Outdoor Microalgae Cultivation for Wastewater Treatment

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1 Introduction

In algae cultivation, one of the important economic constraints for biomass production is the cost of the nutrient media. Therefore, endeavors are being made to exchange the costlier nutrient media with less expensive supplemental sources. Among current solutions is the utilization of various sorts of wastewater for biomass production. Thus, in recent decades algae research has gained interest mainly oriented to the cultivation of algae in wastewater for cost-effective microalgal biofuel production and waste remediation (Diniz et al. 2016; Salama et al. 2017; Shchegolkova et al. 2018; Wu et al. 2014). The commercial use of algal cultures with application to WWT spans about 70 years. Currently, great interest is being evidenced all over the world including the USA, Australia, Taiwan, Mexico, and Thailand. The utilization of algae in WWT systems for nutrient removal was investigated earlier by Oswald et al. in 1957; wastewaters mainly provide water as well as a large number of necessary nutrients for algae cultivation. Algae are a photosynthetic microorganism with the capacity of converting solar energy into biomass and taking up nutrients such as nitrogen (N) and phosphorus (P) from wastewater (Lam and Lee 2012; Salama et al. 2017). The photosynthetic process produces O_2, which is essential in wastewater to allow aerobic bacteria to break down organic contaminants.

Many studies have shown that nutrient removal from wastewater using microalgae is applicable to various wastewater types including municipal (Álvarez-díaz et al. 2017; Liu and Ruan 2014), domestic (Cabanelas et al. 2013; Dahmani et al. 2016), agro-industrial (Posadas et al. 2014a), piggery (Yuan et al. 2013), and food processing (Lu et al. 2015).

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Coupling WWT with algae cultivation has been reported to be a good economically viable opportunity (Delrue et al. 2016, 2018) for cost reduction for biomass production as well as for water treatment, which provides an opportunity for clean water production in areas of limited water supply, with a very realistic possibility for utilization in the desert environment and hot arid regions (Fig. 1).

To mass develop microalgae with WWT, a variety of issues, mostly technical and economic, must be considered: finding suitable strains of algae, assessing nutrient content to determine readily available forms for algal uptake, determining the presence of biological inhibitories such as fungal and viral infection, solids content and turbidity for wastewater, competition between different microorganism populations, and environmental factors.

Well-balanced formulas of wastewater are necessary to realize maximal growth potential and high removal rate; with most necessary nutrients, municipal wastewaters offer a suitable option as artificial media for algae cultivation. However, inhibitory and toxic substances in the wastewater may smother the development of algal species. Furthermore, not all cultures are suitable for mass cultivation in open systems because of contamination, thus limiting species that can be used for cultivation on a large scale. Finding strains well adapted to cultivation in open ponds through screening of indigenous species or acclimation could provide a solution to this problem. Additional carbon sources (carbon dioxide, for example) from flue gases and different nutrients might be essential for unbalanced wastewater nutrition, especially the N/P ratio.

WWT using various algae strains has been extensively studied indoors at a laboratory scale, needing to be scaled up to outdoors applications to provide an inexpensive process; however, many factors such as illumination, temperature, and seasonal changes can be a challenge (Rawat et al. 2013; Zhu et al. 2016). To the best of our knowledge, only a few pilot plants have been successfully constructed throughout the world, mainly located in high-irradiance areas (Fig. 2).

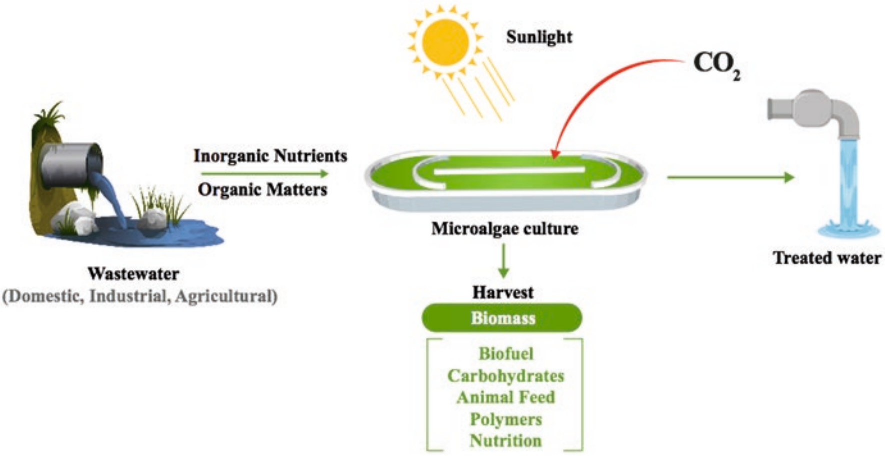

Fig. 1 Principles of microalgae production integration with wastewater treatment (WWT)

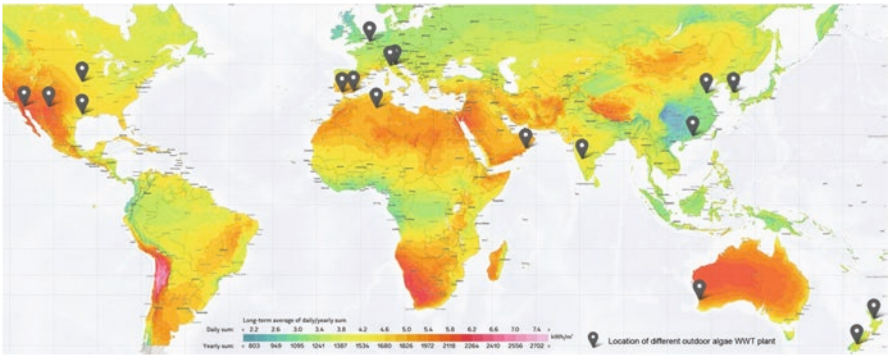

Fig. 2 Location of different outdoor algae wastewater treatment plants and direct normal irradiation (kWh/m^2) (solargis. info)

2 Algae Culture from the Laboratory to Outdoors

Research on microalgae culture in wastewater has increased. The larger part of this exploration is done on a laboratory scale using artificial light, competent technicians, and controlled growth conditions. However, the transfer of results from laboratory research to outdoor production needs further investigation to adjust and optimize the growth kinetics and nutrient uptake parameters.

Biomass productivity and photosynthetic activity are dependent not only on the total amount of solar energy impinging on the culture surface, but also on the various key factors such as pH, nutrients, salinity, and temperature (Cabello et al. 2015; Mahdy 2016). At the laboratory scale, culturing algae for WWT using real and different types of wastewater, many strains have been identified and high nutrient removal efficiency achieved. Even though a strain may grow well in laboratory cultures under particular incubation conditions (e.g., room temperature and relatively low light intensities), there is no warranty that it will grow efficiently in outdoor systems (García et al. 2017), as these are subject to variable seasonal water temperatures and fluctuations in light throughout the day (Tan et al. 2015). In any case, it is difficult to exploit results obtained in the laboratory to determine outdoor operating conditions. Table 1 shows some experiements conducted under outdoor conditions using various media and systems.

3 Challenges in Using Microalgae for WWT

The use of microalgae or microalgae–bacteria symbiosis has been demonstrated to provide a good treated water quality by removing organic matter and nutrients including nitrogen, phosphorus, hazardous contaminants, heavy metals, and also even for the removal of a specific pollutant (Ma et al. 2014; Olguín 2012).

Table 1 Microalgae grown in outdoor condition

Strain	Culture system	Medium	Outdoor location	References
Chlorella	Batch reactor 0.25 m^2 (10 l)	BG11 and F	Florence, Italy (July to September)	Guccione et al. (2014)
Nannochloropsis sp.	Green wall panel photobioreactors (<110 l)	BG11 and F	Livorno, Italy	Rodolfi et al. (2009)
Dunaliella salina	Paddle wheel tanks (20 m^2)	Artificial medium contain	Puerto Santa Maria, Cadiz, Spain	García-González (2013)
Chlorella pyrenoidosa	Photobioreactor (175 l)	Effluent of anaerobically digested activated sludge	Shandong Province, China	Tan et al. (2015)
Chlorella vulgaris	Open photobioreactors (3 l)	Piggery wastewater diluted 10- and 20-fold	Valladolid University, Spain	González-Fernández et al. (2016)
Chlorella zofingiensis	Open plastic pond (100 l)	Dairy wastewater	Foshan City, China	Technology (2016)
Ulothrix sp.	Batch reactors (12 m^3)	Aquacultural wastewater	Inagro, Roeselare, Belgium	Van Den Hende et al. (2014)
Nannochloropsis gaditana	Outdoor bubble columns (100 l)	Real centrate from an urban wastewater	Almería, Spain	Villegas et al. (2017)
Chlorella pyrenoidosa	Closed rectangular tanks (175 l)	Alcohol wastewater	Shandong Province, China	Tan et al. (2017)
Algae–bacteria	(8 m^3)	Domestic wastewater	Hamilton, New Zealand	Park et al. (2011)
Galdieria sulphuraria	Enclosed photobioreactors (700 l)	Urban wastewater	Las Cruces WW Treatment Plant in southern New Mexico	Henkanatte-gedera et al. (2017)
Chlorella pyrenoidosa	Airlift circulation photobioreactor (890 l)	Starch-processing wastewater	Shandong, China	Chu et al. (2015)
Nannochloropsis gaditana	Tubular photobioreactors	Anaerobic digestion of municipal wastewater	Almería, Spain	Ledda et al. (2015)
Microalgal consortium	Fiberglass paddlewheel-driven raceway ponds (1 m^2)	Anaerobic digestate of piggery effluent	Murdoch University, Australia	Ayre et al. (2017)
Indigenous algal consortium	HRAP (100 l)	Municipal wastewater	Daejeon, South Korea	Kim (2014)

(continued)

Table 1 (continued)

Strain	Culture system	Medium	Outdoor location	References
A mixed culture: two *Scenedesmus* sp.	Open circular ponds of 30 l capacity (1 × 1.5 m)	Pulp and paper mill effluent	Karnataka, India	Kim (2014)
Indigenous isolate: *Scenedesmus* sp.	Open pond (4 m × 80 cm) With 38 cm deep,	Produced water	Sultanate, Oman	Winckelmann et al. (2015)
Chlorella, Scenedesmus, Spirulina	Twelve open tanks (2270 l)	Treated wastewater (post-clarifier	Houston, Texas	Bhattacharjee and Siemann (2015)

The main challenges for the application of microalgae for WWT are the variation of cultivation condition and the harvesting of the algae biomass, the result of the settling characteristics and operational conditions. The concentration and composition of the organic material in the influent subjected to daily and seasonal variation must be considered in the design: the hydraulic retention time must be optimized and reduced to face environmental and nutrient fluctuations. Selection of the desired species, and finding and fixing an optimal ratio of algae/bacteria, and micropollutants removal, and a need for external CO_2 present additional obstacles.

Although there are several studies on this topic, additional research is needed to prove the effectiveness of microalgae-based WWT systems at full scale as perspectives for coming years and a promising alternative for the WWT process.

4 Effect of Wastewater Composition

The raw sewage/untreated wastewater composition and characteristics vary depending on the source and the location, and contain in their composition the main nutrients necessary for the growth of microalgae (Table 2).

In recent years, the cultivation of various microalgae strains in various raw wastewaters based on agricultural, municipal, and industrial sources was successful (Álvarez-díaz et al. 2017; Ayre et al. 2017; Zhu et al. 2013) (Fig. 3).

Urban/municipal wastewaters are a mix of a small percentage of industrial with domestic influents, mainly containing small amounts of suspended and dissolved organic and inorganic solids. Among the organic substances present in sewage are carbohydrates, lignin, fats, proteins, soaps, synthetic detergents, and various natural and synthetic organic chemicals from the processing industries. Most of the municipal wastewater is rich in nutrients such as phosphorus, ammonia, nitrogen, and a variety of inorganic substances, for example, calcium, chlorine, sulfur, magnesium, phosphate, and potassium. However, a number of potentially toxic elements such as chromium, silver, mercury, iron, gold, zinc, lead, copper, cadmium, nickel, arsenic, tin, selenium, aluminum, cobalt, manganese, and molybdenum could be present where algae are able to accumulate these toxic elements, when they are present in small concentrations.

Table 2 Different wastewater treatments for outdoor microalgal cultivation

Source of wastewater	Wastewater characteristic (mg/l)		Removal efficiency (%)		Strain	References
Domestic	COD	426	COD	78	*Chlorella pyrenoidosa*	Dahmani et al. (2016)
	TN	1.15	TN	95		
	TP	3.22	TP	81		
	COD	156 ± 79	COD	66	*Stigeoclonium* sp., *Chlorella* sp. *Monoraphidium* sp., *Chlorella* sp. *Stigeoclonium* sp.	Matamoros et al. (2015)
	TN	81 ± 9	TN	99		
	TP	N. R.	TP	N. R.		
	COD	N. R.	COD	N. R.	*Mucidosphaerium pulchellum* (H.C. Wood) C. Bock, Proschold & Krienitz	Sutherland et al. (2014)
	TN	N. R.	TN	79		
	TP	N. R.	TP	49		
	BOD	115.5 ± 71.5	BOD	50	N. R.	Craggs et al. (2012)
	TN	24.2 ± 9.5	TN	65		
	TP	N. R.	TP	19		
	COD	575 ± 84	COD	84	*Scenedesmus* sp.	Posadas et al. (2015a)
	TN	64 ± 15	TN	79		
	TP	9 ± 3	TP	57		
	COD	167 ± 64	COD	89	*Acutodesmus* sp., *Aulacoseira* sp., *Chlorella* sp., *Desmodesmus quadricaudatus*, *Limnothrix redekei*, *Nitzschia* sp., *Planktothrix* cf. *prolifica*, *Pseudanabaena limnetica*, *Synechocystis aquatilis*, *Woronichinia* sp.	Posadas et al. (2014b)
	TN	106 ± 9	TN	92		
	TP	12 ± 3	TP	96		
Urban and municipal	COD	79.62 ± 3.17	COD	N. R.	(TPBR) *Scenedesmus obliquus*	Arbib et al. (2013)
	TN	25.00 ± 1.80	TN	91. 4		
	TP	2.23 ± 0.12	TP	86. 5		
	COD	79.62 ± 3.17	COD	N. R.	(HRAP) *Scenedesmus obliquus*	Arbib et al. (2013)
	TN	25.43 ± 0.54	TN	65. 6		
	TP	2.23 ± 0.12	TP	56. 2		
	COD	N. R.	COD	N. R.	*Actinastrum, Scenedesmus* *Chlorella, Spirogyra* *Nitzschia, Golenkinia,* *Micractinium, Chlorococcum,* *Closterium, Euglena*	Woertz et al. (2009)
	TN	51	TN	96		
	TP	2.1	TP	99		

(continued)

Table 2 (continued)

Source of wastewater	Wastewater characteristic (mg/l)		Removal efficiency (%)		Strain	References
Agro-industrial	COD	3000 ± 28.1	COD	89	*Scenedesmus* sp.	Usha et al. (2016)
	TN	20.09 ± 1.3	TN	65		
	TP	1.55 ± 0.01	TP	71		
	COD	342 ± 26	COD	56	N. R.	de Godos et al. (2010)
	TN	18.8 ± 9.4	TN	97		
	TP	N. R.	TP	15		
	COD	526 ± 97	COD	76	N. R.	de Godos et al. (2009a)
	TN	59 ± 22	TN	88		
	TP	N. R.	TP	N. R.		
	COD	678 ± 249	COD	77	N. R.	Posadas et al. (2015b)
	TN	31 ± 10	TN	83		
	TP	19 ± 5	TP	94		
	TOC	1247 ± 62	TOC	50	*Chlamydomonas*, *Microspora Chlorella*, *Nitzschia*, *Achnanthes Protoderma*, *Selenastrum*, *Oocystis*, *Ankistrodesmus*	de Godos et al. (2009b)
	TN	656 ± 37	TN	100		
	TP	117 ± 19	TP	86		

N. R. not reported, *BOD* biochemical oxygen demand, *COD* chemical oxygen demand, *TOC* total organic carbon, *TN* total nitrogen, *TP* total phosphorus, *HRAP* high-rate algal pond

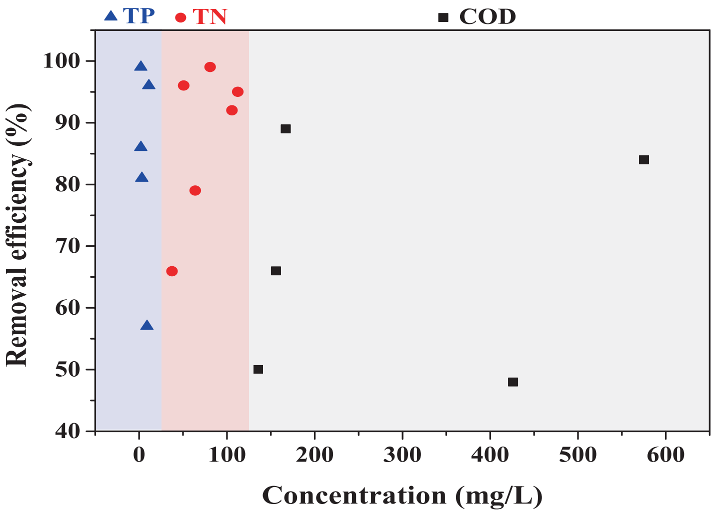

Fig. 3 Removal efficiency from domestic wastewater (outdoor microalgae cultivation based on recent research)

Accumulating processes take place by diverse mechanisms that depend on the species of microalgae and heavy metal ions; diverse factors significantly influence the fixation of heavy metals including the chemical substance concentration, pH, temperature, and accessibility of nutrients in the medium (Kumar et al. 2015; Talebi 2016).

It should be noted that several studies have shown that microalgae biomass has very effective performance for the reduction and elimination of various kinds of pollutants. A range of microalgae strains has been reported to have a potential efficiency of removal of a heavy metal; for example, *Chlorella vulgaris* is able to remove almost 100% of Cu and Fe and more than 60% of Zn, Pb, and Ni when cultured in urban wastewater (Kumar et al. 2015). The elimination of heavy metals in microalgae is mediated by two simultaneous mechanisms: passive and active. Passive mechanisms such as ion exchange, chemisorption, and adsorption occur at the cell surface, are frequently reversible and kinetically fast (Chojnacka et al. 2005). However, active mechanisms for heavy metal removal imply intracellular and extracellular (Ozturk et al. 2014).

Dishwashing liquids and shampoos are mainly constituted of surfactants, discharged into sewage wastewater. A few studies have reported their effect on algae growth (nutrient uptake); inhibition and toxic effect was observed for cationic and anionic surfactants with a concentration exceeding 1 mg/l. However, toxicities of non-ionic surfactant range from 0.003 to 18 mg/l; for example, 0.17–0.69 mg/l of sodium dodecyl sulfate is a minimum inhibitory concentration of *Chlamydomonas reinhardtii* (Groot 1990).

Utilization of microalgae for municipal WWT could be very effectively implemented for small communities where the variations in the effluent concentration could be minimized and controlled.

5 Agricultural Wastewater

Ranges of wastewaters are produced by the agriculture industry. The nutrient concentrations are wholly source dependent; a few studies showed the feasibility of the algal treatment of piggery, dairy, and poultry wastewater.

Agriculture wastewater is characterized by high nutrient concentrations, which may lead to algae being unable to grow. WW is very rich in pollutants such as ammoniacal nitrogen and phosphorus as well as having significant chemical oxygen demand (COD) concentration. The majority of studies report the important need for dilution (50%) for appropriate microalgae growth (Ayre et al. 2017) and a freshwater supply. High ammonia concentrations and high turbidity can be among the major challenges for long-term outdoor microalgae cultivation.

In poultry wastewater, total phosphorus (TP) and total nitrogen (TN) are generally more than 280 mg/l, with a COD exceeding 6000 mg/l; outdoor culture requires diluted poultry media and selection of the right algal strain to avoid photoinhibition. Studies conducted indoors showed that *Arthrospira platensis* cannot survive and grow and was significantly inhibited in 20× and 25× diluted poultry WW; however, *Chlorella vulgaris* displayed better growth in 10× diluted media (Markou et al. 2016).

The outdoor culture of *Scenedesmus* sp. using diluted effluent from a chicken manure biogas plant (25×) has been reported; ammonium and orthophosphate uptake were achieved at 90% but COD and nitrate at more than 50% (Lu et al. 2015).

Increasing use of pesticides, insecticides, antibiotics, and other fertilizers in agriculture will have a significant impact on microalgae growth and the kinetic nutrient uptake process for large-scale application.

6 Industrial Wastewater

Industrial WWT is not simple because its composition fluctuates and may contain poorly biodegradable components and high organic matter content (Dvořák et al. 2014). The characteristics of industrial wastewater and the presence of toxic components vary according to the type of activity that generated the pollution. Wastewater from slaughterhouses includes copper and zinc; petroleum industry wastewater contains benzene, naphthalene, nonylphenol, toluene, and xylene. Plastic plant waste contains octylphenol and chromium; the textile finishing sector produces waters rich in tribulphosphate, naphthalene, xylene, copper, and zinc. The manufacture of biocides or phytosanitary products pollutes the water with arsenic, chromium, copper, and zinc.

Industrial wastewater generally contains many heavy metals compared to municipal wastewater, but less phosphorus and nitrogen. For this purpose, it is necessary to use microalgae species or strains that effectively adsorb heavy metals.

Research studies are devoted to the removal of heavy metals and organic pollutants from microalgae grown in industrial WW. However, the high concentrations of organic toxins and heavy metals present in industrial wastewater tend to limit the possibilities of microalgae cultivation following the inhibition of microalgae growth.

A very few studies have reported outdoor culture using industrial wastewater because of its complex composition. Usha et al. (2016) studied the removal of organic pollution and nutrient rejects from paper and pulp mill effluent in an open outdoor pond diluted with distilled water (60%) using a *Scenedesmus* sp. strain; removal of up to 89% and 75% of COD and biological oxygen demand (BOD), respectively, was achieved. NO_3-N and PO_4-P removal up to 65% and 71.29%, respectively, were observed at the end of 28 days.

The scaleup for outdoor removal in industrial wastewater needs further investigation; indoor cultured studies showed removal efficiency.

7 Systems for Outdoor Microalgae Cultivation

Microalgae cultivation systems are one of the important aspects of biomass productivity. For this, numerous types of algal cultivation systems are developed (Fig. 4); the majority of them are based on closed or open systems. Based on the literature, it is impossible today to compare the performance of different outdoor systems because the reactors are not located in the same places, the mode of operations and measurements are different, and there are several cultivated strains.

7.1 Open Systems

A stabilization pond of wastewater is a basin at a designed depth in the ground for the treatment of wastewater. They are used to treat a variety of WW under broad weather conditions and are cost-effective to provide WWT when land is available. Moreover, their operation is very easy it requires minimum maintenance. These ponds are generally used preferably in areas with a warm climate. Raceway ponds are widely used for large-scale algal biomass production.

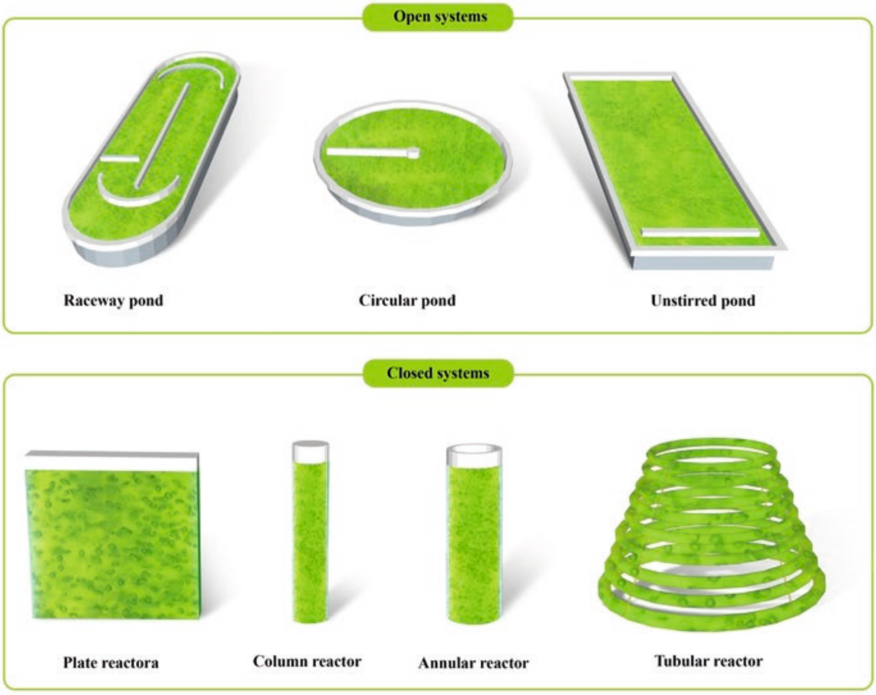

Fig. 4 Examples of different types of pond systems for outdoor microalgae cultivation

The algae ponds used in the WWT plants are constructed in a suitable form for the location; the mixing is generally achieved by a gravity flow. The Werribee WWT plant in Melbourne is one of the largest algal ponds for WWT with a surface area of 11,000 ha. Winckelmann et al. (2015) reported a successful study in an arid desert for cultures of indigenous algae using wastewater in open ponds (38 cm deep, 80 cm wide, 4 m long). However, Ayre et al. (2017) reported outdoor cultivation of microalgae on undiluted anaerobic digested waste using 1 m^2 fiberglass paddlewheel-driven raceway ponds during the winter. Many disadvantages have been observed, such as ammonia lost to the atmosphere or an undesirable contamination of the culture during long-term growth.

Major wastewater pond systems are large shallow ponds, tanks, raceway ponds, and circular ponds (Ugwu et al. 2008). The most popular type for microalgae cultivation and WWT is the raceway pond (de Godos et al. 2014). However, some major limitations can affect biomass productivity in open ponds, such as evaporative losses, easily contaminated cultures, photoinhibition in the summer, light used by the cells, and diffusion of CO_2.

However, the working depth is one of the most important design parameters of a raceway pond. Working with a shallow depth can exposure the algae to high temperature, especially during the summer, but a too great pond depth can prevent efficient light penetration. The optimal depth can be determined according to the quality and quantity of light penetrating, taking into account wastewater turbidity, which induces light-scattering processes and attenuation.

The hydraulic retention time (HRT), defined as the time that a wastewater remains inside the pond before it is evacuated, is an important factor that determines the efficiency and the cost-effectiveness of wastewater treatment. Many authors have suggested keep the HRT long enough to reach a maximum algae growth rate with high nutrient uptake and prevent nutrient limitation (Kim 2014). The HTR should be connected to seasonal variation and WW composition. HTR in the summer must be shorter than in the winter, because for a high nutrient reduction in the summer compared to the winter studies have reported that the removal efficiency of phosphorus and nitrogen with *Scenedesmus obliquus* was 20% higher in the summer (Lu et al. 2016).

7.2 Closed Systems

Various closed systems that have been designed for algae culture include tubular, flat-plate, and bag reactors; usually photobioreactors have better light penetration. Most of the closed systems are attractive for cultivation of algae for high-value product. High biomass productivity with minimal contamination has been reported, but a closed system not adapted for algae WWT use because of the high cost of maintenance and the need of more technical knowledge.

The design principles of most bioreactors used for WWT are derived from and similar to the pure culture systems: closed photobioreactors (PBRs) are generally considered to be too complicated to promote to a large scale. Tubular bubble column photobioreactors of 40 l were used for *Chlorella zofingiensis* outdoor culture in artificial wastewater in southern China with temperature varying between 20.6 ° and 30.8 °C; a biomass yield of 17.4 g m^{-2} day^{-1} was achieved (Zemke et al. 2013).

Vertical flat-plate PBR supplemented with municipal wastewater have been reported. Although the experiment was performed indoors under controlled conditions of illumination and temperature, 99% of TN and TP was removed with *Chlorella vulgaris* and *Scenedesmus obliquus* (Salama et al. 2017).

8 Limiting Factors in Outdoor Cultivation

Two competitive processes are responsible for the production of algal biomass: respiration and photosynthesis. However, the outdoor cultivation for nutrient removal from wastewater may depend on various abiotic and biotic factors such as algal species, nutrients, location, season, temperature, and irradiance, amount of rain, and/or wind and turbidity.

To maximize algal productivity, the different growth factors must be maintained. Nutrients can be controlled by adding wastewater as a medium into the culture. However, light intensity, temperature, and evaporation depend on solar irradiation, location, and season, and therefore cannot be controlled during outdoor cultivation.

The following section describes how the different growth factors predict algal productivity in outdoor cultivation.

8.1 Light Limitation and Photoinhibition

Outdoors, depending on the location of the crop and because of seasonal changes, the algae are exposed to irradiation that changes with time, different levels of illumination, and daily fluctuations. The effectiveness of photosynthesis is influenced by the fluctuation in light conditions with regard to intensity, wavelength, and duration. It is essential to evaluate each parameter to determine the optimal light condition that yields maximal productivity.

The intensity and availability of light is the major factor in the growth and productivity of photosynthetic microorganisms. Low light intensity leads to growth limitation whereas light intensity can inhibit the growth process: this phenomenon is known as photoinhibition (Barber 1992; Han 2002). Excess light intensity imposes a serious limitation on photosynthetic efficiency, particularly when coupled with high oxygen level and/or a temperature that is not optimal (Tredici and Zittelli 1998).

In a single day, light intensity changes from zero to saturated or oversaturated light levels. Therefore, the outdoor production of microalgae is light limited at the beginning and end of the day and light saturated in the middle of the day. Furthermore, light intensity also varies throughout the year, leading to complex operational processes in outdoor culture.

Microalgae have developed protective mechanisms to accommodate the changes in light intensity. Photo-acclimation is a process in which the microalgae reduce their photosynthetic pigment content as a way to protect the photosynthetic apparatus against increased irradiance (Sousa et al. 2013). The chlorophyll content per cell varies in relationship to the surrounding light environment (Sousa et al. 2013). Chlorophyll increases in the light-limited phase until the cells become optically dark and decrease under the light-saturated phase, resulting in more transparent cells (Simionato et al. 2011; Sousa et al. 2013). When exposed to strong illumination, microalgae protect their photosynthetic capacity by decreasing chlorophyll content and increasing carotenoid content in their pigmentation. This phenomenon occurs only with a limited number of microalgae, such as *Haematococcus pluvialis* and *Dunaliella salina*.

In most cases, however, chlorophylls and carotenoids decrease when microalgae are exposed to high light intensities, and as a result, the cells gradually turn from green to yellow to orange because the degradation of chlorophylls is usually more rapid than that of carotenoids. The increase in carotenoid content allows algae to dissipate excited chlorophyll energy and remove reactive oxygen species (ROS), thus maintaining the photosystem structure and functions. At very strong illumination, protective mechanisms will not be able to overcome the excess of electrons, ROS accumulation, and singlet oxygen formation, resulting in the death of algal cells (Simionato et al. 2011; Sousa et al. 2013).

In an outdoor culture system, in addition to the total amount of solar energy received on the growing surface that is important for biomass productivity, the amount of energy available at the cell level is necessary for good production (concepts of “light regime” and “light per cell”) (Chaumont 1993). However, the decrease in photosynthetic activity is explained by an enzymatic inactivation caused by high temperature, high irradiance, or both. *Scenedesmus obtusiusculus* showed a linear response of photosynthetic activity for irradiances in the range of 8–300 μE m^{-2} s^{-1}, which remains constant until an irradiance of 970 μE m^{-2} s^{-1}. At high irradiances (1600–2360 μE m^{-2} s^{-1}), photoinhibition was observed at temperatures above 35 °C. At high irradiance (1000 μE m^{-2} s^{-1}), photoinhibition has been reported for *Synechococcus, Haematococcus*, *Chlorella*, *Phaedolactinum*, and an *Scenedesmus almeriensis* strain (Revah and Morales 2015).

Photosynthesis of most algal species is saturated at a solar radiation level of 200 μE m^{-2} s^{-1}, which is about 10–17% of summer/winter maximum outdoor light intensity (Park et al. 2011).

The culture density and algal strain affect the light saturation level; the algal productivity from incident solar radiation can be estimated from the maximum efficiency of photosynthetic conversion of algae. For this, it is necessary to study the influence of light on the growth of any particular strain. Algal productivity could be determined from the average solar radiation photoinhibitory process that can result in a loss in biomass productivity and nutrient removal efficiency. This problem can be solved by shading the culture such that the incident photon flux decreases and by avoiding a culture of microorganisms in the heating systems.

8.2 Temperature Limitation

In the closed system, especially in the photobioreactor, the temperatures increase with increasing solar radiation and air temperatures. The heat is transferred mainly through radiation, air radiation, direct and diffuse solar radiation; heat is transferred to the medium through natural or forced convection in case of mixing.

In the outdoor culture system, geographic location will define the maximum temperature that algae may be exposed to and be able to grow and survive; in high insolation, temperature can reach 40 °C.

Economically, temperature control for outdoor large-scale ponds is impossible, as temperature varies during the day and with season; thus, the algal strain should be chosen to tolerate a broad range of temperature as well as showing high productivity and nutrient removal during the year in summer when the pond achieves a high temperature in arid areas, especially at night or in the winter when the pond may freeze over.

8.3 Evaporation

High evaporation rate is one of the main problems of outdoor algae ponds and is most often seen as a limitation. Evaporation is a surface process, mainly influenced by air temperature, relative humidity, and wind. Evaporation from algae ponds can be estimated from standard evaporation ("Pan A"). The factors affecting evaporation rate are temperature, surface area, humidity of air, and wind speed. However, algae ponds are much shallower and mechanically mixed, and thus are expected to increase in evaporation rates. In most of the areas considered suitable for algae culture the evaporation rate is found to be high, which can affect the "blow-down" ratio (BDR) and induce salinity and nutrient concentration variations. Freshwater evaporation rate in some tropical regions is found to be 0.01 $m^3\ m^{-2}\ day^{-1}$, or 10 mm per day (Chisti 2012). On the other hand, the composition and nature of the wastewater affect the evaporation rate. The evaporation rate decreases as the solids and the concentrations chemical increase. Under the same environmental condition, the evaporation rate of seawater and wastewater from a pond is generally a little less than the evaporation rate of freshwater.

In reality, a microalgae strain can survive and grow at peak summer temperatures but it develops less well during winter days. For this, the solution is to deploy different strains of microalgae under different climatic variations of the year (different seasonal crops). We can assume the use of alternate microalgal cultivation with seasonal changes for seamless continuity in biomass production over the year (Kenny and Flynn 2017).

9 Conclusion

Much knowledge on the use of microalgae for WWTs has been validated and proved. The removal efficiency of a nutrient or specific pollutant from a different type of wastewater by several microalgae strains has been tested and studied. Although few data on outdoor cultivation performance are available, it appears that selecting the locations for algal cultivation using WWT indeed provides economic and environmental advantages that will, therefore, require careful optimization and assessment. However, further research for performing an algae-based WWT process is still needed to answer to the future challenges such as technical and economic feasibility at an outdoor large scale, optimization of hydraulic retention time, contamination control, and harvesting of algae biomass.

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