh/Year	Cost per kWh (£)	Total cost (£)
Lights	0.17	8	32.64	228.48	992.80	11,913.60	0.2729*	3251.22
Air Pump	0.075	1	1.80	12.60	54.75	657.00		179.30
Monitoring	0.002	1	0.05	0.34	1.46	17.52		4.78
Heater/Chiller system	0.242	1	5.81	40.66	176.66	2119.92		578.53
Total Electricity			40.30	282.07	1225.67	14,708.04		**4013.82**
Labour cost								18,200.00
Nutrients								657.00
Other consumables, repair and maintenance								3500.00
Total OPEX								**26,370.82**

* Considering Industrial Small band prices (https://www.gov.uk/government/statistical-data-sets/gas-and-electricity-prices-in-the-non-domestic-sector)

Table 4
Potential economic scenarios projected for the cube PBR system based on two harvests per week and a running period of 11 months per year.

1000 L Cube PBR		Units
Biomass production (Average)	1.08	g/L
Harvest	50 %	week
Period	11.00	months
Total biomass	51.70	kg/year
Biomass price[1]	20.00	£/kg
Revenue	1034.03	£
Electricity costs[2]	-3679.34	£
Other operational costs (labour, nutrients, consumables)	-22,198.55	£
Profit	**-24,843.86**	£
Pigment price[3]	10,000.00	£/kg
Total pigments[4]	7.15	kg/year
Additional production costs[5]	-1372.86	£
Revenue from pigments	71,503.18	£
Profit	**45,286.46**	£
Total running costs	-27,250.75	£
Profit from biomass	-24,843.86	£
Profit from phycocyanin	45,286.46	£
Payback (Biomass)	-	years
Payback (Phycocyanin)	1.68	years

[1] Average price for resale biomass according to Costa et al. (2019)
[2] Considering Industrial Small band prices (Department for Energy Security and Net Zero (2023), https://www.gov.uk/government/statistical-data-sets/gas-and-electricity-prices-in-the-non-domestic-sector).
[3] Conservative price of food-grade C-PC (£10,000 kg^{-1})
[4] Percentage of phycocyanin detected in our system (13 %)
[5] Production cost ($249.70 kg^{-1},~£192 kg^{-1}) of phycocyanin according to Chaiklahan et al. (2018)

sunlight, any seasonal factors or variations are negated. For example, highs and lows in atmospheric temperature are removed from production considerations as the entire production system could easily be housed in an empty warehouse or building and would require less footprint than a tennis court. For a continuous production run, harvesting 50 % of the reactor volume twice weekly is sensible (Ishika et al. 2021; Liu et al., 2018) and can actually improve the vitality of the resulting algae. Under this production schedule, a total biomass of 51.7 kg per year is generated. In an ideal environment, the resale value of spirulina biomass should be £20–30 kg^{-1}. Prices for biomass vary according to region with a global average price of £96.23 kg^{-1}, a minimum of £0.78 to £7.8 kg^{-1} in China, and a maximum of £203 to £290 kg^{-1} in Brazil (Costa et al., 2019). In this scenario, with a price of £20 kg^{-1}, losses of £24,843.86 are obtained from potential biomass sales alone. To break even in this scenario, then a sale price of ~£500 kg^{-1} would be required and is not feasible based on the higher global estimates for Spirulina retail prices. Obviously, to sell into these markets would require additional costs for shipping and duties etc. not factored here. Ultimately, when considering capital investment, there is no money to be made in this scenario. If the scenario was reimagined to include a situation where the energy costs were negated, i.e. onsite generation or co-location with industry that has excess energy available, then there is still no realistic way to make money.

Obviously, the biomass can be further processed to extract proteins or pigments and obtain products of higher added value. According to the maximum extraction results in this study, annual pigment production could be 7.15 kg $year^{-1}$. Mao et al. (2024) suggest that food grade PC pigment selling price is $0.13 mg^{-1} (£100,000 kg^{-1}) and analytical grade is $15 mg^{-1} (~£11.5 million kg^{-1}). Using this figure for food grade material, then the profit from pigment sales would be ~£690,000. However, pigment prices can vary. Literature data suggests the price for food grade pigment can be anywhere from £20,000 to £170,000 (Borowitzka (2013); Fernandes et al. (2023); Lauceri et al. (2023)). Pigment price depends on many factors such as market demand, purity, and the final product application. However, in most cases literature estimates do not reflect the real market value. Several retail companies (weblinks, 2025) selling food grade phycocyanin were evaluated and the cost was found to be in the range £16,800 kg^{-1} to £26,200 kg^{-1}. Thus, for the purpose of the scenario, a conservative estimate for phycocyanin cost was made as £10,000 kg^{-1}.

In this scenario, a profit from pigment sales would result in a net profit of £45,286.46. This assumes that all of the phycocyanin generated is harvested. However, a simple series of scenarios can be generated for less recovery efficiency leading to profits of £31,260 (80 % recovery), £24,247 (70 % recovery), and £17,234 (60 % recovery). In all cases, the plant demonstrates a positive bottom line. However, the phycocyanin content in algae can achieve up to 20 % of the dry weight (García-López et al., 2020; Dianursanti et al., 2018; Jaeschke et al., 2021), which would lead to a much better economic scenario than the 13 % used here. Another sensible potential bulk product from the PBR would be protein. Data related to the sale price of protein is very sparse in the literature. However, current bulk sale market prices for vegan or vegetarian protein are in the region of £40–120 kg^{-1} for vegetal proteins from pea, fava, or soybeans and was taken as a reference. Thus, if a biorefinery approach were taken and co-extraction technology for both pigments and protein could be realised for a similar production cost, then this would realise an additional profitable revenue stream. Any residual biomass could then be sold as a fertilizer, low quality biomass, or animal feed and provides a small additional revenue. With a capital investment required of £75, 898.10, this could mean a breakeven point of only 1.7 years. The 80 %, 70 %, and 60 % recovery scenarios would take the payback period to 2.4, 3.1, and 4.4 years respectively. Obviously, in this rather idealised scenario not all costs were not included, but the economics do lead to a rather favourable outcome and attractive business proposition. Similarly, the described process runs on recycled carbon dioxide, but other required nutrients, such as nitrates and phosphates, could also come from industrial wastes. This would generate a holistic circular economy and could add further revenue from carbon credits, while negating feed costs, and render the resulting process as highly sustainable as well as assisting in the race to net zero.

The cube PBR design is a first concept and clearly improvements can be made to make the economics even better than those reported. The real benefit of the design is that the PBR is easily transportable to any location, it is very cheap when compared with other designs, the PBR is easily scalable, is easy to use, and can be applied to different commercial industries or as a solution to waste issues. This provides companies with a sustainable and eco-friendly alternative to waste disposal. As the design is remarkably cheap, this can facilitate transfer of microalgae cultivation technologies to many industries and provide cultivation alternatives in countries where solar hours are scarce. In each case considered, the cube PBR would deliver reliable and robust production at all global latitudes, avoiding issues with weather, as well as other variables, that impact externally illuminated reactor performance. This will ultimately benefit society and lead to new products being available in a true integrated circular economy.

4. Conclusions

The successful cultivation of *Arthrospira platensis* CCMP1295 in a novel low cost internally illuminated cube PBR at 1000 L was demonstrated. The production run consisted of three cycles of production, i.e. growth and harvest, over 78 days. Effective control of the reactor parameters, temperature and pH, ensured optimal growth conditions were maintained. The system demonstrated a robust growth profile and stability over time with no observed lag phase. Growth metrics for each production cycle showed positive trends with exponential increases in biomass culture density measured by both optical density and dry weight. Peak biomass density or culture concentration was measured as 1.37 g L^{-1}, the average maximum specific growth rate was 0.419 $days^{-1}$, and the maximum biomass productivity was 80.5 mg L^{-1} day^{-1}. Aerial production rates from this new reactor were compared to that for existing raceway production systems and were found to be significantly higher, 92.1 kg $year^{-1}$ m^{-2} for optimal conditions compared to 4.2 kg

year^{-1} m^{-2} for the raceway.

The composition of the biomass was analysed by FTIR and indicated that the maximum protein content achieved during cultivation was 54.1 % on a mass basis. The biomass profile was compared with fresh biomass obtained from other reactors and commercially obtained. The results showed in all cases that the biomass generated in the study was as good as, if not better, in quality than that of the other sources. Phycocyanin pigment levels were also determined and found to be 78.27 mg g^{-1} of biomass, which was also significantly higher than the levels seen in the alternative algae sources.

An economic appraisal of the PBR performance was made and production was shown to require a capital investment of £12,776.60 m^{-3} reactor operational volume, which is very cheap when compared to other PBR designs or fermenters. At an operational volume of 1000 L, the initial capital costs for conventional systems exceed £50,000. In contrast, our novel design reduces these initial costs by a factor of five while maintaining a high-quality level of biomass production, as demonstrated in this study.

Basic operational parameters were also evaluated, and the reactor was shown to require 1009.76 kWh kg^{-1} algae as built. The major energy demand in these figures comes from the LED lights required for illumination to facilitate photosynthesis and the heater/chiller required for thermal control. A conservative business case was developed and demonstrated that direct sales of Spirulina biomass from the reactor would not be sensible. However, several scenarios using a biorefinery approach suggest that the reactor can be highly profitable if proteins and pigments are the focus. When this is the case, annual revenue from the reactor could be as high as £45,286.46 leading to a payback period of only 1.7 years.

A review of the literature showed that many performance characteristics required to commercially analyse algal productions systems, such as culture density, productivity, and composition, are obtained at particularly small reactor volumes in the region of 250 mL to 10 L. Care needs to be taken when using such data for business case development as the performance estimates obtained are often unachievable at scale and can lead to significantly optimistic business cases that will ultimately fail when exposed to real world production values.

Overall, the Cube PBR has been demonstrated to support high-quality biomass production, with pigment and protein extraction offering promising economic benefits. The new reactor is reliable and provides a cost-effective, scalable solution that can be adapted to various commercial and environmental conditions, promising to be a viable option for diverse applications in the microalgae industry. This has the potential to be a step-change in algal production technology leading to carbon capture and utilisation, which will deliver a sustainable future and has the potential to realise a true circular economy.

CRediT authorship contribution statement

José Ignacio Gayo-Peláez: Writing – original draft, Methodology, Formal analysis, Data curation. **Darren L. Oatley-Radcliffe:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization. **Alla Silkina:** Writing – original draft, Methodology, Data curation. **Andrew R. Barron:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

All authors declare that they do not have any financial or personal relationships with other people or organizations that could inappropriately influence or bias their work.

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The authors report no commercial or proprietary interest in any product or concept discussed in this article.

Data availability

Data will be made available on request.

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