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REVIEW

Development of thin-layer cascades for microalgae cultivation: milestones (review)

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Abstract

In this work, the key moments of the development of the so-called thin-layer cascades (TLC) for microalgae production are described. Development started at the end of the 1950s when the first generation of TLCs was set-up in former Czechoslovakia. Since, similar units for microalgae culturing, which are relatively simple, low-cost and highly productive, have been installed in a number of other countries worldwide. The TLCs are characterized by microalgae growth at a low depth (< 50 mm) and fast flow (0.4–0.5 m/s) of culture compared to mixed ponds or raceways. It guarantees a high ratio of exposed surface to total culture volume (> 100 1/m) and rapid light/dark cycling frequencies of cells which result in high biomass productivity (> 30 g/m^2/day) and operating at high biomass density, > 10 g/L of dry mass (DW). In TLCs, microalgae culture is grown in the system of inclined platforms that combine the advantages of open systems—direct sun irradiance, easy heat derivation, simple cleaning and maintenance, and efficient degassing—with positive features of closed systems—operation at high biomass densities achieving high volumetric productivity. Among significant advantages of thin layer cascades compared to raceway ponds are the operation at much higher cell densities, very high daylight productivities, and the possibility to store the culture in retention tanks at night, or in unfavourable weather conditions. Concerning the limitations of TLCs, one has to consider contaminations by other microalgae that limit cultivation to robust, fast-growing strains, or those cultured in selective environments.

Introduction

Microalgae can be grown in controlled mass aquaculture (algaculture) using various cultivation units. These primarily photosynthetic microorganisms represent a diverse group of tremendous ecological importance since they inhabit all major Earth ecosystems—aquatic and terrestrial, oceanic and freshwater, from arctic regions, through alkaline or saline habitats, to hot springs. Thus, they can tolerate a wide range of light intensities, temperatures, pH values and salinities. Microalgae are efficient biomass producers responsible for a substantial part of global primary production, being at the base of the food chain. Annually, they assimilate 30–50% of the inorganic carbon and produce about 50% of oxygen from/to the atmosphere (Chapman 2013; Yan et al. 2013). In 2017, annual worldwide microalgae production reached about 30,000 tons of dry biomass based on companies' reports (lecture by Claudia Grewe at the 6th Congress of the International Society for Applied Phycology 2017, Nantes, France), but a qualified estimate is several times higher due to ample production in China. Microalgae production for human use has become scarce to cover current annual demand. Microalgae in general are a potent source of natural substances such as proteins, carotenoids, oils, antioxidants, prebiotics and other compounds that are widespread used as food and feed supplements, in agriculture as biofertilisers, biostimulants and biopesticides, in wastewater treatment, as well as cosmetics and pharmacology, and most recently as a feedstock for biofuels (Khan et al. 2018).

Initiated in the 1950s (Burlew 1953), numerous cultivation systems have been designed for microalgae growth (Chaumont 1993; Borowitzka 1999; Tredici 2004; Zittelli

Dedicated to the memory of Prof. Ivan Šetlík

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et al. 2013; Masojídek et al. 2015; Acién et al. 2017). The choice of a suitable cultivation system and the adjustment of the cultivation regime must be worked out for each individual microalgae strain, purpose, or product. Two basic approaches to mass production are used: one applies to cultivation in open reservoirs (with direct contact of the microalgae culture with the environment), while the other involves closed or semi-closed vessels—photobioreactors (PBRs) with no direct contact between the culture and the atmosphere. There are major operational differences between open reservoirs and PBRs and, consequently, the growth physiology of the microalgae is different between the two systems (Grobbelaar 2009). Important variables of a cultivation system are the light intensity, temperature, length of optical path mixing, light acclimated state of the organism, nutrient availability, and gas exchange (supply of CO_2 and O_2 degassing). At present, open reservoirs are the most widely used cultivation systems for commercial large-scale production of tons of biomass due to their low cost of construction, maintenance and operation (Zittelli et al. 2013; Costa and de Morais 2014; Masojídek et al. 2015; Acién et al. 2017). Unfortunately, the use of open ponds is restricted to a small number of microalgae species due to the limited control of cultivation conditions and biological contamination. Hence, open systems are suitable for "robust" microalgae strains (mostly belonging to the *Chlorophyta*) that grow rapidly (Doucha et al. 2005; Doucha and Lívanský 2009; Masojídek et al. 2011a), or under very selective conditions (e.g., the cyanobacteria *Spirulina*—*Arthrospira*, or the green microalga *Dunaliella*) (Belay 2013; Borowitzka 2013).

Open cultivation systems represented by artificial ponds, tanks, raceways (shallow race-tracks mixed by paddle wheels) and sloping cascades (i.e., inclined-surface systems). An overview of open culture systems used for the mass cultivation of microalgae outdoors has been published recently (for a recent review, see Zittelli et al. 2013; de Vree et al. 2015; Acién et al. 2017). Productivity in these open systems is usually low, ~ 1 g/m^2/day of dry mass (DW), due to limited light, the lack of mixing, and CO_2 supply and the cultures are usually grown at a biomass density ranging between 0.5 and 1 g/L depending on the culture depth. To improve mixing, open systems are mixed by impellers, rotating arms, paddle wheels, or by a stream of air enriched with CO_2 supplied into the culture. The culture depth may vary between 10 and 30 cm.

The aim of this review is to review the milestones of thin-layer cascade (TLC) technology starting from the early development in the 1960–1970s, through "hard" times in the 1980s, its resurrection in the 1990–2000 up to today when various modifications have been used worldwide. The history of microalgae cultivation systems in former Czechoslovakia is presented in more detail, including construction characteristics of three TLC generations, their productivity, and advantages and limitations of TLC system.

Initial period of microalgae mass culturing

In the 1940s, cultivation trials with microalgae were carried out by the Carnegie Institution for Science. The possibility of growing the unicellular green microalga *Chlorella* (*Chlorophyta*) was considered in large-scale units. The early cultivation studies of several research groups were summarized in the "bible" of microalgae biotechnology edited by John S. Burlew of the Carnegie Institution in Washington, DC (Burlew 1953). The first attempts for large-scale microalgae cultivation and design of early pilot plants focused on closed systems in order to isolate cultures from the natural environment as to prevent contamination of cultures by other microorganisms. One of the productive pilot plants for mass cultivation of *Chlorella* was devised and tested at Arthur D. Little, Inc. in Cambridge, Massachusetts, in collaboration with the Carnegie Institution in 1952 (Fig. 1). In Germany, Gummert and co-workers experimented with large-scale cultures of *Chlorella* grown in open cultivation units using concrete trenches (reservoirs) with a plastic lining (Gummert et al. 1953). In Israel, a small-scale pilot plant to produce biomass of *Chlorella* or *Scenedesmus* as green fodder for cattle was set-up as a closed, mixed reservoir mounted in a greenhouse taking advantage of climatic conditions with a year-round availability of sunlight (Evenari et al. 1953). In Japan, at the Tokugawa Institute of Biological Research, an early attempt was made to use a shallow trough covered with transparent polyethylene sheets (Mituya et al. 1953).

By the mid-1950s, it was proven that open outdoor cultures were feasible and that they would probably not suffer from contamination if fast-growing strains (e.g., *Chlorella* or *Scenedesmus*) were cultivated. Once the feasibility of open cultures for outdoor cultivation was confirmed, it substantiated this concept for outdoor microalgae culture. This is without

Fig. 1 One of the first pilot-scale units (about 4000 L) was devised and tested at Arthur D. Little, Inc. in Cambridge, Massachusetts in collaboration with the Carnegie Institution in 1951. The photobioreactor was formed by a loop of thin-walled polyethylene tube of a 7–8-cm depth with continuous circulation of the culture by a pump

doubt due to the much simpler design of the open type units, whose construction is therefore much cheaper.

The overall goal of these early researchers strictly was to produce protein for potential human and animal consumption.

Start of thin-layer culturing in the 1960–1970s

In former Czechoslovakia, the concept of "cascade" cultivation units which was quite different from "deep" open ponds emerged in the late 1950s. The first pilot units were built at the Botanical Garden of the Slovak Academy of Sciences in the city of Košice (Eastern Slovakia) in 1958 by the research team lead by Ivan Šetlík. Soon after in January 1960, the research team joined the Laboratory for Algae Research of the newly established Institute of Microbiology, Czechoslovak Academy of Sciences in Třeboň. At the laboratory in Třeboň, the research was aimed at defining the scientific basis for commercial exploitation of microalgae cultivated on a large scale. In the 1960s, the efforts of the laboratory were focused mostly on the technological development of microalgae mass culture.

The idea was to grow microalgae on a system of shallow sloped troughs of about 2–5 cm deep arranged one below another, to form a cascade of hydraulic jumps, over which microalgae cultures were continuously circulated to secure sufficient mixing for solar irradiation attenuation and gas exchange to cells. As compared to mixed ponds, a specific feature of cascade design was the difference between day and night operation modes which is used until now. The culture was kept circulating only during hours when there was sufficient photosynthetically active radiation. During the dark or unfavourable weather periods, the culture was kept in a retention tank in which it was aerated and mixed. In principle, the culture was continuously recirculated to the highest trough from the lowest by pumping so that the culture cascaded by gravity in a thin layer. In these culture units, it was expected that cultures could reach higher biomass density as well as productivity. A number of outdoor production units (Fig. 2) was developed and constructed in Třeboň as well as cultivation procedures based on the principle of complex approach to study microalgae production, instrumentation development, biotechnology, physiology, cell biology, genetics and ecophysiology. The major drawback of this construction was that the culture motion in the troughs was not turbulent enough to prevent the sedimentation of cells completely. This concept was not appropriate for application on a larger scale due to slow eddy motion, sedimentation of microalgae cells, high energy consumption for pumping due to high mean slope, or high CO_2 desorption caused by the jumps.

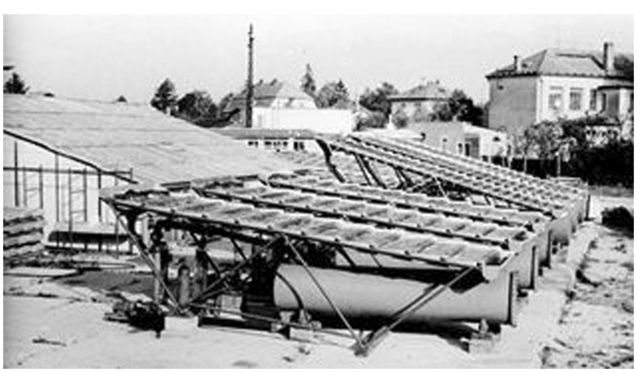


Fig. 2 First generation of outdoor cascades built in Třeboň in early 1960s

As a next step, the design of cascade cultivation units was modified in order to establish a steady and uniform flow of relatively thin layer of culture. In 1962–1963, the second generation of original cultivation units was constructed, as a unique outdoor pilot plant with 2 U of 50 m^2 (volume of 2500 L) and one of 900 m^2 (culture volume of 40,000 L) were built on the new laboratory campus "Opatovický mlýn" (Fig. 3) (Šetlík et al. 1970) which was one of the first large-scale plants for microalgae cultivation in Europe. These highly productive units were based on sloping planes, which are known worldwide as the Třeboň type units—cascades (Fig. 3). The principle of microalgae cultivation was to maintain turbulent flow of a relatively thin culture layer using a plain surface fitted with transversal baffles. The pilot plant was constructed as dual-purpose units that were used during the winter as a greenhouse for hydroponic vegetable and flower cultivation and in summer for microalgae biomass production.

As compared to open reservoirs (ponds, raceways) with a depth of suspension in the range of 15 to 30 cm where diluted cultures of microalgae (0.5–1 g/L DW) were grown under limited mixing and gas exchange, the main advantage of the cascade system constructed at the Laboratory for the Algae Research in Třeboň was the growth of well-mixed dense (10–

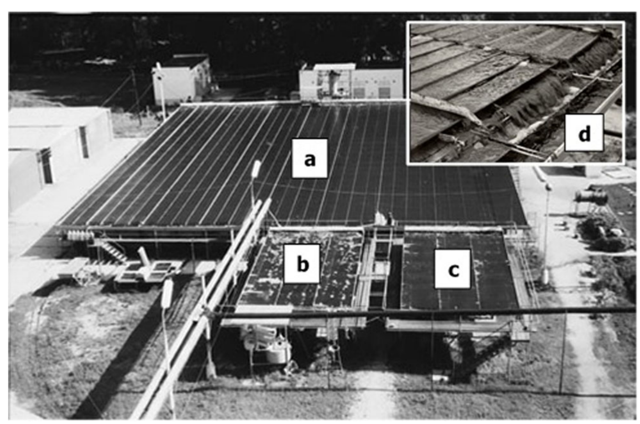


Fig. 3 The 2nd generation of outdoor cascades units for microalgae cultivation installed in Třeboň consisted of a pilot unit of 900 m^2 (a) and of two experimental units (b and c), each of 50 m^2. Insert (d): microalgae culture flowing over baffles to the collecting trough. The units were based on sloping glass planes with a slope of 3%, supported by a steel structure. The surface was fitted with transverse baffles, 3.5 cm high and 15 cm apart. The principle was to maintain turbulent flow of a relatively thin layer for high biomass productivity

15 g/L DW) microalgae culture in a relatively thin layer (< 5 cm) (Masojídek and Prášil 2010). In deep raceway ponds, the low turbulence of microalgae culture may result in lower efficiency of solar energy use due to photoinhibition of the microalgae cells in the upper layer exposed to high irradiance. Also, a much lower volume of dense microalgae suspension can be treated at harvest. Novel, thin-layer cultivation units were constructed of glass plates with a slope of 3%, supported by a steel structure. Additionally, the cultivation planes were fitted with transverse baffles 3.5 cm high and 15 cm apart to create intensive turbulence in the microalgae suspension which flows at a velocity of 7 cm/s. At the lower end of the culture area, the suspension was collected by a trough to a reservoir and conveyed by the return tube to the circulation pump. Then, it was lifted back to the upper part of the culture surface. The culture was circulated over the surface during the day while during the night was kept in the aerated retention tank to reduce heat losses, or during rainfall to avoid dilution by rainwater. As compared to deep ponds, the thin layer system was characterized by an intensive mixing which prevented culture sedimentation and increased the frequency of light/dark periods. Due to sufficient culture turbulence, high average irradiance per cell and good gas exchange can guarantee higher areal productivity for thin-layer systems.

In outdoor cultivations experiments as well as in most laboratory studies, the green microalgae *Scenedesmus* and *Chlorella* were used. Later, in the 1970s, thin-layer cultivation systems were constructed in Poland, Cuba and Italy to compare growth under various climatic conditions. A large-scale cultivation plant with a total area of 3000 m^2, using thin-layer sloped platforms placed on the ground, was constructed in Rupite in southwest Bulgaria (Becker 1994). The concrete platforms over which microalgae flowed had a slope of 3% and were fitted with transversal baffles to increase culture turbulence (Fig. 4). The advantage of the Rupite location was a high number of sunny days and a source of CO_2 from carbonic mineral springs.

The cultivation units in Třeboň were operated during the cultivation seasons from 1963 to 1970. After the Soviet invasion in 1968, large-scale microalgae cultivation was intentionally suppressed for almost 20 years. A partial resurrection of large-scale microalgae culturing in Czechoslovakia came at the end of the 1980s.

Thin-layer cascades in the 1990s

Only after political changes in 1989, support for Czech microalgae biotechnology was renewed and steady development has occurred since. In September 1993, the 6th International Conference on Applied Algology was held at Třeboň which helped to renew collaborations broken in the 1960s. Since the 1990s, the industrial cultivation of microalgae to produce biofuels and bioproducts has increased dramatically worldwide (Khan et al. 2018).

Fig. 4 The large-scale cultivation plant with a total area of 3000 m^2 made of concrete was constructed in Rupite, southwest Bulgaria. It consisted of thin-layer sloped platforms placed on the ground over which microalgae would flow. The platforms had a slope of 3% and were fitted with transversal baffles to increase culture turbulence

Starting at the end of the 1980s and early 1990s, the third generation of thin-layer units was developed and various cultivation units were tested in Třeboň—from experimental to large scale (Fig. 5). Each unit of 225 m^2 was constructed as a cascade of four planes arranged symmetrically in a mirror-like design. The microalgae culture is pumped from the retention tank to a distributor tube at the high point of the upper roof-like platforms and moves down along the surface to troughs which transfer it to lower platforms. Then, it flows in the opposite direction and at the end is collected back to the

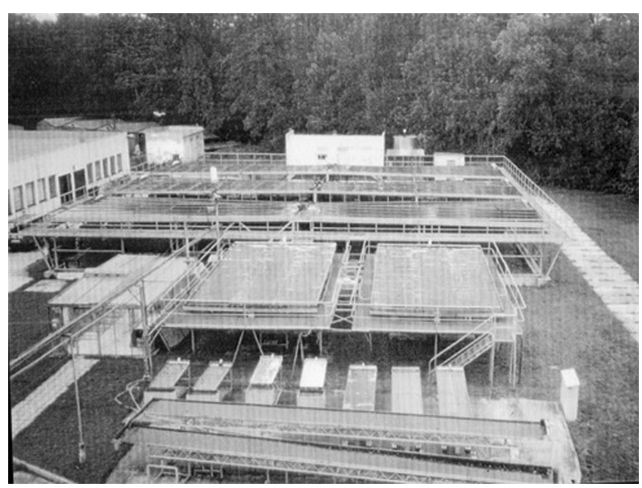


Fig. 5 In the 1990s, various thin-layer cultivation units with an area from 2 to 225 m^2 were tested. An important improvement was that in the largest units, the system of transversal baffles was replaced with plastic rods (diameter of 13 mm) mounted over a distance of 150 cm perpendicularly to the culture flow. This guaranteed high turbulence due to the flow of suspension under and over the rods with an average layer thickness of about 10–15 mm

retention tank located just under the unit. In this way, the dark volume in distribution tubing and in the retention tank can be minimized due to the short distances (Masojídek et al. 2011a).

Compared to the previous generation, some modifications were made: a reduction of surface inclination from 3 to 1.7%, mixing baffles were removed, but cultivation planes were divided into parallel lanes to support lateral mixing (Fig. 6). The main disadvantage of the previous, second-generation thin-layer units was the presence of baffles which required laborious installation and difficult cleaning of the cultivation surface. Thus, based on the experience from the TLCs used in the 1970s, the third generation of TLCs employs a much thinner layer of microalgae—less than 10 mm. Instead of densely spaces baffles, plastic rods with a diameter of 13 mm were placed 1.5 m apart and thus the flow velocity could be increased to 0.4–0.5 m/s (Doucha and Lívanský 1995; Grobbelaar et al. 1995). The rods were placed 3 mm the above cultivation surface perpendicularly to the culture flow. High turbulence was reached due to culture flow under and over the rods with an average layer thickness of about 10 mm.

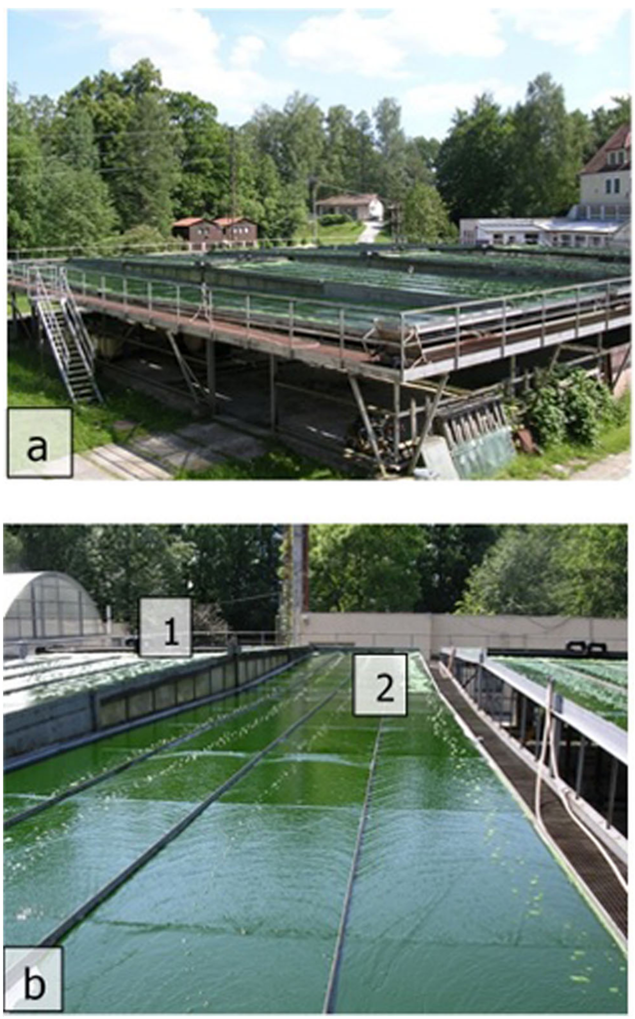


Fig. 6 At the beginning of the 1990s, a cultivation plant of about 660 m^2 was constructed which is still under operation. It consists of 3 units (**a**) with each (220 m^2) being made of glass plates glued in metal frames and arranged as a cascade of four planes arranged symmetrically in a roof-shape design (**b**). The culture is pumped to the distributor of upper cultivation planes (1), flows along to the end and turns back to the lower cultivation planes to be collected by a gutter (2) to a retention tank

Later, experiments in Rupite and Třeboň demonstrated that microalgae cultures can be grown on a smooth inclined surface (glass plates glued in metal frames) without any baffles or rods (Fig. 6). The thickness of culture layer could be decreased only to 6–8 mm with a flow speed of about 0.4–0.5 m/s reaching high biomass density, 15 g/L or more (Lívanský et al. 1993; Doucha and Lívanský 1995) as compared to 1–2 g/L in the culture grown in 40–50 mm with baffles (Figs. 3 and 4). The high flow velocity of the culture in thin layer resulted in a Reynolds number of about 4500, while the value characteristic for laminar flow in open channels is only about 500 (Masojídek et al. 2011a). The detailed principle of thin-layer cascade functioning is described in the legend of Fig. 7. Due to the high ratio of the exposed surface to total volume (> 100 1/m), the cell average irradiance is high even at high biomass densities under short light/dark cycles which support high productivity. The highest photosynthetic activities were achieved in cultures of 6.5–12.5 g/L DW (when operated in semi-batch regime), which was reflected in a maximum daylight productivity of up to 55 g/m^2/day of dry biomass under optimal conditions during few favourable days. The culture growth can result in maximal biomass densities up to 50 g/L DW (Masojídek et al. 2011a). First and foremost, the cleaning and maintenance of smooth surface units are much simpler, as compared with the baffled system. Another advantage of TLCs is temperature self-regulation as the culture is quickly heated by solar irradiance on cold mornings, and on the other hand cooled by increased evaporation when overheating. In this respect, TLC is more effective compared to deep culture systems due to the large surface and thin layer operation. Experiments have shown that the modification of this type of unit can make production more efficient, using semi-batch regimes in the range of biomass density between 15 and 35 g/L, simplifying cleaning and maintenance.

Starting in the mid-1990s, modern techniques like chlorophyll (Chl) fluorescence were introduced for microalgae culture monitoring to support semi-empirical methods (Knoppová et al. 1993; Torzillo et al. 1996). Photosynthesis measuring techniques were applied to monitor photosynthetic activity of microalgae mass cultures in situ. The pilot experiments were carried out in cascades and closed photobioreactor systems in the Czech Republic, Italy and Israel. Photosynthesis measuring techniques have been used to optimize culturing regimes and to estimate adverse environmental conditions—high irradiance, temperature extremes, high dissolved oxygen concentration and their synergism on

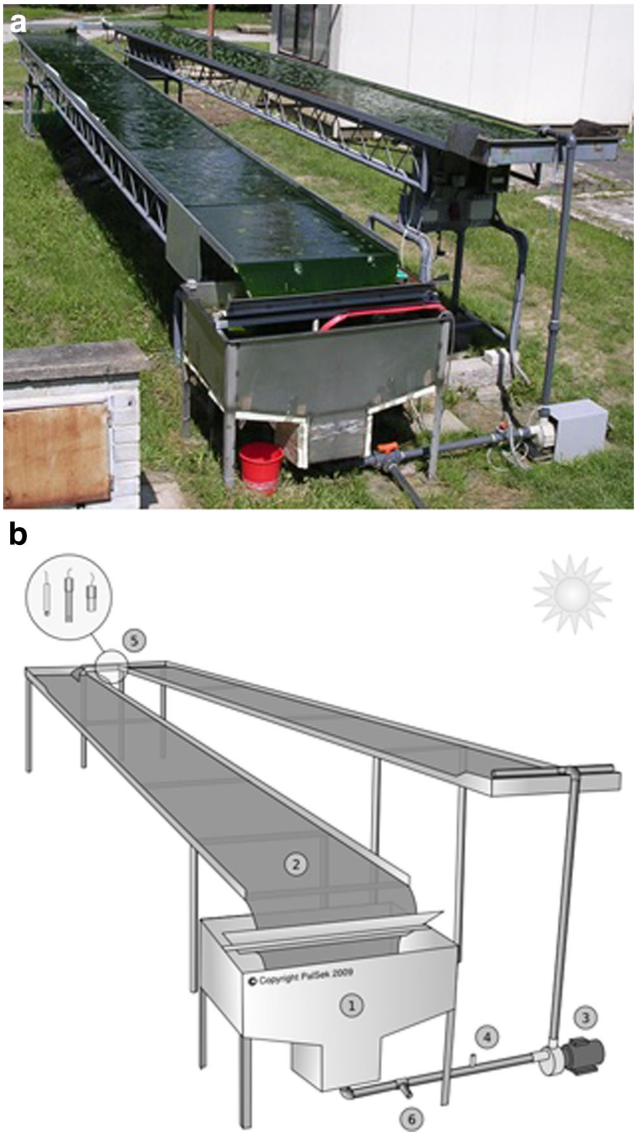


Fig. 7 (**a**) Experimental thin-layer cascade (total surface 24 m^2, working volume 220 L, flow speed 0.4–0.5 m/s, culture layer thickness 6 mm) was built in 1989 and served to verify construction and function of large thin-layer cascades. As soon as the first functional tests had been performed with this unit, the construction of the large culture unit started (Fig. 6). The experimental unit (surface of 24 m^2; volume 225 L) consists of two declined cultivation lanes made of glass plates (declination of 1.7%) supported by a scaffolding (Institute of Microbiology, Academy of Sciences, Třeboň, Czech Republic). (**b**) Schematic diagram of a cascade where a thin layer (6 mm) of suspension flows along declined surface. The microalgae culture is pumped (3) from the retention tank (1) and evenly distributed to the high point of the upper plane, flows down along the surface to the trough which transfers the culture to the upper part of lower lane (2), and at the end, the culture is collected back to the retention tank located under the unit. As the culture falls on a plate in a thin layer and then passes through a filter screen, efficient degassing takes place. As the start of one lane and the end of the other are close, the dark volume in distribution tubing and retention tank can be minimized. CO_2 is supplied in the tubing before the pump (4) based on pH stat system (5).

microalgae growth (for a review see Masojídek et al. 2011b; Malapascua et al. 2014).

Productivity of thin layer cascades

From the 1960s, large-scale microalgae cultivation in Třeboň has mostly been aimed at biomass production for use in nutrition—health food and supplements for human and animal nutrition in collaboration with commercial companies. A portion of the produced biomass has also been used for cosmetic purposes and isolation of bioactive compounds.

Starting in 1963, when the second generation of thin-layer cascades was set in operation, biomass productivity steadily increased from 7 g/m^2/day in 1963 to 12 g/m^2/day in 1967 (Fig. 8) (Šetlík et al. 1967; Nečas and Lhotský 1968). Important to note is that the data was calculated as an average figure during cultivation season between May and September, for about 150 days. Fast-growing robust microalgae *Scenedesmus obliquus* and two strains of *Chlorella* were cultured for production. Microalgae cultures were circulated on the cultivation surface only during the day (about 10–13 h) and during the night, the suspension was kept in an aerated tank. The decreased temperature during the night reduced the biomass consumption caused by respiration. The lower daily yield values during this year were probably caused by a reduced supply of CO_2 adopted on a practical economical basis (Nečas and Lhotský 1969). The growing season in 1970 is the last archived before large-scale production was virtually out of operation for several years. The microalgae increments were periodically harvested by centrifugation every 2 to 4 days, and the final product was mainly spray-dried. In 1964, 1.5 ton of dry biomass was produced which was sent to other institutes for further analysis. The overall objective was to determine the potential of microalgae as a replacement for human high-protein diet and as a feed for cattle and poultry, due to the

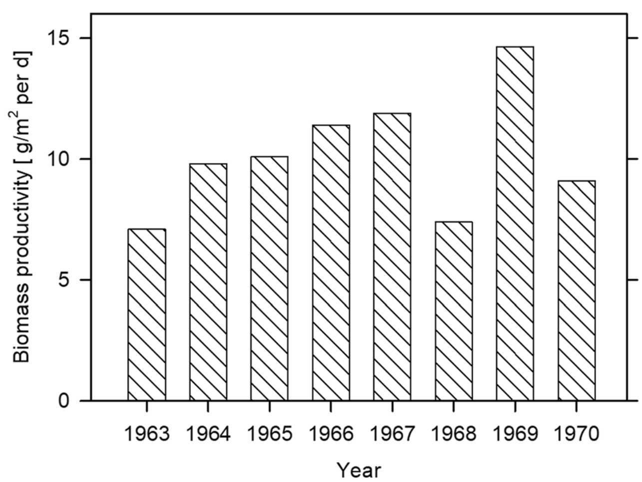


Fig. 8 Overview of averaged biomass productivity (g/m^2/day) of the second generation of thin-layer cascades (area 900 m^2) in Třeboň in the period of 1993–1970. The data were evaluated as an average during cultivation season between May and September regardless of the strain (Šetlík et al. 1967; Nečas and Lhotský 1968; Nečas and Lhotský 1969; Nečas and Lhotský 1973)

expected food crisis. After the political turmoil in 1968, research activities were substantially inhibited.

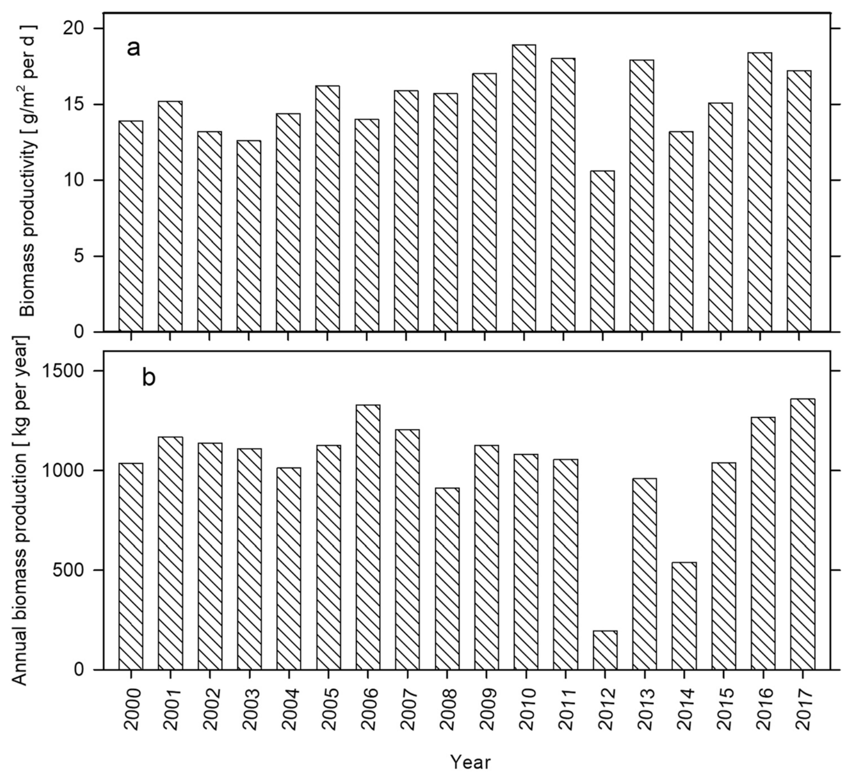
The productivity of the third generation of thin-layer cascades (area of 650 m^2) was monitored between 2000 and 2017 (Fig. 9a). We observe the trend of increasing averaged biomass productivity from about 14 g/m^2/day in 2000 to about 17–18 g/m^2/day in 2016–2017. It is important to note that biomass productivity in moderate climate zones varies according to weather conditions in the particular year, and it is responsible for decreased productivities to about 10 g/m^2/day in 2012 and to about 13 g/m^2/day in 2014 which also caused low biomass harvest (Fig. 9b).

Fig. 10 Outdoor thin-layer cascade for cultivation of microalgae constructed from stainless steel. It has an area of 90 m^2 and can be operated with a volume between 500 and 1500 L. The surface-to-total-volume ratio can be operated in the range between 60 and 180 1/m corresponding to a layer thickness of suspension between 5 and 15 mm

After the turn of millennium

Compared with the units used in the 1990–2000, the latest generation of TLC has been innovated to improve the cultivation process and ease of maintenance (Fig. 10) (Masojídek et al. 2013; Masojídek et al. 2015). The latest unit has an area of 90 m^2 and is made up of two identical platforms where microalgae culture is exposed to sunlight in a north-south orientation. Compared to the previous generation made of fragile glass plates glued to metal frames, the cultivation surface was made of stainless steel plates which can be easily cleaned and maintained, avoiding corrosion and damage by hailstone or snow. The slope of each platform is (independently) adjustable between 0 and 3% which in combination with a variable flow (20–50 cm/s) maintained by a pump make it possible to set-up suspension layer thickness between 5 and 15 mm. Thus, we can adjust layer thickness in order to study the optimal conditions at varying biomass densities regulating optimum culture irradiance. The shape of the retention tank was designed to minimize the dark volume of the microalgae culture which can be lower than 10%. The exposed surface to total volume ratio (S/V) can be maintained in the range of 60–180 1/m in this unit. All

Fig. 9 Overview of biomass productivity (g/m^2/day; panel **a**) and total biomass harvest (kg/year; panel **b**) of the third generation of thin-layer cascades (area 660 m^2) in Třeboň in the period of 2000–2017. The data were calculated as an average during cultivation season between May and September



materials used for construction are biocompatible (stainless steel, PVC, PE, zinc-galvanized parts). The construction is durable to climate conditions and corrosive environmental factors for long time periods.

Compared to cultivation surfaces made of glass or polypropylene, the great advantage of stainless steel cultivation surface was solidness, easy cleaning and resistance of the material to harsh weather or culture conditions (UV radiation in summer, frost in winter). The use of this demonstration unit was intended for microalgae production as food and feed additives, especially enriched in certain bioactive compounds (e.g., carotenoids, polyunsaturated fatty acids, etc.) or chemical elements (Se, Cr, I, Fe, Zn) as well as for wastewater treatment or marine strain culturing.

In the 1990–2000s, massive development of microalgae biotechnology has occurred and returned to the stage again due to the potential shortage of fossil fuels for transportation and their ever-increasing costs. The aim should be at least partial replacement of fossil fuels, especially oil and natural gas by renewable biofuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Agro-fuels (i.e., bio-diesel and bio-ethanol) for transport vehicles have been produced almost exclusively from food and feed crops grown on agricultural land such as rape, sugar beet, corn, or wheat which has a number of very serious negative consequences: Huge monocultures, high water consumption and the use of high doses of fertilizers and pesticides also increase food price. Microalgae have been considered as one of the potential sources of biofuels (Chisti 2008). Although the economic feasibility of biofuel production has still been questionable, it has also supported massive research and development of microalgae biotechnology. The quest for cheap production of biomass led to use of wastewaters as nutrient sources—animal wastes, municipal wastewaters and industrial wastes (including CO_2 from flue gases) for biomass production. Thin-layer cascades as high production cultivation units were tested for this purposes.

A special, multiple cascade pilot plant was built in Třeboň to produce microalgae biomass as a potential source of the third-generation biofuels (Doucha et al. 2005). The cascade was similar to a semi-closed system as it was placed in a greenhouse to use solar radiation as the source of light and heat and CO_2 was obtained from flue gas in order to decrease production costs. In this system, the culture was circulated over the cascade of 8 polypropylene lanes connected by troughs (total area of 56 m^2 and total volume of 500–600 L) which were arranged in a meandering way with an inclination of 1.6% (Fig. 11). The flow velocity was about 0.5 m/s with culture layer thickness of 6–7 mm. It was estimated that about 50% of flue gas decarbonisation can be attained in the photobioreactor and 4.4 kg of CO_2 is needed for the production of 1 kg (dry mass) microalgae biomass. A scheme of a combined farm unit was proposed including anaerobic digestion of organic agricultural wastes, production and combustion of biogas, and utilization of flue gas for production of microalgae biomass, which could be used in animal feeds. The placement of the cultivation unit in a greenhouse can partially prevent cross-contamination by invasive microorganisms and also control culture conditions, especially during bad weather days.

Fig. 11 A multiple-lane cascade system constructed as a semi-closed system placed in a greenhouse (Institute of Microbiology, Třeboň, Czech Republic). The culture was circulated over the cascade of 8 smooth polypropylene platforms arranged in a meandering way with a declination of 1.6% (total area of 56 m^2 and total volume of 500–600 L) which were connected by troughs. The flow velocity was about 0.5 m/s with a culture layer thickness of about 6–7 mm

A similar labyrinth-like thin-layer system of 32 m^2 with total volume of 1200 L was constructed at the University of Almería, Spain (Fig. 12) (Morales-Amaral

Fig. 12 Labyrinth-like thin-layer system of 32 m^2 in area with total volume of 1200 L was constructed at the University of Almeria, Spain (Morales-Amaral del Mar et al. 2015). The layer thickness of culture is about 20 mm. The culture is pumped from the aerated tank to the first layer using a low-stress centrifugal pump; then it is circulated by gravity at 0.2 m/s until it returns back to the tank

Fig. 13 A large-scale plant consisting of cascade raceway modules of 1500 m^2 (volume of 100,000 L) was installed in Pataias, Portugal, for the BIOFAT project (designed and built by the company A4F EU). The unit consists of two sloped platforms (declining of 0.5%), 10 m wide and 75 m long which form a cascade-like system running in opposite directions. This facility is a hybrid between a raceway pond and a sloping cascade since the layer thickness is about 40 mm, resulting in the S/V ratio of about 15 1/m. In this case, the operating biomass density is about 4 g/L DW (courtesy by Luis Costa)

del Mar et al. 2015). The culture was pumped from the aerated tank to the first layer and back to the tank using a low-shear centrifugal pump at a flow velocity of about 0.2 m/s. The layer thickness was about 20 mm. Side-by-side in one greenhouse, this system was compared with a raceway pond of the same area having a layer thickness of 120 mm circulated by a paddle wheel. Both systems were used to study outdoor production of the freshwater microalga *Scenedesmus* sp. using centrate from anaerobic digestion as a sole nutrient source. The aim was to estimate feasibility of large-scale microalgae production systems reducing the biomass cost by the use of waste instead of expensive fertilizers and disposing abundant, nutrient-rich wastes (Morales-Amaral del Mar et al. 2015; Acién et al. 2017). Such a combination may help to increase the possibility of producing commodities, like biofuels, from microalgae biomass by coupling their production to treatment processes.

A large-scale production plant, consisting of two cascade raceway modules of 1500 m^2 (total volume of 100,000 L), was built within the FP7 EU project BIOFAT (www.biofat-project.eu) by the company A4F in Pataias, Portugal, in 2011–2016 (Fig. 13). Each unit consisted of two sloped platforms, 10 m wide and 75 m long (declining by 0.5%), which form a cascade-like system running in opposite directions. This facility is a hybrid technology between raceway pond and sloping cascade since the layer thickness is about 40 mm, resulting in the S/V ratio of about 15 1/m. In this case, the operating biomass density is about 4 g/L DW. The unit was used for production of biomass for nutritional use.

In 2016, an international team led by the University of Almería opened the EU H2020 project SABANA (http://www2.ual.es/sabana/project), the goal of which is to develop and demonstrate an integrated microalgae-based sustainable biorefinery to produce a range of value-added products

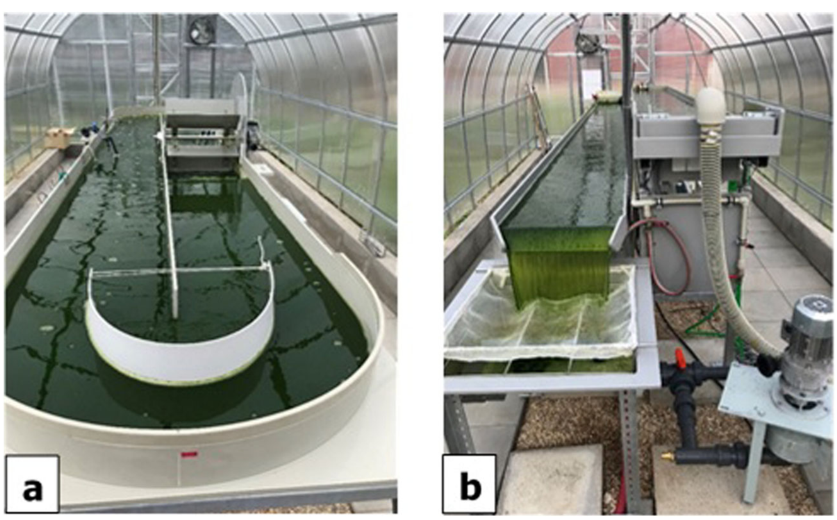


Fig. 14 (**a**) Thin-layer raceway pond mixed by a paddle wheel of 5 m^2 with a working volume of 100–600 L, culture layer thickness between 15 and 60 mm, and flow velocity about 0.2 m/s (manufactured by F&M s.r.l., Firenze, Italy). (**b**) Thin-layer cascade of 5 m^2 with working volume 60–70 L, culture depth 7–10 mm and flow velocity 0.5 m/s (manufactured by Agrico Ltd., Třeboň, Czech Republic) placed in a polycarbonate greenhouse with temperature control by electric fans and heaters for better control of suitable cultivation conditions (Centre Algatech Třeboň). The raceway set-up (panel **a**) was modified to enable cultivation in a thin layer (minimum of about 15 mm), as a paddle wheel was placed in a sump to secure gentle mixing (flow speed about 0.2 m/s) giving a total surface to volume ratio about 40 1/m. In the thin-layer cascade (panel **b**) with adjustable surface declination (0–3%) where the ratio of total surface to volume is about 80 1/m, various types of pumps have been tested for culture circulation. Both units are equipped with automatic addition of CO_2 on the basis of pH (8 ± 0.2)

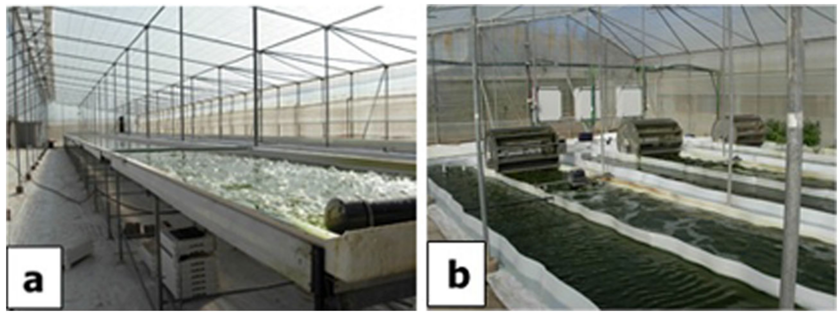


Fig. 15 R&D facilities consisting of two thin-layer cascades (**panel a** ;area of 60 and 140 m^2, layer thickness of 15–20 mm, 2400 and 3400 L, respectively) and three raceway ponds (**panel b**; 13.5-cm culture depth, each 80 m^2 and 12,000 L) mounted in a PE greenhouse were constructed by the University of Almería, Spain

(biostimulants, biopesticides and aquafeed additives) and low-value products (biofertilizers, aquafeed) for agriculture and aquaculture recovering nutrients from wastewaters (sewage, centrate, or pig manure). The final goal to achieve is to build and operate a demonstration facility for producing biofertilizers/biopesticides and aquafeed on a 5-ha scale.

Two experimental cultivation systems—raceway ponds and thin-layer cascades—have been tested at Algatech Centre Třeboň for their suitability to culture selected microalgae strains. In laboratory and pilot-scale trials, the most promising freshwater microalgae strains with biostimulating and biopesticide activities were characterized and examined for large-scale production in different cultivation systems—raceway ponds and thin-layer cascades which basically differ only in circulation devices—paddle wheel or centrifugal pumps (Fig. 14). It is an important point when either single-cell or filamentous microalgae are cultured as concerns their sensitivity to friction. Both experimental units—raceway ponds and thin-layer cascades—were placed in greenhouses to protect cultures from cross-contamination and maintain the same cultivation conditions. Monitoring techniques based on physiological behaviour and photosynthetic activity (Chl fluorescence techniques, dissolved oxygen measurements) to optimize culture growth in outdoor large-scale units were also tested in these trials.

Lately, experimental R&D systems of raceway ponds and thin-layer cascades have been constructed at the University of Almería to test microalgae cultivation based on seawater and wastewaters (Fig. 15). Large-scale DEMO plants of 1 ha including biomass processing technology will be finished in 2019.

Conclusions

Thin-layer, cascade-like systems have been used in a number of countries worldwide (Bulgaria, Italy, Germany, Portugal and Spain) as relatively simple, low-cost and highly productive units for microalgae culturing. TLCs bring together the advantages of open systems (direct sun irradiance, evaporative self-cooling, simple cleaning, low maintenance costs and increased interface for light dilution and oxygen stripping) with the positive features of closed systems (operation at high biomass densities achieving high productivity). Among the most significant advantages of TLCs compared to raceway ponds are the operation at much higher cell concentrations, very high daylight productivities, and the possibility to store the culture in a retention tank at night or in the case of unfavourable weather conditions. Among the limitations of these systems is the possibility of contamination by other microalgae resulting in growing preferentially fast-growing strains or those cultivated in selective environments to overcome this. The other is limited control of cultivation regime which depends on ambient conditions, but this problem can be overcome when cultivation units are located in countries with favourable climatic conditions.

TLCs are usually characterized by low depth (< 10 mm) and fast flow (0.4–0.5 m/s) of culture. It guarantees a high ratio (> 100 1/m) of exposed surface to total culture volume and high turbulence of cells compared to mixed ponds or raceways. Rapid light/dark cycles of cells (frequency of 10–100 Hz) are required to match turnover of the photosynthetic apparatus in order to utilize irradiance efficiently and facilitate higher biomass productivity (Hu et al. 1998; Richmond 2013; Zarmi et al. 2013). Applied intermittently to the individual cells in turbulent cultures, high irradiance is diluted by being available in smaller doses to more cells within a given time span and volume. Thus, the light is used more effectively, compared with poorly stirred cultures.

These mentioned features of thin-layer systems are important from a biotechnological point of view in order to optimize the growth of outdoor microalgae mass cultures under varying climatic conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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