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Cultivation of *Haematococcus pluvialis* for astaxanthin production on angled bench-scale and large-scale biofilm-based photobioreactors

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Abstract:

The green microalga, *Haematococcus pluvialis*, is currently cultivated for natural astaxanthin in suspended systems. Immobilised cultivation in a twin-layer (TL) porous substrate bioreactor is a potential revolution in microalgal biotechnology worldwide. For the first time in Vietnam, small-scale (0.05 m^2) and large-scale (2 m^2) biofilm-based photobioreactor systems arranged at an angle of 15^0 were successfully designed, assembled, and operated; the temperature, humidity, air, and light conditions for *H. pluvialis* cultivation were successfully controlled. Studies were conducted of both systems to determine the optimal storage time of algae after harvest from suspension before inoculation into the TL system, carbon dioxide supply method, light intensity, and initial cell density. In the 0.05 m^2 and 2 m^2 systems, dry biomass productivity reached 12 g m^{-2} d^{-1} (3% astaxanthin content in the dry biomass) and 11.25 g m^{-2} d^{-1} (2.8% astaxanthin) after 10 days of cultivation. The 2 m^2 biofilm-based photobioreactor system provides many advantages in scaling up astaxanthin production from *H. pluvialis*.

Keywords: **astaxanthin production, biofilm-based photobioreactor, *Haematococcus pluvialis*, twin-layer porous, twin-layer system.**

Classification number: **3.5**

Astaxanthin from *H. pluvialis* and algae suspended cultivation for astaxanthin harvest

Astaxanthin is a keto-carotenoid that is mainly used as a supplementary pigment in feedstock for salmon and shrimp cultivation feedstock; it is sometimes also applied in poultry farming to implant colouration in egg yolks [1]. Recent studies have shown the strong anti-oxidant activity of astaxanthin in a rat model [2] with benefits to the immune system, cardiac muscles, reducing risks of various cancers, and human skin-ageing treatments [3-8].

The green alga *H. pluvialis* is the most common natural astaxanthin producer at the commercial scale. This alga species is able to accumulate astaxanthin pigment up to 5.9% of its dry biomass [1, 9, 10]. The *H. pluvialis* life cycle includes one biflagellate green cell stage, one non-motile green cell (palmella) stage, and one thick-walled cyst (akinete) stage (Fig. 1). Changes in cell states are driven by environmental conditions. The most notable life-history stage of *H. pluvialis* is the cyst-forming period with its distinctive cell enlargement and increase of astaxanthin production which causes the change in algal color from green to red [11].

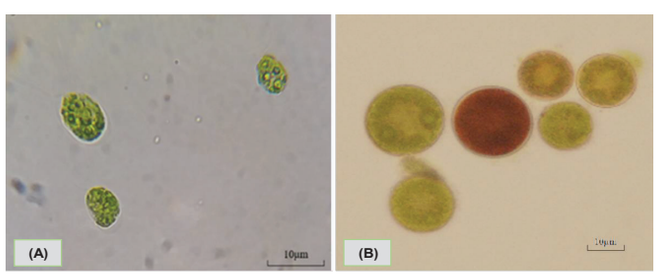

Fig. 1. Microscope image of different *H. pluvialis* life stages: (A) Two-flagellated cells; (B) Immobilized green cells and thickened wall red cysts (x40).

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To attain maximal astaxanthin production, *H. pluvialis* is mainly cultured in two-phase cultivation systems. The first phase, known as the green phase or growth phase, is optimised for vegetative growth to achieve a high cell density. In suspended cultivation, a maximum light intensity of 150 µmol photons m^{-2} s^{-1} should not be exceeded in order to maintain cell growth and divisions, and environmental parameters such as temperature, carbon dioxide (CO_2) levels, and pH need to be closely monitored [1, 12]. As the required biomass is attained, the second phase, known as the stressed or red phase, is switched on to stimulate astaxanthin accumulation [1, 12].

In the two-phase system, each growth phase requires different cultivation conditions and technologies, high energy consumption, and prolonged cultivation time [13, 14].

Currently, suspended cultivation of *H. pluvialis* is more common for the production of astaxanthin at the commercial scale. Suspended cultivation is applied in open ponds or closed photobioreactors. Open-pond cultivation is utilised only for the stressed phase with a short cultivation time (4-6 days) to minimise contamination and apply stressed conditions [12]. The closed photobioreactor can minimise contamination and control culture parameters better but it has drawbacks such as of high assembly and maintenance cost [15-17]. Moreover, suspended systems have very low biomass concentration (0.05-0.06% of cultivated liquid) and the harvest of algae thus demands additional costs of energy and labour [18].

Previous studies of astaxanthin accumulation in *H. pluvialis* in Vietnam: studies of *H. pluvialis* and astaxanthin production in Vietnam have just been conducted since 2010. The Institute of Biotechnology (Vietnam) managed to select one *H. pluvialis* HB strain (own isolate) with a high astaxanthin accumulation capability (4.8% in dry biomass). This strain's favourable growth conditions include RM culture medium [19], a temperature of 25^0C, light intensity of 30 µmol photon m^{-2} s^{-1}, and nitrate as a nitrogen source [20]. A maximum cell density of 4.02×10^6 cells ml^{-1} was obtained by increasing the nitrate concentration in the RM medium four-fold and switching the light cycle from 12 light/12 dark hours to 16 light/8 dark hours with nutrient supply by exchange of the culture medium [21, 22].

To stimulate astaxanthin accumulation, other than the limited nutrient condition, it is important to note that the carbon source is a limiting factor in *H. pluvialis* astaxanthin synthesis [23]. With supplementation with 100 mM bicarbonate, the HB strain switched to the cyst stage within 3 days and accumulated astaxanthin amounting to 3.96% in the dry biomass [23]; however, this experiment was only conducted at the scale of a 500 ml conical flask containing 350 ml algae liquid cultivated in two separate phases, with sedimentation by gravity and centrifugation to harvest the algal biomass. Cultivation at the 10 l scale resulted in an increase in cell density (4.12×10^6 cells ml^{-1}) though astaxanthin synthesis at this scale has not been investigated [23].

Trinh, et al. (2017) [24] recently conducted a study using two-phase suspended cultivation. In the algal growth phase, the algal cell density increased by only 3.5 times (from an initial density of 2.10^5 cells ml^{-1}) after 18 days of cultivation. In the astaxanthin synthesis induction phase in a 5 l culture medium bioreactor, cell density did not increase after 10 days of cultivation and the astaxanthin content was very low (194 µg l^{-1}).

At a larger scale, there are studies using two-phase suspended cultivation in closed systems of 26, 50, and 100 l with a long cultivation period (~25 days) and a relatively complicated process involving multiple centrifugations to increase algal density and exchange the culture medium [21, 22]. In the 50 and 100 l systems, the cell density did not improve significantly and there was no report of the astaxanthin content in the dry biomass.

Immobilised cultivation of *H. pluvialis* in a vertical TL biofilm photobioreactor

The TL biofilm photobioreactor was invented by Melkonian and coworkers in Cologne [25, 26] for microalgae biomass cultivation. This system is able to hold eight twin-layered modular units (each with a ground size of 1 m^2). The algae growth area is 2×0.67 m^2 for both sides in one unit [27]. The twin-layered structure includes one layer of vertically arranged non-woven glass fiber (80 g m^{-2}, Isola AS Eidanger, Norway) attached to source layers to maintain a continuous medium flow by means of gravity with a flow rate of 6-10 l h^{-1} m^{-2} using an agriculture drip-irrigation system (Netafim, Frankfurt, Germany) operating at a maximum pressure of 0.8 bar. The prepared culture medium (80-100 l) is stored in closed containers or reservoirs and is distributed all over eight twin-layered structures by two independent pumps (gamma/5b, ProMinent Dosiertechnik GmbH, Germany). After flowing through all these structures, the medium is collected below and directed back into the reservoirs. The medium is exchanged once after 6 days [27].

Above the source layer a substrate layer is attached by self-adhesion (both layers are hydrophilic). The substrate layer can be made of common printing paper (45-60 g m^{-2},

for instance 'Kölner Stadt-Anzeiger', Dumont Schauberg, Cologne, Germany) and is used as carrying agent to immobilise algal cells. This substrate layer prevents cells from infliltrating the culture medium and source layer but allows the source layer to control the growth of the immobilised biomass via diffusion of the culture medium [26]. Before inoculation into the TL system, algal cells are harvested from the liquid medium by centrifugation at 1,000 g. The suspended liquid is inoculated into substrate layers using a paint roller at the density of 2 g dried biomass m^{-2}. The roller is also used to transfer algae from one TL module to another [27].

The TL system has been used to to cultivate various algal species, including *H. pluvialis* [10, 25, 28-30]. These studies has investigated the influence of many parameters such as the inoculum temperature, light intensity, and nutrient concentration on the immobilised cultivation of *H. pluvialis*; however, these studies were limited by continuous illumination at a maximum intensity of 230 µmol photon m^{-2} s^{-1}. The immobilised cultivation in these studies was applied the stressed phase of *H. pluvialis* and not to the whole cultivation process, including cell multiplication [10, 28, 30, 31].

The TL photobioreactor has recently shown a great promise, achieving production of both biomass and astaxanthin of *H. pluvialis* in only a one-phase system at high light intensity was achieved in a TL photobioreactor recently [32]. The algae were cultivated under light intensities ranging from 20 to 1,015 µmol photon m^{-2} s^{-1} with 1-10% CO_2 added in the gas phase. Dried biomass production reached 19.4 g m^{-2} d^{-1} and the final dry biomass, 213 g m^{-2}, after 16 days of cultivation. During the whole process, the astaxanthin content increased with light intensity and astaxanthin production reached 0.39 g m^{-2} d^{-1}, with a final amount of astaxanthin of 3.4 g m^{-2}. The astaxanthin content was 2.5% in the dry biomass. In comparison with two-phase cultivation using the same TL photobioreactor, one-phase cultivation provided a similar amount of total astaxanthin with half of the cultivation time. It was also more convenient than two-phase suspended cultivation [32].

Until recently, immobilised cultivation using the TL system included two set-ups: a bench-scale system and a pilot system. Both systems are vertically oriented which increased aerial efficiency eight-fold. However, the productivity in each unit decreases as mutual shading by the modules decreases the light intensity inside each unit; the investment, maintenance, and harvesting costs also increase per module [27].

Immobilised cultivation of *H. pluvialis* on angled a TL biofilm-based photobioreactor for astaxanthin production in Vietnam

The use of a vertical biofilm-based photobioreactor for *H. pluvialis* immobilised cultivation in Vietnam involves several difficulties, including higher investment and maintenance costs and the unavailability of several materials (stable non-woven fiberglass and high quality paper) in Vietnam. Hanging the modules vertically requires the membranes to be strong enough to withstand gravity. The larger the surface area of the culture, the greater the gravity because the mass of the membranes and the water increase. Therefore, the vertical system is impractical to use in Vietnam, especially when use of ground area is not an issue. Accordingly, in Vietnam, the TL biofilm-based photobioreactor should be angled at 15-20^0 on a solid surface to support the gravity of the membranes.

The bench-scale TL biofilm-based photobioreactor (0.05 m²) for *H. pluvialis* immobilised cultivation includes the following components: chamber, supply system, nutrient circulation system, air circulation system (with or without CO_2), steel frame, and light supply system.

The cultivation chamber is made of acrylic glass because this material allows 90% of light to be transmitted (this is determined by measuring light intensity before and after it passes through the acrylic glass). It is also easy to handle and is more durable than silica glass. Each acrylic plate is 5 mm thick and is attached via cyanoacrylate glue and sealed by thermal glue. Fig. 2 presents the technical parameters of the chamber. The cultivation chamber contains supplying elements for immobilised algae: source layer, substrate layer, and air conducts. This chamber minimises contamination from the external environment.

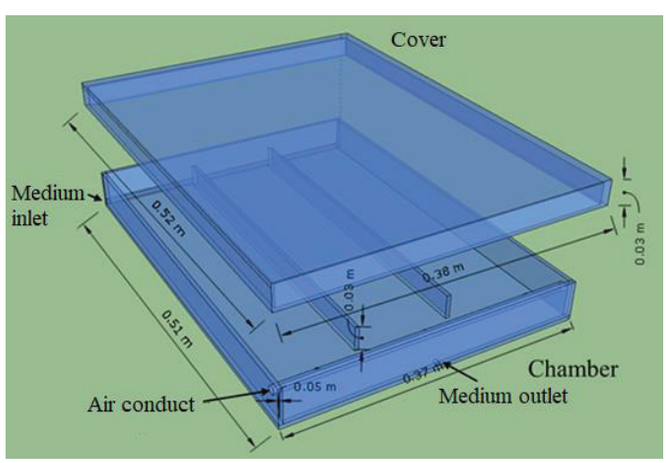

Fig. 2. Design of bench-scaled system.

The provision of nutrients requires a sufficient supply of medium liquid to maintain the wetness of the two layers. The dripping nutrient irrigation system is described in Fig. 3A. The medium is stored in a 20 l container located below the system and is continuously pumped into the dripping system via a pumping system with a flow rate of 1.2 l min^{-1}. The dripping system is assembled from a pressurised Capinet dripper with a flow rate of 5 ml min^{-1}, plastic ducts (outer diameter: 8 mm, thickness: 2 mm), and various joints. The medium flows through the chamber, wets the layers, and is collected in the reservoir via the duct system.

Fresh air (with or without a CO_2 supplement) is supplied via the system depicted in Fig. 3A. The main components include a air pump (160 W, 115 l min^{-1}) and an air filte-air is compressed by the pump to a pressure of 0.033 Mpa and flows through the filter. The CO_2 can be supplemented by air ducts (outer diameter: 10 mm, thickness: 2 mm) leading into the filter; pressurised valves are used to mediate the air pressure to evenly distribute the air to all the chambers. Fig. 2 indicates the location of the duct system which leads the air into the chambers.

A steel frame is designed and assembled as indicated in the diagram in Fig. 3B. The material used is holed 3x3 cm V-shaped steel of 3 mm thickness with an electrostatic coating. The components are assembled using bolts and screws designed for holed steel assembly.

Light system: the experiment utilises many different light sources; the lamps are assembled as show in Fig. 3C. The lamps are automatically switched on and off by a timer with light cycle of 14 hours light/10 hours dark. The light intensity depends on each experiment and was measured using a Lutron LX-1108 (Taiwan) photometer.

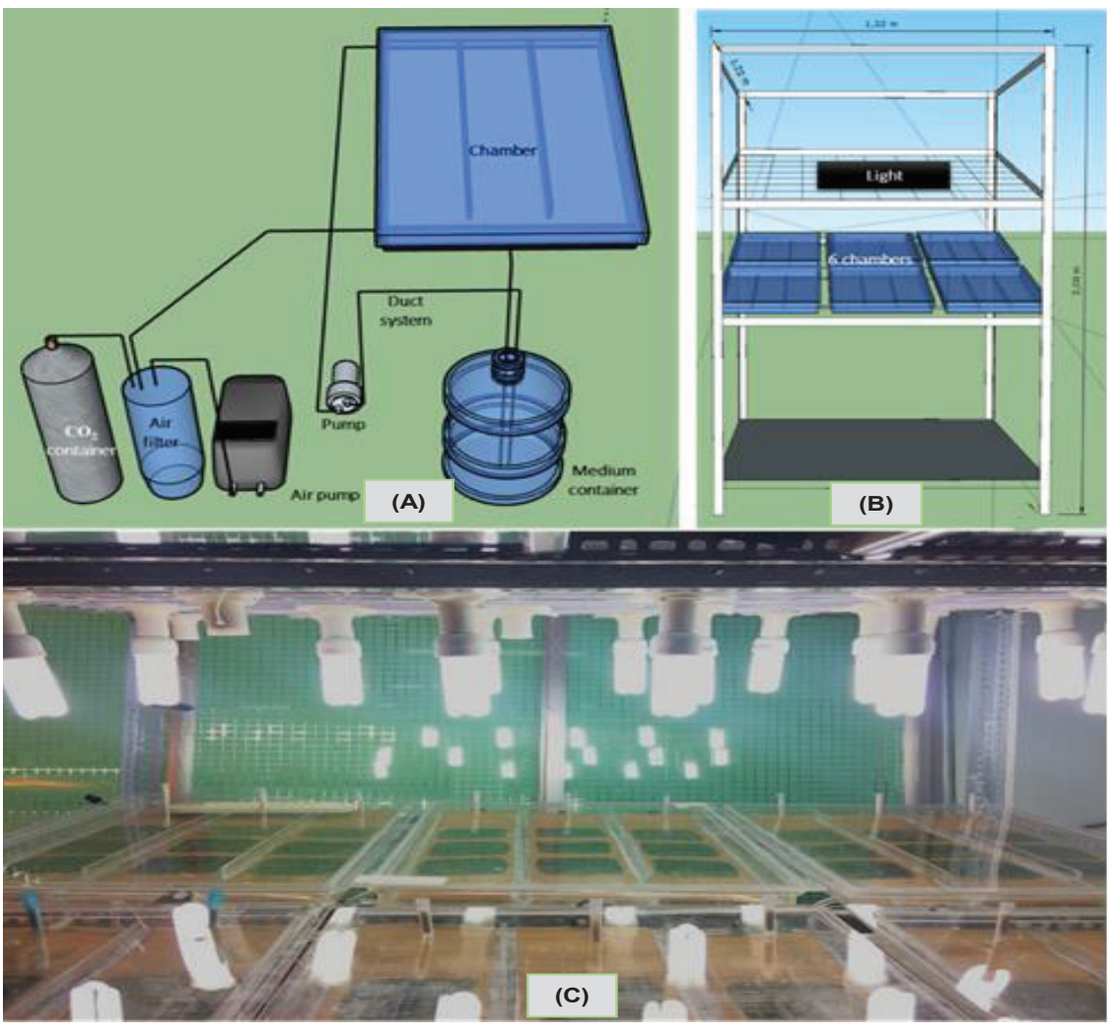

Fig. 3. (A) Nutrient and air supply system for cultivation chamber of bench-scale system; (B) Positioning of chambers and lights in bench-scale system; (C) The bench-scale system in use with *H. pluvialis* on the biofilm.

Suitable layer materials for conditions in Vietnam: the materials for algae attachment need to be durable, inexpensive, widely available, and non-toxic; material which can enhance biofilm yield should be preferred. Non-woven fiberglass and printing paper are most often used as a source layer and substrate layer, respectively, in TL photobioreactor for algae cultivation.

The source layer is made of non-woven fiberglass (0.5x0.1 m). Experiments with *substrate layers* show that there are only two suitable materials: Whatman filter paper and kraft paper (70 g m^{-2}, Vietnam). These materials are durable with a suitable pore size for keeping the algae in place after immobilisation. They were then tested in algae cultivation experiments to compare dry biomass growth in order to select the most appropriate material for use in later studies.

The results of the *H. pluvialis* cultivation experiment show that dry biomass growth in filter paper and kraft paper is not significantly different (filter paper: 6.81 g m^{-2} d^{-1}, kraft paper: 6.63 g m^{-2} d^{-1}, $p>0.05$) at the same inoculation density of 5 g dry biomass m^{-2} after 10 days. The kraft paper was then selected as the substrate layer since (1) it provides biomass growth similar to that of filter paper, (2) kraft paper is much cheaper than filter paper, (3) kraft paper is widely available in Vietnam, and (4) kraft paper has high physical durability and is easy to handle during cultivation and harvesting (unpublished data).

Large-scale biofilm-based photobioreactor (2 m^2): in order to scale up the angled TL photobioreactor system, the biotechnology research team of Nguyen Tat Thanh University successfully designed, assembled, and is optimising the angled biofilm-based biophotoreactor for *H. pluvialis* cultivation at a scale of 2 m^2.

The 2 m^2-scaled biofilm-based photobioreactor for *H. pluvialis* immobilised cultivation uses the same component set as the bench-scaled one. The large-scale photobioreactor utilises four chambers assembled in the same system; each chamber provides a 0.5 m^2 area for algae growth.

The technical parameters of the large-scale chamber are described in Fig. 4. These are the result of several experiments and modifications to suit real-life conditions: (1) Kraft paper and fiberglass plate size of 1x0.6 m; (2) Size and weight of chamber for convenience in handling; (3)

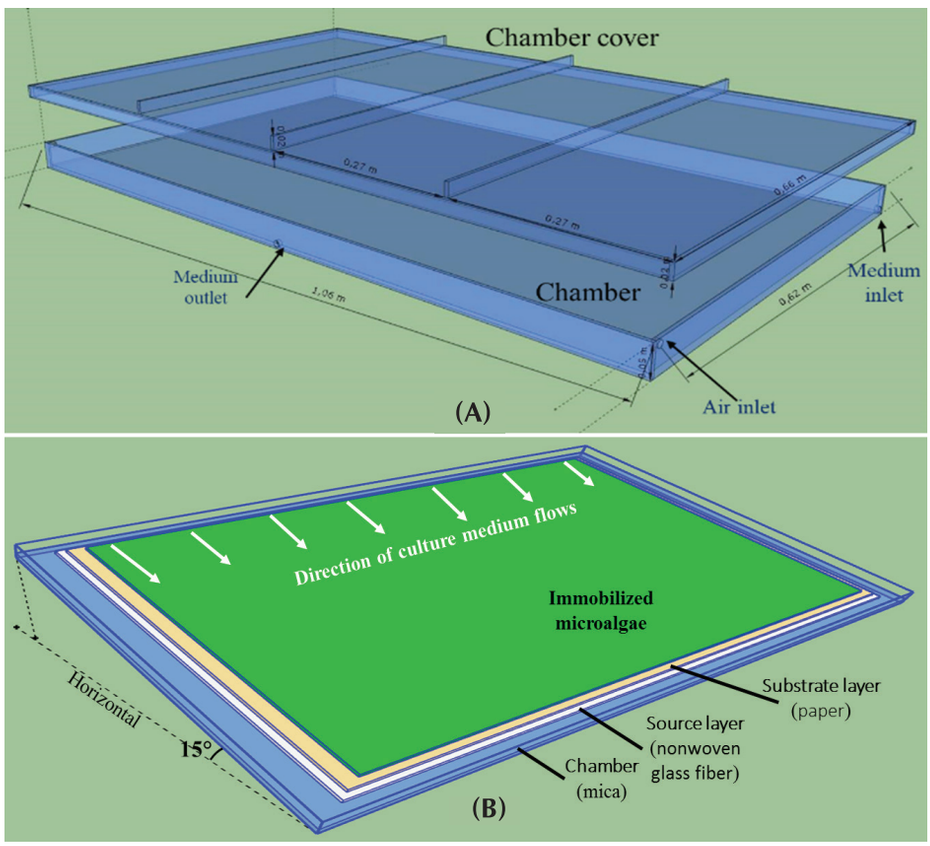

Fig. 4. (A) Design of large-scale system chamber; (B) Components of the TL photobioreactor system.

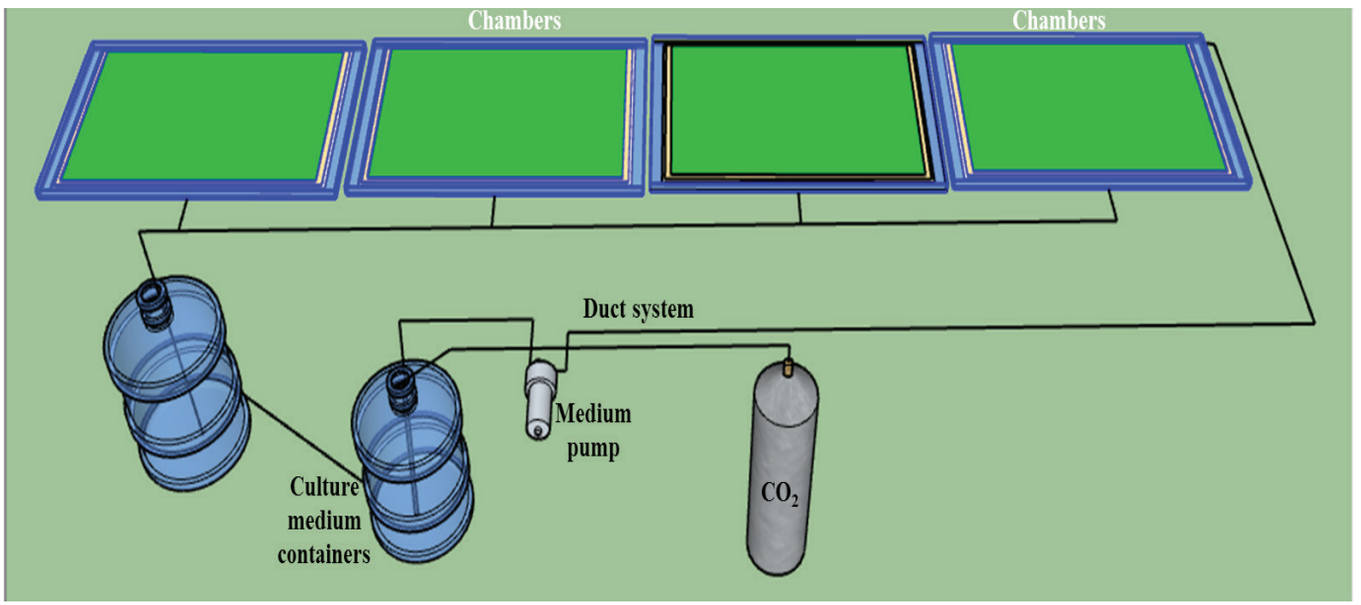

Fig. 5. Design of nutrient and air supply system for 2 m² system chambers.

Suitable size to correspond to the light power of the lamps to achieve maximum efficiency; and (4) An appropriate chamber size for manipulation and maintaining a culture below 28^{0}C inside the chamber.

The nutrient supply system is similar to the bench-scale system (Fig. 5). The large-scale system has its own modifications, for example, a suitable number of drippers in the larger cultivation size (15 drippers/chamber); the drippers are positioned 6 cm away from each other.

A Harwin HP 2500 pump (5-12 W, flow rate: 1.2 l min^{-1}) is used to circulate the medium in the four chambers. The duct system is made of soft polyethylene (PE) 16 mm pipes with a 1.2 mm thickness. Fresh air (with or without CO_2 supplement) is supplied via the system described in Fig. 5. The main components are an air pump: 160 W, 115 l min^{-1}; and an air filter: air is compressed by the pump to a pressure of 0.033 Mpa and flows through the filter. The CO_2 can be supplemented by air ducts (outer diameter: 10 mm, thickness: 2 mm) leading into the filter and pressurised valves.

The steel frame is designed and assembled as in indicated in the diagram in Fig. 6. The material used is holed 3x3 cm V-shaped steel of 3 mm thickness and with electrostatic coating. The components are assembled using bolts and screws designed for holed steel assembly.

The light source for the 2 m² system includes: (1) a light system that provides 300-1,300 µmol photon m^{-2} s^{-1}

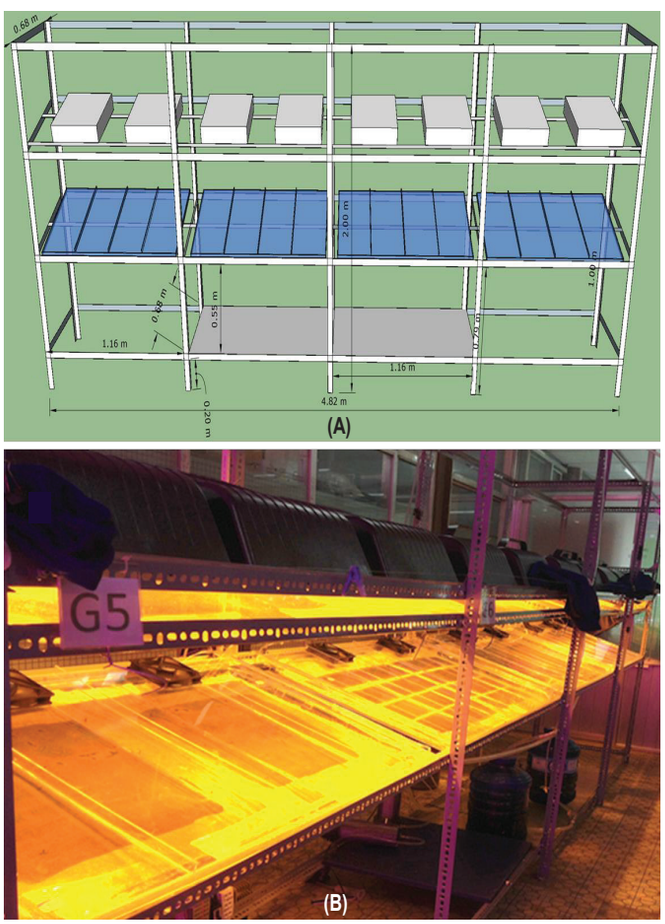

Fig. 6. (A) Diagram of chamber and light source positioning in 2 m² system; (B) The 2 m² system in use with *H. pluvialis* on the biofilm.

intensity (provided by eight 400 W Philips high pressure sodium lamps) or (2) a light system that provides 300-1,150 µmol photon m^{-2} s^{-1} intensity (provided by ten 250 W Philips high pressure sodium lamps). The lamps are assembled according to Fig. 6. The light intensity differed in each experiment and was measured using a Lutron LX-1108 (Taiwan) photometer.

Cultivation of *H. pluvialis* in the astaxanthin accumulation phase on an angled bench-scale TL biofilm-based photobioreactor

Immobilised algae cultivation for astaxanthin harvest was carried out at bench scale to investigate the factors influencing the growth rate and astaxanthin accumulation of *H. pluvialis*.

Experiments on the angled 0.05 m^2 bench-scale system include: (1) Investigation of the most suitable CO_2 supply method; (2) Investigation of the most suitable light intensity (intensities from 200 to 1,150 µmol photon m^{-2} s^{-1} were investigated); (3) Investigation of most suitable initial cell density (2.5, 5, 7.5, 10 g dried biomass m^{-2}); and (4) investigation of the influence of green algal biomass storing time on biomass growth and astaxanthin accumulation (storing algae at 4^0C over 1, 3, 5, and 7 days after centrifugation).

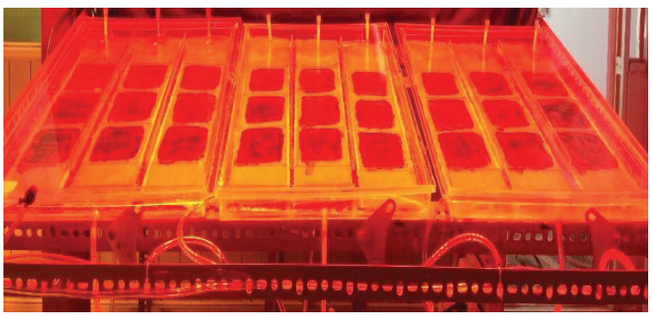

Fig. 7. The microalgae *H. pluvialis* on bench-scale system after 10 days of cultivation at an initial dry biomass density of 7.5 g m^{-2}.

The result shows that the most suitable CO_2 supply method is aerating fresh air with 1% CO_2 supplement into the culture medium to supply dissolved CO_2 and to maintain a pH favourable for algae growth. The most suitable light intensity for dry biomass and astaxanthin accumulation is 600-700 µmol photon m^{-2} s^{-1}. The most suitable storing time is less than 24 hours after centrifugation; a longer storing time causes a higher cell death rate and decreases algae growth after immobilisation. With an initial density of 7.5 g m^{-2}, average dry biomass production reached 12 g m^{-2} d^{-1} after 10 days of cultivation and the astaxanthin content amounted to 3% of the dry biomass (Fig. 7).

Cultivation of *H. pluvialis* in the astaxanthin accumulation phase on an angled large-scale TL biofilm-based photobioreactor (0.5 m^2 x 4 = 2 m^2)

The experiment was managed to establish the protocol for immobilised high productivity *H. pluvialis* cultivation on an angled large-scale system. The system is designed to maintain a temperature of 24-26^0C, and humidity below 80% via a cooling and dehumidifying system to maintain algae growth. The system operated continuously for 10 days with 14 light/10 dark hours cycle.

For experiments on the biofilm, cultures of *H. pluvialis* CCAC 0125 (Culture Collection of Algae at the University of Cologne, Germany) were expanded to 10 l PE bags with 6 l of BG11 medium [19] and placed in 23-25^0C. Algae were exposed to a light intensity of 50-60 µmol photons m^{-2} s^{-1}, a photoperiod of 14/10 hours light/dark cycle and were aerated with fresh air. Microalgae were collected from the logarithmic growth phase after 16 days with a Hettich ROTANA 460 centrifuge (Germany). The percentage of flagellate cells after centrifugation was 85%, and the maximum storage time of the inoculum was 24 hours at 4^0C. At the industrial scale, the inoculum of *H. pluvialis* will be cultured in 80-100 l PE bags. The step required to harvest a large number of flagellate cells in suspension is still being solved.

Initial algae density on biofilm was 5-7.5 g dry biomass m^{-2}. The fixation of algae on biofilm has been tested with many different methods. However, using a large brush to fix the algae shows many advantages. On average, the time needed to paint 1 m^2 of biofilm is 5 minutes. The density and quality of the algae are checked immediately during fixation.

An appropriate CO_2 supply method is aerating fresh air with 1% CO_2 supplement into the culture medium to keep pH in 6.5-8. The culture medium used is BG11 [19] (100 l for 10 days) which is diluted daily to keep electrical conductivity value in the range of 1,800-2,000 µS cm^{-2}. The light system providing the highest biomass growth and astaxanthin content has an intensity of 300-800 µmol photons m^{-2} s^{-1}.

Optimisation of high productivity *H. pluvialis* cultivation on a large-scale horizontal system produced some results. Average productivity of 11.25 g m^{-2} d^{-1} and an astaxanthin content of 2.8% of the dry biomass was obtained from the 2 m^2 system in the above-described conditions. Contamination was controlled during the cultivation period (Fig. 8). The 2 m^2 system provided slightly lower yields than the 0.05 m^2 system. However, astaxanthin productivity was higher in both suspended and immobilised systems than in most previous studies (Table 1).

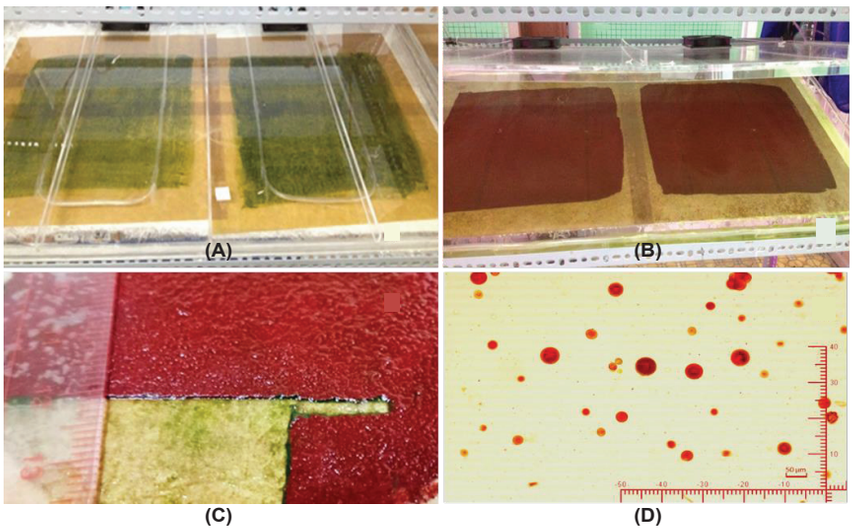

Fig. 8. Surface of *H. pluvialis* biofilm (A) and after 10 days of cultivation (B and C) on a 2 m^2 system; (D) Microscope image of *H. pluvialis* after 10 days of cultivation (x40).

Table 1. Comparison of *H. pluvialis* cultivation results on an angled biofilm-based photobioreactor system with other cultivation system based on surface area.

System	Strain	Medium	Temp (°C)	CO_2 (%)	Light condition (µmol photon m^{-2} s^{-1})	Stess factor	Cultivation period (green phase + red phase) (days)	Astaxanthin content (% dried biomass)	Astaxanthin productivity (mg l^{-1} day^{-1})	Astaxanthin productivity (mg m^{-2} day^{-1})	Dried biomass productivity (g m^{-2} day^{-1})	References
Outdoor tube (50 l)	Isolated	BG11	25	For controlling pH	Sunlight 400-1600	Intense light	4 (Red phase)	3.6	7.2	136.8[a]	3.8[a]	[33]
Outdoor open pond	ZY-18	NIES-N	28	None	Sunlight Max. 1000	Intense light + N limited	20 (Green phase + red phase)	1.7	-/-	40[a]	2.34[a]	[29]
Indoor open pond	26	BG11	20	For controlling pH	20-350 14/10 hour	Intense light	12 (Green phase + red phase)	2.79	4.3	61[a]	2.2[a]	[34]
Indoor bubble column	ZY-18	NIES-N	28	None	250 Continuous	Intense light + N limited	12 (Green phase + red phase)	3.6	-/-	237.6[a]	6.6[a]	[29]
Indoor bubble column (0.5 l)	K-0084	Modified BG11	25	1.5	350 Continuous	Intense light + N limited	5 (Red phase)	4.0	11.5	528[a]	13.2[a]	[13]
Indoor closed container (10 l)	HB (isolated)	Modified RM	25	For controlling pH	85 16/8 hour	Intense light, N limited, high C/N, + bicarbonate	30 (green phase) + 3 (Red phase)	4.88	2.75	92[a]	1.88[a]	[23]
Indoor bubble column (5 l)	-/-	RM	25	40 ml/min	60 16/8 hour	N limited, High C/N	22 (Green phase + red phase)	-/-	0.009	0.264[a]	-/-	[24]
Indoor immobilised biofilm (0.08 m^2)	NIES-144	NIES-N	25	None	150 Continuous	N limited	12 (Green phase + red phase)	1.3	-/-	65.8	3.7	[28]
Indoor immobilised biofilm (0.08 m^2)	SAG 34-1b	BG11	25	1.5	100 Continuous	N limited or exhausted	7 (Green phase + red phase)	2.2	-/-	143	6.5	[10]
Indoor immobilised biofilm (0.05 m^2)	CCAC 0125	Modified BG11	26	1	650 14/10 hour	Intense light + N, P limited	7 (Green phase + red phase)	3.5	-/-	371	10.6	[32]
Indoor angled immobilised biofilm (0.05 m^2)	CCAC 0125	Modified BG11	26	For controlling pH	600-700 14/10 hour	Intense light + N, P limited	10 (Green phase + red phase)	3.0	7.2	360	12	This study
Indoor angled immobilised biofilm (2 m^2)	CCAC 0125	Modified BG11	26	For controlling pH	600-700 14/10 hour	Intense light + N, P limited	10 (Green phase + red phase)	2.8	6.3	315	11.25	This study

[a]: the values are converted to 'per surface area'.

Conclusions

Angled immobilised cultivation systems for *H. pluvialis* were successfully designed and operated. The dry biomass productivity and microalgal astaxanthin content of the 2 m^2 system reached 11.25 g m^{-2} d^{-1} and 2.8%, respectively, which are similar to or higher than that of other systems. Both biomass and astaxanthin production can likely be improved by optimisation of the cultivation process. The data show that these systems can be applied for production at a larger scale. Further studies will be rewarding to improve the dry biomass and astaxanthin productivity of *H. pluvialis* cultivated on an angled TL biofilm-based photobioreactor system.

Angled immobilised cultivation on the TL-biofilm-based system provides remarkable advantages compared with traditional suspended cultivation, such as in term of water, energy, and cultivation time-saving. The angled system is also likely easier to scale up than the vertical TL system and perhaps more cost-efficient (for further discussion of vertical vs horizontal TL systems, see Podola, et al. (2017) [35]). However, understanding the underlying processes (light, nutrient, and air distribution, etc.) in the TL system is still limited relative to suspended systems, although some progress has recently been made [36-39].

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