Article Title: Outdoor microalgae-based urban wastewater treatment: recent advances, applications and future perspectives

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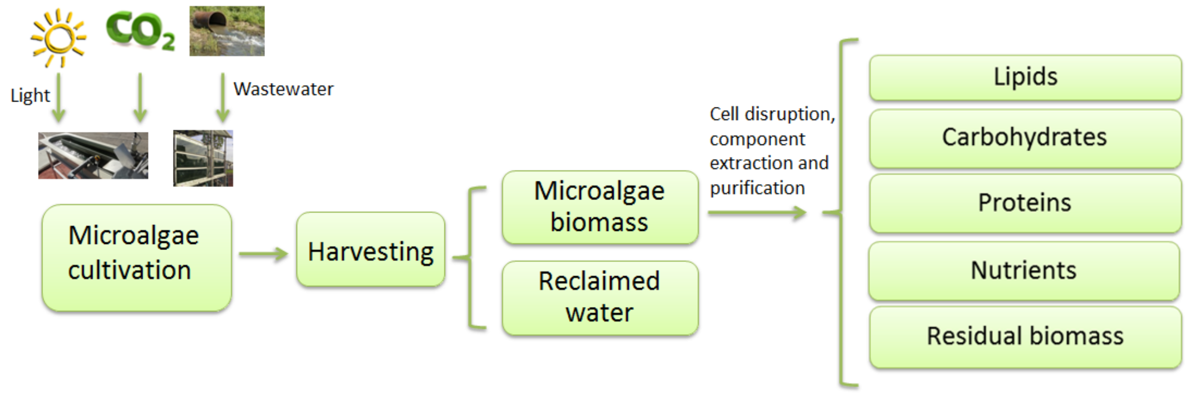
Abstract

Although microalgae-based wastewater treatment has been traditionally carried out in extensive waste stabilisation ponds (WSP), recent trends focus on the use of microalgae to apply the circular economy (CE) principles in the wastewater treatment sector due to the capacity of algae to absorb carbon dioxide while recovering nutrients from sewage. To this aim, the development of new intensive microalgae-based systems with higher efficiency and level of process control are required. Results obtained for these systems at lab-scale are generally promising. However, upscaling to outdoor conditions is often uncertain. Some advances have been made in terms of applying open systems at large scale. However, there are still some issues related to land requirements and the economic feasibility and robustness of the process that have to be overcome to widely implement these systems.
This article aims at describing the main design and operating factors regarding outdoor microalgae cultivation. It will also explain some microalgae cultivation technologies to treat wastewater, showing their advantages, disadvantages, and the possibility to treat different wastewater streams with microalgae cultures. Future perspectives of this biotechnology will be commented as well.

Graphical/Visual Abstract and Caption



1. INTRODUCTION

Microalgae cultivation has long been used to treat urban wastewater. By the mid-1950´s, W.J. Oswald and his collaborators investigated the ability of microalgae-bacteria consortia to treat and aerate wastewater in extensive waste stabilisation ponds (WSP) (Oswald et al., 1953; Oswald and Gotaas, 1957). Later, the oil crisis in the 1970´s and in the 2000´s boosted the development of microalgae biomass as potential third generation of bio-fuels (Paddock, 2019). However, the increasing human population, the economic development and the change in consumption patterns have raised the need for a transition in the wastewater treatment sector from its traditional vision (based on extensive technologies which focus on reducing pollutants) to a new paradigm (based on developing intensive wastewater treatment technologies which frame within circular economy (CE) principals). Circular economy is related to sustainable development where the virgin resource consumption is limited (or hindered) by enhancing the resource recovery from by-products such as wastewater (Ubando et al., 2020).

The emerging scientific interest in microalgae biotechnology observed during the last decade (Garrido-Cárdenas et al., 2018) is mainly due to their capacity to integrate wastewater treatment and resource recovery while producing valuable biomass that presents a wide variety of applications (Cuevas-Castillo et al., 2020; Goswami et al., 2020; Hussain et al., 2021). This way, wastewater is no longer treated as waste but as a source of energy, nutrients, and reclaimed water (Robles et al., 2019), thus applying the CE principals to the wastewater sector. Consequently, traditional wastewater treatment plants (WWTPs) shift to novel water resource recovery facilities (WRRFs) (Seco et al., 2018). In addition, microalgae cultivation has been reported to reduce the environmental impacts and to consume up to 50% less energy than conventional treatment methods based on activated sludge (Acién et al., 2018; Kohlheb et al., 2020; Nagarajan et al., 2020).

However, the development of intensive efficient full-scale microalgae-based systems is still in early stages. There are some issues related to land requirements and the economic feasibility and robustness of the process that have to be investigated to improve the feasibility and applicability of this technology at large scale.

In this review, the following topics related to microalgae cultivation are assessed: i) the main factors related to the design and operation of outdoor microalgae cultivation processes; ii) microalgae cultivation technologies; and iii) configurations and perspectives of microalgae-based wastewater treatment systems.

2 MICROALGAE CULTIVATION

Microalgae usually refer to a wide group of microscopic organisms which are capable of carrying out the oxygenic photosynthesis. This includes eukaryotic microalgae and prokaryotic cyanobacteria (Acién et al., 2021; Umamaheswari and Shanthakumar, 2016). Generally, microalgae use the photoautrotophic metabolism to grow, i.e., they use an inorganic carbon source, light as energy source and nutrients to produce carbohydrates, lipids, proteins, etc. (Behera et al., 2018). However, some microalgae can also use organics as carbon source, being heterotrophic and/or mixotrophic (Javed et al., 2019; Zabed et al., 2020). Since photoautotrophic growth is the most frequent (Assunçao and Malcata, 2020; Cuevas-Castillo et al., 2020), this study will only focus on photoautotrophic cultivation, which includes light and dark reactions (Reynolds, 2006).

Microalgae are versatile microorganisms that have shown the capacity of recovering nitrogen and phosphorus from sewage to values that can accomplish legal requirements (González-Camejo et al., 2020a). They can adapt to wide ranges of pH, irradiance intensity, temperature and nutrient concentrations (Mantovani et al., 2020; Soares et al., 2019). Specifically, green microalgae genera (mainly *Chlorella, Scenedesmus* (see Figure 1) and *Chlamydomonas*) have been extensively reported as ideal for efficient wastewater treatment due to their adaptability to such medium (Pachés et al., 2020). In fact, many authors have tested microalgae cultures for wastewater remediation under lab-scale conditions using sewage from different streams (Table 1). The adaptability of microalgae to different wastewater media enables them to be used in different WWTP configurations depending on the goal of the microalgae cultivation process. This will be further discussed in Section 4.

Table 1. Biomass productivities and nutrient removal efficiencies in different lab-scale urban wastewater streams.

Species	Wastewater	Reactor (volume)	Conditions	Operation	Influent concentration ($mg \cdot L^{-1}$)	BP	NRE (%)	PRE (%)	Reference
Chlorella vulgaris	Secondary effluent	Erlenmeyer (500 mL)	L = 0.98 $\mu W \cdot m^{-2}$ L/D = 24/0 T = 20ºC pH = non-controlled	Batch (28 d)	N: 66.9 P: 26.0	-	56	12	AlMomani and Örmeci (2016)
Neochloris oleoabundans	Secondary effluent	Erlenmeyer (500 mL)	L = 0.98 $\mu W \cdot m^{-2}$ L/D = 24/0 T = 20ºC pH = non-controlled	Batch (28 d)	N: 66.9 P: 26.0	-	57	6	AlMomani and Örmeci (2016)
Mix indigenous microalgae	Secondary effluent	Erlenmeyer (500 mL)	L = 0.98 $\mu W \cdot m^{-2}$ L/D = 24/0 T = 20ºC pH = non-controlled	Batch (28 d)	N: 66.9 P: 26.0	-	67	31	AlMomani and Örmeci (2016)
Cyanobacteria + green algae	Centrate + secondary effluent	Cylindrical (2.5 L)	L = 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ L/D = 12/12 T = 27ºC pH = 8.5	Continuous SRT = 10 d HRT = 6 d	N: 71.6 P: 20.0	120	58	83	Arias et al. (2019)
Microalgae consortium	Primary effluent	Duran bottles (200 mL)	L = 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ L/D = 12/12 T = 15ºC pH = 8	Batch (8 d)	N: 49.4 P: 3.1	-	83	100	Delgadillo-Mirquez et al. (2016)
Chlorella sorokiniana	Raw sewage	Flasks (2 L)	L = 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ L/D = 16/8 T = 22ºC pH = 7	Batch (15 d)	N: 52.6 P: 8.5	-	87	68	Gupta et al. (2016)
Scenedesmus obliquus	Raw sewage	Flasks (2 L)	L = 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ L/D = 16/8 T = 22ºC pH = 7	Batch (15 d)	N: 52.6 P: 8.5	-	99	98	Gupta et al. (2016)

Chlorella sp.	Dilluted Centrate	Erlenmeyer (500 mL)	L = 150 μmol·m^{-2}·s^{-1} L/D = 24/0 pH = 8	Semi-continuous	N: 60.0 P: 18.1	100	95	85	Ledda et al. (2015)
Chlorella spp. + *Scenedesmus* spp.	Centrate + secondary effluent	Column (12 L)	L = 230 μmol·m^{-2}·s^{-1} L/D = 12/12 T = 9-20ºC pH = 7.2-8.0	Batch	N: 120-250	-	> 90*	-	Marazzi et al. (2017)
Scenedesmus obliquus	AnMBR effluent	Flasks (2 L)	L = 250 μmol·m^{-2}·s^{-1} L/D = 14/10 T = 20-25ºC pH = 7.5	Batch (8 d)	N: 67.9 P: 4.6	48	97	100	Pachés et al. (2020)
Chlorella vulgaris	AnMBR effluent	Flasks (2 L)	L = 250 μmol·m^{-2}·s^{-1} L/D = 14/10 T = 30-35ºC pH = 7.5	Batch (13 d)	N: 48.7 P: 5.4	42	85	100	Pachés et al. (2020)
Scendesmus obliquus	Secondary effluent	Flat-panel PBR (4.5 L)	L = 250 μmol·m^{-2}·s^{-1} L/D = 14/10 T = 20ºC CO_2 = 5%	Continuous HRT = 2.8 d	N: 34.9 P: 3.6	380	87	98	Ruiz et al. (2013)
Scenedesmus LX1	Secondary effluent	Erlenmeyer (500 mL)	L = 200 μmol·m^{-2}·s^{-1} L/D = 14/10 T = 25ºC pH = 7.8	Batch (13 d)	N: 27.4 P: 2.3	450	72.6	~100	Wu et al. (2017)
Haematococcus pluvialis	Secondary effluent	Erlenmeyer (500 mL)	L = 200 μmol·m^{-2}·s^{-1} L/D = 14/10 T = 25ºC pH = 7.8	Batch (13 d)	N: 27.4 P: 2.3	350	73.7	~100	Wu et al. (2017)
S. LX1 + H. pluvialis	Secondary effluent	Erlenmeyer (500 mL)	L = 200 μmol·m^{-2}·s^{-1} L/D = 14/10 T = 25ºC pH = 7.8	Batch (13 d)	N: 27.4 P: 2.3	530	85.0	~100	Wu et al. (2017)

**NH_4 removal efficiency (includes nitrification)*

AnMBR: anaerobic membrane bioreactor; BP: biomass productivity; L: light; L/D: light:dark cycle; NRE: nitrogen removal efficiency; PBR: photobioreactor; PRE: phosphorus removal efficiency; T: temperature.

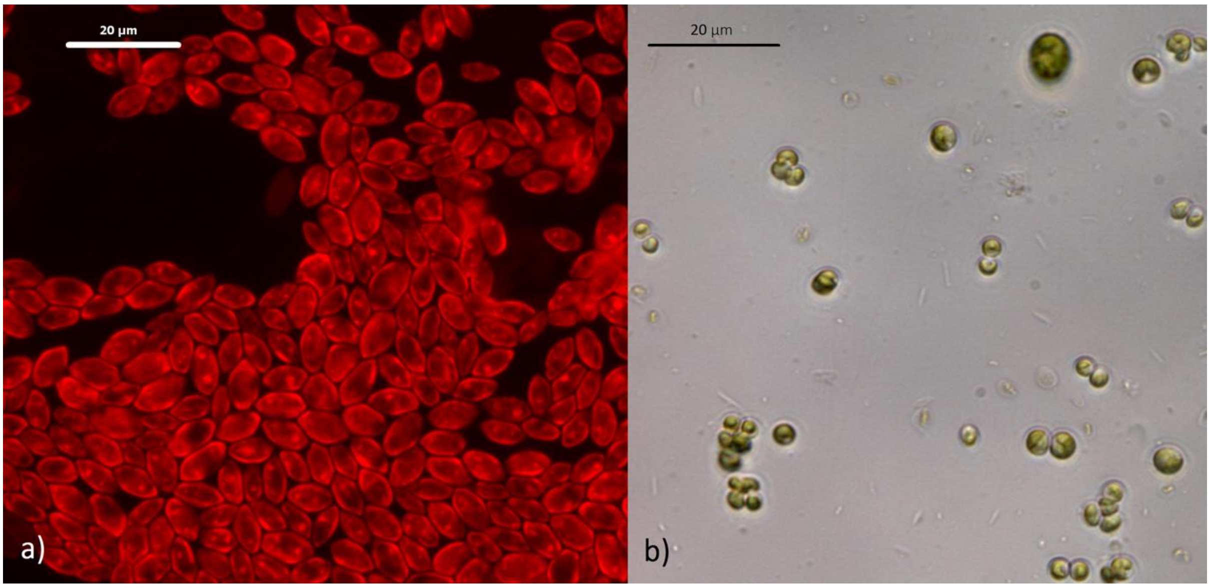


Figure 1. Common green microalgae for wastewater treatment: a) Scenedesmus; b) Chlorella.

It must be noted that pure cultures can only be cultivated in highly controlled lab-scale conditions (Gao et al., 2016; Luo et al., 2018), while outdoors, contamination of microalgae with other microorganisms is expected (Galès et al., 2019; Shahid et al., 2020). These polycultures can increase microalgae productivity due to their better adaptability to variable conditions and their higher robustness, resistance, and efficiency in the use of resources (AlMomani and Örmeci, 2016; Rossi et al., 2020). For these reasons, mixed cultivation is the only feasible option for outdoor microalgae cultivation. There are two main approaches regarding microalgae polycultures, depending on the goal of the treatment process:

i) *microalgae-bacteria consortia*: this mixed culture enables the simultaneous removal of organic matter and nutrients due to symbiotic interactions between microalgae and bacteria (Robles et al., 2019). During photosynthesis, microalgae produce oxygen that is used by bacteria to oxidise the organic matter. Consequently, carbon dioxide is produced, which can be used by algae as inorganic carbon source (Chai et al., 2021; Delgadillo-Mirquez et al., 2016; Shahid et al., 2020).

ii) *microalgae as dominant organism*: when the biodegradable concentration of the wastewater stream is low (for instance, in effluents from aerobic biological reactors or anaerobic digestion), microalgae tend to dominate the interaction with bacteria. In this case, the bacteria activity will be aimed to be as low as possible since microalgae-bacteria can also present some competitive interactions such as nutrient competition and the release of toxic compounds that can negatively affect microalgae growth (Day et al., 2017; González-Camejo et al., 2019a). Moreover, the bacteria biomass present in the consortia increases the shadow effect of the culture, therefore decreasing the light availability of algae.

2.1 Design and operating factors related to microalgae cultivation

When a microalgae cultivation system is selected for outdoor sewage treatment, the following aspects have to be considered:

2.1.1 Climatic conditions

Microalgae are highly dependent on environmental conditions such as solar radiation and temperature, as well as on their diurnal and seasonal variations (Morillas-España et al., 2020; Wallace et al., 2016). Hence, the selection of the microalgae cultivating place is essential to obtain maximum microalgae performance. Many authors have developed life cycle assessments (LCA) to predict the best sites to cultivate microalgae and they generally agree that warm regions with high solar radiation are the most appropriate. In this respect, Jonker and Faaij (2013) obtained significantly lower energy consumption in the production of microalgae in Bissau (Guinea-Bissau) and Huelva (Spain) than in Uppsala (Sweden). In addition, Díez-Montero et al. (2018) studied the energy balance of a hypothetical microalgae-based wastewater system in thirteen Spanish geographic locations, obtaining the most favourable cultivating conditions in Seville and Almeria (the places with the highest solar radiation).

2.1.2 Open/closed systems

Microalgae cultivation systems are basically divided in open systems such as natural stabilisation ponds or raceway reactors (Fernández et al., 2016; Mara, 2004; Umamaheswari and Shanthakumar, 2016) or closed photobioreactors (PBRs) like tubular or flat-panel PBRs (Assunção and Malcata, 2020). Open systems are usually the most economical option (Acién et al., 2018;2021). However, as they have no physical barriers between the culture and the atmosphere, they are significantly more affected than closed PBRs by the contamination of competing organisms and grazers, which negatively affects microalgae performance (Day et al., 2017; Galès et al., 2019). They are also more affected by climatic conditions and present high stripping losses of carbon (in the form of CO_2) and nitrogen as ammonia (NH_3) and/or nitrogen gas (N_2) (Faleschini et al., 2012; Mantovani et al., 2020).

In the case of microalgae cultivated in closed PBRs, despite being more protected from climatic conditions and outer contamination, the oxygen produced during photosynthesis can accumulate. This is controversial as oxygen concentrations over 400% of saturation has been reported to be inhibitory (Chisti, 2007). Examples of open and closed systems will be given in Section 3.

2.1.3 Horizontal/vertical reactors

Open ponds must be horizontal reactors while closed PBRs can be either horizontal or vertical (De Vree et al., 2015). Outdoor horizontal reactors receive higher sunlight radiation since it is applied to the reactor perpendicularly. This makes horizontal PBRs usually be more efficient regarding productivity and environmental impacts (Pérez-López et al., 2017). However, higher radiation increases the risk to suffer from photoinhibition which would reduce microalgae growth and photosynthetic efficiency (PE), especially in summer or at noon (Straka and Rittman, 2018). On the other hand, in vertical PBRs, light radiation is applied with a certain angle which implies that microalgae are exposed to lower photon flux. This avoids excessive energy exposure and results in less dissipated energy in the form of fluorescence and/or heat (De Vree et al., 2015). Consequently, PE is higher in vertical systems. In fact, De Vree et al. (2015) compared the PE of pilot-scale raceway pond, vertical flat-panel PBR and horizontal and vertical tubular PBRs, obtaining 1.5%, 3.8%, 1.8% and 4.2%, respectively.

2.1.4 Suspended/attached cultures

Microalgae cells are usually cultivated free in the wastewater media, growing as individual cells or small coenobia and being suspended in the reactor (Assunção and Malcata, 2020; Mohsenpour et al., 2021). In suspended systems, microalgae assimilate nutrients more efficiently as they have all (or most) of their membrane surface free. In addition, as no other cells surround them, they present better illumination than when they form flocs (Felipe Novoa et al., 2020). For this reason, suspended systems have reported higher biomass and oxygen production than attached cultures (Lin-Lan et al., 2018).

Another approach is cultivating microalgae in biofilms or granules. Microalgae biofilm is formed by a consortium of microorganisms including microalgae, bacteria, and protozoa, which are wrapped by cations, inorganic compunds, soluble microbial products (SMP) and extracellular polymeric substances (EPS) (Mohsenpour et al., 2021). Biofilms normally develop on attaching materials with different shape and structure (Li et al., 2019). These attached systems can ease biomass harvesting except for membrane filtration, where the fouling characteristics of the SMP and EPS matrix hinders the process (Kumar et al., 2020; Luo et al., 2019). Advantages and disadvantages of some harvesting methods are discussed in Table 2.

Attached systems are generally divided in two groups: i) fixed-bed systems in which a stationary matrix (i.e. artificial or natural porous matrices, fibres or surfaces) is needed for biomass immobilisation; and ii) fluidised bed systems where biomass is immobilised on a floating substratum, increasing the surface:volume (S/V) ratio and improving light distribution (Wollman et al., 2019)

Table 2. Advantages and disadvantages of microalgae harvesting technologies.

	Advantages	Disadvantages	References
Sedimentation	- Simple - Low capital and operation costs - High space requirements	- Poor settling rate - Low quality of effluent - Biomass losses - Time consuming - Diluted biomass concentration	Razzak et al. (2017) Soares et al. (2019)
Flocculation	- Faster settling rate than sedimentation - Better quality of effluent	- Use of chemical reagents (metal salts mainly) - Extra cost - Metal can disable microalgae - Needs to be combined with other separation methods	Rajesh-Banu et al. (2020) Soares et al. (2019)
Flotation	- Low capital costs - Faster than sedimentation - High efficiencies	- Use of reagents - Possible disruption of microalgae	Razzak et al. (2017) Rajesh-Banu et al. (2020)
Centrifugation	- Rapid - Capable of harvesting most algal cell types	- Very energetically costly - Shear stress - Low EPS removal	Acién et al. (2018) Razzak et al. (2017)
Filtration	- High-quality permeate - Higher biomass concentration - Low space requirement - No chemicals needed during filtration. - Easy to scale-up	- Air-sparging costs - Membrane fouling.	Acién et al. (2018) Rajesh-Banu et al. (2020) González-Camejo et al. (2020a) Razzak et al. (2017) Seco et al. (2018) Zhang et al. (2019)

2.1.5 Light path

Light path is a critical factor which influences the light availability of the system (González-Camejo et al., 2020b). Due to microalgae biomass and their pigments, the incident light which illuminates the reactor´s surface exponentially decreases along the culture (Wagner et al., 2018). Consequently, microalgae in the deepest places of the reactor remain light-limited or even in complete darkness, reducing microalgae productivity (Fernández et al., 2016; Raeisossadati et al., 2019). For this reason, light path in microalgae reactors tends to be short, i.e., around 15-30 cm for raceway ponds (Acién et al., 2021; Arbib et al., 2017) and in the range of 2-10 cm for closed PBRs (González-Camejo et al., 2020a; Slegers et al., 2011). However, it must be considered that the shorter light path, the higher risk of overheating, which can be detrimental for microalgae growth (see Section 2.1.12).

Light path is also associated to the S/V ratio, which is another relevant factor affecting microalgae performance. Generally, the higher the S/V ratio, the higher amount of light photons are supplied to the reactor surface, thus improving biomass productivities (Assunção and Malcata, 2020; Morillas-España et al., 2020). However, as aforementioned in Section 2.1.3, excessive radiation can cause microalgae photoinhibiton.

2.1.6 Non-photic volume

Another factor related to the light availability of the culture is the non-photic volume. This refers to the reacting volume that is not exposed to light such as connecting pipes and distribution tanks. If a membrane system is used to separate microalgae from the wastewater stream and recirculated it to the reactor as in membrane photobioreactors (MPBRs) (González-Camejo et al., 2020a; Luo et al., 2018) or in membrane-couple algal ponds (Robles et al., 2019), the volume of the membrane tanks should be minimised to increase microalgae performance. In this respect, Viruela et al. (2018) obtained 15%, 67% and 41% higher nitrogen removal rate (NRR), phosphorus removal rate (PRR) and biomass productivity (BP), respectively, when the membrane tank´s volume was reduced from 27.2% to 13.6% of the total reacting volume.

2.1.7 Orientation

Microalgae reactors must be properly oriented to maximise microalgae performance. In raceway ponds, they have to be north-south oriented to minimise the shadow produced by the reactor´s walls and baffles (Romero-Villegas et al., 2018a).

Regarding PBRs, north-south orientation has been reported to be more favourable for latitudes over 35º N, while closer to the equator, the east-west orientation contributes to increase the photosynthetic rate due to higher solar radiation (Romero-Villegas et al., 2018b).

2.1.8 Cultivation mode

Cultivation mode influences microalgae growth rate and productivity (Barbera et al., 2020; Behera et al., 2018). Lab-scale studies have been traditionally based on batch cultivation (Almomani and Ormeci, 2016; Gupta et al., 2016). However, in outdoor systems batch cultivation is not feasible as it would require huge surface areas. On the other hand, continuous cultivation has been reported to obtain higher microalgae activity as they are maintained in the exponential growth phase for longer (Assunção and Malcata, 2020; Umamaheswari and Shanthakumar, 2016). According to Yadav et al. (2020), semi-continuous feeding is the most suitable option for large-scale microalgae cultivation for its simplicity and ease of implementation. In this respect, González-Camejo et al. (2019a; 2020a) operated a pilot-scale MPBR system semi-continuously, only feeding the culture during light hours to increase microalgae activity.

2.1.9 Operating conditions

Operating conditions, i.e., solids retention time (SRT), hydraulic retention time (HRT) and dilution rate (inverse of HRT) can also play a significant role in microalgae performance as they affect microalgae biomass productivity, nutrient recovery, and the activity of competing organisms (Barbera et al., 2020; González-Camejo et al., 2020b). There are two main ways to operate microalgae-based wastewater treatment systems:

i) without biomass retention. In this case, HRT is equal to SRT (Galès et al., 2019; Romero-Villegas et al., 2018b). Since microalgae usually present lower growth rates than bacteria, i.e., in the range of 0.4-0.9 d^{-1} (Pachés et al. 2020; Ruiz et al., 2013), relatively long HRTs are needed to avoid microalgae washout: 3.3-10 d (Arbib et al., 2017; González-Camejo et al., 2019a). In addition, low HRTs will increase the loading rates to the reactor, which can be detrimental for the wastewater treatment process due to excessive concentration of nutrients and/or other pollutants that can inhibit microalgae growth partially or totally (Assunção and Malcata, 2020). In this respect, González-Camejo et al. (2020a) observed a decrease in MPBR performance when operated at 1-d HRT due excessive nutrient loading rates which promoted nitrifying and heterotrophic bacteria proliferations. Furthermore, Faleschini et al. (2012) reported maximum biochemical oxygen demand (BOD_5) load of 60 kg $BOD_5 \cdot ha^{-1} \cdot d^{-1}$ for a WSP operated in a temperate climate region. On the other hand, long HRT can make the culture be nutrient-limited, thus favouring the proliferation of superior organisms such as protozoa and rotifers which can compete with and/or predate microalgae (Arias et al., 2019; González-Camejo et al., 2019a).

ii) decoupling SRT from HRT. To do so, microalgae biomass needs to be separated from the wastewater stream by a harvesting system (Table 2), being membrane filtration the most common. This separation enables to increase nutrient loads to the reactors, maximising microalgae nutrient uptake. Simultaneously, microalgae biomass remains in the reactors for longer which gives them enough time to grow with the goal of optimising biomass productivity (Gao et al., 2016; Luo et al., 2018).

2.1.10 Mixing

Microalgae reactors based on suspended cultures are usually mixed (either by mechanical mixing or air sparging) due to several reasons: i) when the reactor is well-mixed, the microalgae culture move rapidly from dark to illuminated zones (Kwon et al., 2019), reducing the shadow effect and thus increasing microalgae performance (Barceló-Villalobos et al., 2019); ii) to maintain culture homogenisation (Acién et al., 2021); iii) to improve the CO_2-mass transfer (Assunção and Malcata, 2020); iv) to prevent microalgae sedimentation (Huang et al., 2017); v) to reduce biofouling in the inner walls of the PBRs; and vi) to avoid excessive oxygen accumulation. However, excessive mixing will increase shear stress, which can damage microalgae (Vo et al., 2019).

Mixing also plays a significant role in determining the operating costs of the treatment systems. In this respect, raceway ponds (Section 3.1.2) are usually mixed by paddlewheels which energy requirements are less than 10 W·m^{-3}, while air sparging of closed PBRs (Section 3.1.3) can consume up to 400 W·m^{-3} (Acién et al., 2021).

2.1.11 pH control

Microalgae activity implies a pH rise due to the carbon fixation during photosynthesis (Eze et al., 2018). pH can reach values over 10, which despite being beneficial for pathogen removal in open systems (Chai et al., 2021), can also inhibit green microalgae growth (Iasimone et al., 2018). On the other hand, pH values around 7-7.5 are optimum for green microalgae (Eze et al., 2018). These pH values have been also reported to produce negligible ammonia concentration and phosphorus precipitation (Hussain et al., 2021; Tan et al., 2016). These processes are inconvenient because ammonia can inhibit the photosynthetic process and reduce the nitrogen concentration in the culture due to ammonia stripping (Galès et al., 2019; Tua et al., 2021) while phosphorus precipitation not only lowers the bioavailability of this nutrient, but also diminishes the light dispersion in the microalgae culture due to an increase of the culture turbidity (González-Camejo et al., 2019b). For this reason, an effective pH control system is essential to improve microalgae performance. pH control is often performed by injecting CO_2 (either pure or contained in flue gases), avoiding the carbon limitation of wastewater simultaneously (Acién et al., 2021; Assunção and Malcata, 2020). In this respect, Yadav et al. (2020) reported an increase of 62% of microalgae cell density when CO_2 was added to the culture.

On the contrary, CO_2 addition significantly increases the operating costs, especially in open systems, where significant amounts of carbon dioxide are released to the atmosphere (Acién et al., 2016).

2.1.12 Temperature control

Optimum temperature for microalgae growth is around 20-30ºC (González-Camejo et al., 2019c; Umamaheswari and Shanthakumar, 2016). However, temperatures of only 2-4ºC over the optimum lead to reduction of microalgae performance or even to cell death (Mazzelli et al., 2020).

In the case of open ponds, excessive temperatures are usually regulated by water evaporation. However, excessive water losses from the system can significantly change the ionic composition of the culture, which can in turn affect microalgal growth (Mohsenpour et al., 2021).

On the other hand, in closed PBRs culture temperatures can reach values 10-30 ºC higher than in their surroundings (Yeo et al., 2018; Wang et al., 2012). A possible solution consists of installing heat-exchangers (González-Camejo et al., 2019c), surface water spraying systems, shading nets, pool water immersion, overlapping tubes, or regulate the feed stream to reduce culture temperature (Assunção and Malcata, 2020). However, this increases the treatment costs significantly, making close PBRs unfeasible to be used in wastewater treatment processes.

2.1.13 Artificial lighting

In general, outdoor cultivation systems are light-limited (Barceló-Villalobos et al., 2019; González-Camejo et al., 2020a). To overcome this light attenuation, artificial light sources could be added to the microalgae culture to achieve higher performance (Cuevas-Castillo et al., 2020; Mohsenpour et al., 2021). In fact, some authors have tried to reduce the dark volume by introducing LED lamps in the darkest zone of the PBR (Rebolledo-Oyarce et al., 2019). However, it must be considered that artificial illumination is highly energy-demanding and it is not feasible to treat wastewater unless the energy needed for illumination would be obtained from energy surplus within WRRFs (see Section 4.5) or microalgae *Biorefineries* (see Section 5).

3. OUTDOOR MICROALGAE CULTIVATION TECHNOLOGIES

Although recent lab-scale studies based on intensive microalgae-based wastewater treatment systems usually present promising results in terms of biomass productivity and nutrient removal efficiencies (Table 1), up-scaling to outdoor conditions often reduce microalgae performance significantly (Table 3). This entails an increase of the operating costs and/or land requirements in order to obtain adequate wastewater depuration. To maximise microalgae activity and thus reduce nutrient effluent concentrations, there are plenty of variables to be considered (González-Camejo et al., 2020b). Some key aspects to take into account to accomplish success in microalgae-based wastewater treatment are: i) the selection of robust microalgae strains, capable to grow under variable conditions (Morillas-España et al., 2020). In this respect, native microalgae are usually a preferable option as they are better adapted to the environment (Galès et al., 2019); ii) selection of the most appropriate reactor configuration (Mohsenpour et al., 2021); iii) monitoring, automation and control of microalgae cultivation to implement the process. Many approaches have been already done in this respect (Foladori et al., 2018; Martínez et al., 2019; Robles et al., 2020), although further research is needed to implement industrial-scale microalgae cultivation systems.

Microalgae-based wastewater treatment systems:

Microalgae-based wastewater treatment systems shows great potential to implement circular economy principles to the wastewater sector. However, up-scaling of microalgae cultivation systems to outdoor conditions is often uncertain.

Table 3. Microalgae performance in outdoor microalgae-based wastewater treatment systems.

PBR	Wastewater	SRT/HRT (d)	Productivity (mgVSS·L^{-1}·d^{-1})	NRE (%)	PRE (%)	Reference
HRAP	Secondary effluent	3.1-4.6	87-136	74-82	70-90	Arbib et al. (2017)
Rotating algal biofilm	Open lagoon effluent	-	158	75	23	Christenson and Sims (2012)
Primary Facultative Pond	Sewage	24-31	-	>90*	-	Faleschini et al. (2012)
Flat-panel MPBR	AnMBR effluent	3/1.5	258	85	99	González-Camejo et al. (2020a)
HRAP	Digestate	10	27	41	71	Mantovani et al. (2020)
Membrane HRAP	Synthetic	6/2.5	90	60	66	Robles et al. (2019)
Tubular PBR	Seawater + centrate	3.3	600	>95	>95	Romero-Villegas et al. (2017)
Flat-panel MPBR	AnMBR effluent	4.5	66	7.7	1.2	Viruela et al. (2018)

**Corresponds to ammonium removal.*

HRAP: high-rate algal pond; HRT: hydraulic retention time; MPBR: membrane photobioreactor; NRE: nitrogen removal efficiency; PBR: photobioreactor; PRE: phosphorus removal efficiency; SRT: solids retention time.

Microalgae suspended cultures have been mostly operated in open systems (mainly stabilisation and raceway ponds) and closed PBRs, although other prototypes have recently been tested with the goal to overcome the drawbacks of previous systems.

3.1 Suspended systems

3.1.1. Extensive systems

The first approach related to microalgae-based wastewater treatment was based on extensive waste stabilisation ponds. WSP are large shallow basins (delimited by land embankments) where raw wastewater is treated by natural processes involving microalgae-bacteria consortia. They can be composed of one or more series of ponds or be combined with other processes (Faleschini et al., 2012). According to their depth and their biochemical reactions, stabilisation ponds could be: i) anaerobic (2 - 4 m deep); ii) facultative (around 1.5 m deep); and iii) maturation ponds (around 1 m deep), being facultative and maturation the ponds where photosynthesis take place (Butler et al., 2017; Mara, 2004). The high depth of these ponds makes light distribution be very limited in these systems (see Section 2.1.5).

The main advantage of WSP lies on their low-cost and simplicity to remove organic matter, nutrients, and pathogen from wastewater efficiently. Civil works and energy required to treat wastewater are minimal (Burler et al., 2017; Mara, 2004). On the other hand, the depuration process can be hardly controlled and depends on weather conditions and pollutant loading rates completely, usually entailing low biological activity and odour issues, especially during colder months in temperate climate regions (Faleschini et al., 2012). As a result, HRT is much longer than in intensive treatment processes, i.e., in the range of 11-86 d (Abis and Mara, 2005). Although this high retention time make WSP be very robust, it also implies huge land requirements, being in the order of tens or even hundreds of hectares (Mara, 2004; Wallace et al., 2016). For this reason, WSP are usually used to treat wastewater from small rural communities where land is highly available (Abis and Mara, 2005; Faleschini et al., 2012).

3.1.2. Open ponds

High-rate algal ponds (HRAPs) or raceway ponds (Figure 2a) emerged as an enhanced design of WSP with added operational control to maximise microalgae performance (Chisti, 2007; Paddock, 2019). These open systems are the most used at mid and large scale, mainly due to their cost-efficiency and easiness to operate in comparison to closed PBRs (Assunção and Malcata, 2020; Yadav et al., 2020). According to many authors, raceway ponds represent the only feasible microalgae-based configuration to treat wastewater intensively (Acién et al., 2018; Mohsenpour et al., 2021; Cuevas-Castillo et al., 2020).

As aforementioned in Section 2.1.5, raceway depth is usually in the range of 15-30 cm (wider than closed PBRs). This hinders the culture homogenisation and reduces light availability (Barceló-Villalobos et al., 2019). To overcome this, thin-layer reactors have been developed. They consist of open reactors with short culture depths of 0.5-5 cm (Morillas-España et al., 2020). In this respect, Morales-Amaral et al. (2015) obtained 43% higher biomass production in a 2-cm-deep thin-layer reactor than in a 12-cm-deep raceway pond. However, the volume treated by the thin-layer reactor was 3.7-fold lower than that of the raceway for the same surface. In fact, the main disadvantage of open reactors ponds is the huge surface requirements which can account up to 10 m^2 per equivalent person (Acién et al., 2018). Moreover, they present poor mass transfer and pH and temperature gradients that can affect microalgae performance negatively (Morillas-España et al., 2020).

A recent study showed that both biomass production and nutrient recovery of ponds could be improved if they were operated in series instead of parallel (Sutherland et al., 2020). This could significantly reduce surface requirements and operating costs. In addition, Robles et al. (2019) studied the combination of algal ponds with ultrafiltration membranes, showing promising results. This combination could also help to reduce cultivation area needs significantly by increasing the nutrient loading rate while avoiding microalgae washout. These successful pilot plants clearly show the high potential of these microalgae-based systems for intensive wastewater treatment, although there is still a long way to improve the large-scale implementation of this biotechnology. In fact, most of the existing facilities based on open systems are small or medium scale, i.e., between 1 and 50 hectares (Acién et al., 2021).

3.1.3 Closed photobioreactors

In closed PBRs (Figure 2b), factors affecting microalgae cultivation (pH, temperature, etc.) are usually better controlled than in open reactors. In fact, they are designed to attain higher photosynthetic efficiencies with the goal to increase the biomass productivity and nutrient removal of microalgae (González-Camejo et al., 2020a; Mohsenpour et al., 2021). However, these systems present higher operational costs than open reactors (Assunçao and Malcata, 2020; Vo et al., 2019) which make them unfeasible to be used for sustainable wastewater treatment. Despite this, some authors defend that closed PBRs could be useful as an initial step for adapting microalgae to the wastewater to be treated (Gupta et al., 2019; Javed et al., 2019).

Figure 2. Intensive microalgae cultivation systems: a) Pilot-scale raceway pond; b) Pilot-scale closed photobioreactor.

Different closed PBR configurations have been widely reported with the goal of producing microalgae biomass rather than treating wastewater. Tubular, vertical columns and flat-panel PBRs appear as the most common (Assunçao and Malcata, 2020; Bosma et al., 2014; Huang et al., 2017). Despite usually having larger S/V ratios than open ponds (Umammaheswari and Shanthakumar, 2016), microalgae still need large areas to be cultivated in PBRs. To overcome this drawback, membranes photobioreactors have been developed (Gao et al., 2019; González-Camejo et al., 2019a). As aforementioned, in MPBRs more concentrated microalgae biomass and higher nutrient loads can be achieved (Barbera et al., 2020). In comparison to conventional microalgae cultivation systems, higher quality effluents can be attained in MPBRs at shorter HRTs. For instance, González-Camejo et al. (2020a) accomplished legal requirements when treated anaerobic membrane bioreactor (AnMBR) effluent in a pilot-scale flat-panel MPBR operated at 1.25-d HRT. However, these systems must deal with membrane fouling, which hinders the process and increases operating costs (Seco et al., 2018; Zhang et al., 2019).

3.2. Attached systems

Recent studies have been also interested in upscaling microalgae cultivation based on attached systems to overcome some constraints of the systems based on suspensions such as poor light distribution (Assunção and Malcata, 2020). By way of example, Gross et al. (2015) operated a demonstration-scale rotating algal biofilm reactor (RABR) consisting of rotating cylinders partly immersed into the wastewater to provide the surface for microalgae growth. In addition, Johnson et al. (2018) reported the pilot-scale demonstration of the Algaewheel™ rotating algal contactor, which was used to reduce the ammonium load of centrate by a microalgae-bacteria culture. Although results obtained are promising, these systems are not thought to be widely implemented at industrial scale in the near future.

3.3. Prototypes

Many researchers have made extraordinary efforts on the design of new reactor configurations to overcome the drawbacks of previous microalgae-based wastewater treatment systems, trying to improve their light distribution, photosynthetic efficiency, hydrodynamics, and growth kinetics (Assunção and Malcata, 2020; Olivieri et al., 2014). Some examples of these novel reactors include designs derived from more conventional cultivation systems tried to increase the light available to the culture (Abu-Ghosh et al., 2016). Other authors have mounted baffles or static mixers inside PBRs to enhance mixing and create efficient flashing light effect (FLE) inside the microalgae culture. Some examples of these prototypes are twin-layer PBRs, multi-layer trapezoidal channel bioreactor, high-volume V-shape pond, curved-chamber PBR, alveolar panel PBR, flat-panel airlift PBR, dome-shaped PBR, etc. (Assunção and Malcata, 2020; Kumar et al., 2020; Li et al., 2019).

To the best of our knowledge, these prototypes have not been implemented to treat wastewater at large scale yet as their effectiveness to this aim is controversial.

4. URBAN WASTEWATER STREAMS TREATED BY MICROALGAE

Microalgae cultures are able to treat different wastewater streams, each one with different characteristics: i) raw wastewater after pre-treatment (i.e. fat, sand and grit removal); ii) primary effluent coming from the primary settler (or other separation system) to remove most of suspended particles; iii) secondary effluent obtained from the clarifier once most of the biodegradable organic matter (and sometimes ammonium) are oxidised; iv) the centrate; i.e. the liquid waste obtained from concentrating anaerobic digested sludge (Acién et al., 2016); and v) effluents from anaerobic wastewater treatment. Depending on the wastewater stream, the configuration of the treatment system will be different. Figure 3 shows a general and theoretical design of these wastewater treatment configurations.

4.1 Raw sewage

In case of treating raw sewage with microalgae, the traditional WWTP would be significantly simplified as the microalgae-bacteria consortia would serve as primary, secondary and tertiary treatment (Figure 3a). In this respect, Ling et al. (2019) reported promising results treating raw sewage in outdoor 30-L PBRs (HRT = 6 d), i.e., 84% and 85% of ammonium and phosphorus removal, respectively. Moreover, Faleschini et al. (2012) achieved NH_4 removal higher than 90% when treated wastewater in a full-scale WSP in a temperate climate region (HRT = 24-31 d).

Some industrial-scale intensive raw wastewater treatment plants have been operated in southern Spain. FCC Aqualia inaugurated a demonstration facility in Chiclana de la Frontera (Cádiz, Spain). This 2-ha plant (Cano et al., 2019) has been tested to treat around 2,000 $m^3 \cdot d^{-1}$ and creates a positive energy balance where only about 0.1 $Kwh \cdot m^{-3}$ is used for internal process needs (traditional WWTPs based on activated sludge technology spend up 0.5 $Kwh \cdot m^{-3}$ according to Acién et al. (2018)). The microalgae biomass produced is digested to obtain biogas (FCC Aqualia, 2018). Another demonstration microalgae-based plant has been placed in El Toyo WWTP (Almería, Spain) (Sauco et al., 2019). In this plant, a 3,000 m^2 raceway (2,000 population equivalent) has been continuously operated, obtaining during summertime an overall solids, COD, nitrogen and phosphorus removal of 95%, 94%, 75% and 95%, respectively, and energy savings and greenhouse gases reduction up to 64%. Moreover, the effluent water was reported to meet the legal requirements for irrigation purposes and the microalgae biomass was used to produce biofertilisers.

However, raw wastewater is not the most appropriate cultivation medium for microalgae as it can contain high concentration of organic matter, suspended solids, pathogens and other pollutants that can significantly reduce microalgae growth due to the toxicity of some compounds and the reduction of light availability in the culture (Guldhe et al., 2017). For this reason, loading rates are essential parameters to limit the concentration of these substances in these systems (see Section 2.1.9).

4.2 Primary effluent

Primary effluents are more suitable medium for microalgae cultivation than raw wastewater as solids concentration and turbidity are reduced significantly in comparison to raw sewage. However, primary effluents still have relatively high organic matter concentration so that the use of microalgae-bacteria consortia is needed to reduce nutrient and organic matter concentrations simultaneously (Figure 3b). One industrial-scale example of this configuration was reported by García et al. (2018), who used three full-scale horizontal tubular PBRs (11.7 m^3 each) to treat a mixed of agricultural run-off and treated sewage (this wastewater presented similar characteristics than primary effluents). In addition, Algae Systems LLC designed a microalgae-based system based on floating offshore PBRs of around 2 hectares to treat 50,000 $gal \cdot d^{-1}$ of filtered raw wastewater (similar characteristics than primary effluent). This system was able to remove 75%, 93% and 92% of total nitrogen, total phosphorus and biodegradable organic matter, respectively (Novoveská et al., 2016).

4.3 Secondary effluent

Using microalgae as tertiary treatment to recover nutrients from secondary effluents of aerobic systems is theoretically more suitable to improve microalgae performance than previous options since this water stream contains low amounts of solids and organic matter (AlMomani and Örmeci, 2016; Zhang et al., 2019). However, this microalgae-based configuration (Figure 3c) is not the most appropriate in terms of energy costs and environmental impacts as it is still based on conventional activated sludge system, which is very energetically demanding (Mohsenpour et al., 2021).

In this configuration, the symbiotic interaction between microalgae and bacteria to treat wastewater (see Section 2) does not occur, so in this case microalgae will be intended to be the dominant microorganism of the culture. By way of example, Arbib et al. (2017) tested pilot-scale raceway ponds (1.93 m^2 of surface each) to treat the effluent of *Arcos de la Frontera* WWTP (Spain). In all their experiments, the most restrictive discharge limits of the EU Directive 98/15/EC (10 mg $N·L^{-1}$ and 1 mg $P·L^{-1}$) were accomplished, which corroborates the potential of microalgae to be used as tertiary treatment of aerobic systems.

However, secondary effluents usually contain low nitrogen and phosphorus concentrations, i.e., in the range of around 13-20 mg $N·L^{-1}$ and 0.6-2.4 mg $P·L^{-1}$, respectively (Arbib et al., 2017; Gao et al., 2019). Consequently, microalgae used to treat these streams are expected to be nutrient-limited as nitrogen concentrations lower than 10 mg $N·L^{-1}$ and phosphorus concentrations close to depletion have been reported to reduce microalgae growth (González-Camejo et al., 2019b; Pachés et al., 2020). Another inconvenient is that ammonium, which is the preferred nitrogen source for microalgae (Eze et al., 2018), is almost completely oxidised to nitrate in the biological reactor (Figure 3c). This nitrate is assimilated by microalgae at lower rate than ammonium since it has to be reduced to NH_4 prior to be used (González-Camejo et al. 2019c).

4.4 Centrate

Centrate presents much higher nutrient concentration than other urban wastewater streams. In fact, they can reach up to 1,000 mg $N·L^{-1}$ and 30 mg $P·L^{-1}$ (Acién et al., 2016). If this centrate is recycled to the influent WWTP stream, nitrogen load can be increased by 10-20% (Tan et al., 2016) which significantly raises aeration costs in activated sludge systems coupled with nitrification-denitrification. Consequently, if centrate is treated by microalgae (Figure 3d), the footprint of the overall conventional wastewater treatment process will be reduced (Tua et al., 2021). In this respect, Mantovani et al. (2020) operated an outdoor pilot-scale raceway pond to treat the centrate from the Bresso-Niguarda WWTP (Italy). They calculated that this activated sludge system could reduce the energetic aeration needs by 0.382 $W·m^{-2}$ of biological reactor.

However, centrate also contains high amounts of ammonia, turbidity and other inhibitory compounds that can be toxic for microalgae (Acién et al., 2018; Rossi et al., 2020). Hence, the dilution of the centrate (for instance with secondary effluent or seawater) is often needed. The optimal centrate dilution has to be thus evaluated. By way of example, Romero-Villegas et al. (2018a) reported 20% as optimum centrate dilution with seawater in the cultivation of marine microalgae *Nannochloropsis gaditana*, achieving nutrient removal rates of 28.72 mg $N·L^{-1}·d^{-1}$ and 3.99 mg $P·L^{-1}·d^{-1}$, while biomass productivity accounted for 32.42 $g·m^{-2}·d^{-1}$.

4.5 Anaerobic effluents

As aforementioned, novel WRRFs focus on recovering resources from wastewater instead of only removing pollutants. For this reason, WRRFs are more oriented to anaerobic wastewater treatment than aerobic systems (Song et al., 2018). In this respect, AnMBR technology, which consists of the combination of anaerobic processes and membrane filtration, has been reported to obtain high quality effluents in terms of organic matter and suspended solids (Giménez, 2014). Due to the mineralisation of the organic matter in AnMBR systems and the low capacity of the anaerobic microorganisms to remove nutrients (Dai et al., 2015), AnMBR effluents usually contain higher nutrient concentrations than secondary effluents, i.e., nitrogen concentration (mainly ammonium) can vary between 40-100 mg $N·L^{-1}$, while phosphorus can be around 4-10 mg $P·L^{-1}$ (González-Camejo et al., 2019a). Microalgae-based systems seem therefore ideal for tertiary treatment of AnMBR effluents.

A pilot-scale WRRF prototype has been tested by Seco et al. (2018). It consisted of a primary settling step followed by an AnMBR system (acting as secondary treatment) and an MPBR plant for nutrient polishing. The biomass collected from the primary settler, the AnMBR and the MPBR was then digested in an additional AnMBR system in which biogas was produced. Nutrients could be recovered in downstream processes, while the ultrafiltration membranes enabled to produce reclaimed water (Figure 3e). This pilot WRRF showed promising preliminary results: i) chemical organic matter, nitrogen and phosphorus effluent concentrations only accounted for 45 mg $COD·L^{-1}$, 14.9 mg $N·L^{-1}$ and 0.5 mg $P·L^{-1}$, respectively; ii) 0.44 $kWh·m^{-3}$ of influent wastewater was obtained from biogas production; iii) 26.6% of total nitrogen was recovered as ammonium sulphate, and iv) nitrogen and phosphorus could be potentially recovered as biosolids.

Large-scale applications:

Microalgae are able to treat different urban wastewater streams (raw wastewater, primary or secondary effluent, centrate, AnMBR effluents, etc.). The configuration of the microalgae-based treatment process will be different in each case.

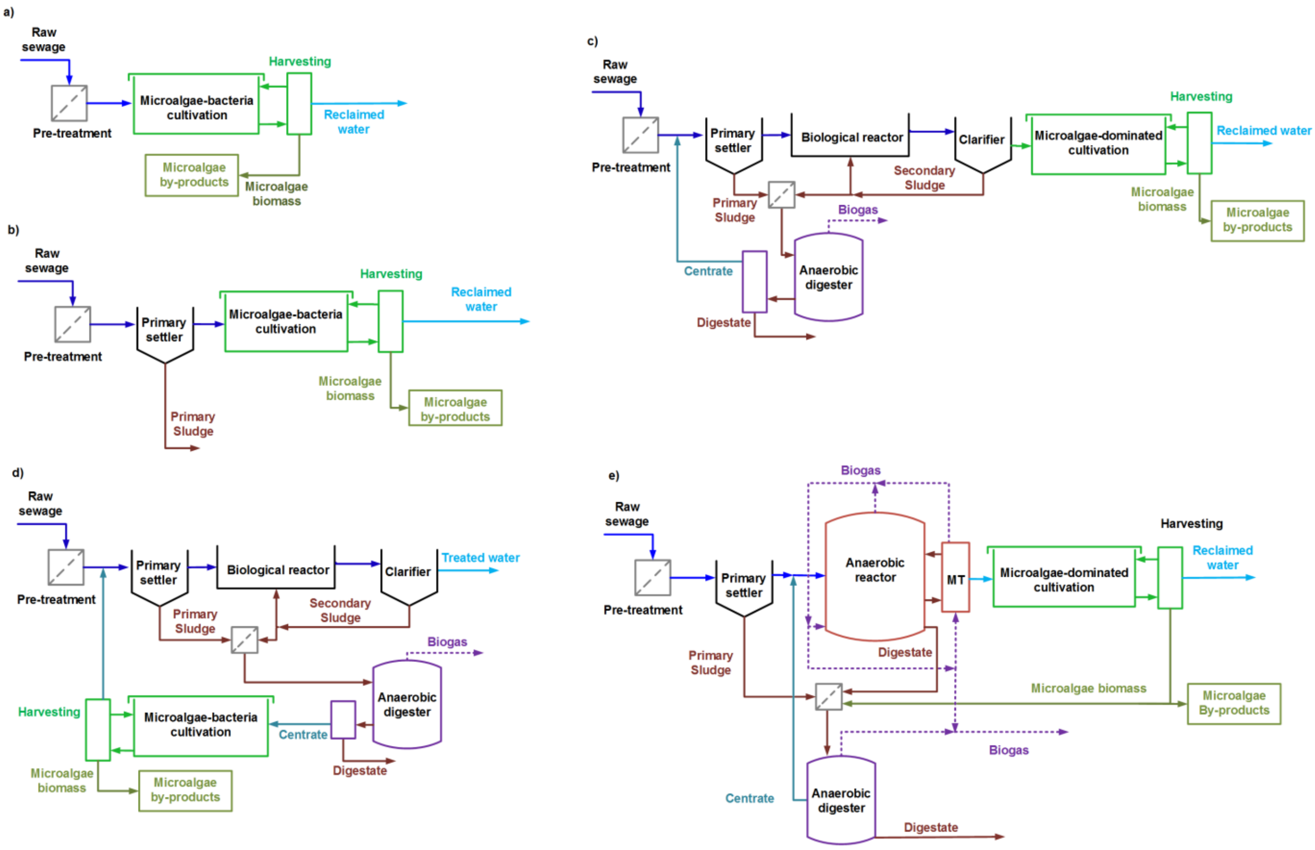


Figure 3. Configurations of microalgae-based wastewater treatment technologies depending on the cultivation media: a) raw wastewater; b) primary effluent; c) secondary effluent; d) centrate; and v) AnMBR effluent. MT: membrane tank.

5. MICROALGAE BIOREFINERY

Despite the plenty advantages of intensive microalgae-based wastewater treatment systems, they are not widely implemented yet due to several challenges such as high capital and operating costs and not being able to assure appropriate water quality in the long-term (Acién et al., 2021; González-Camejo et al., 2020a;2020b). To make microalgae cultivation feasible, the process has to focus on several issues: i) to increase microalgae performance through the optimisation of the cultivation process; ii)to apply circular economy principles by obtaining economic benefits from the microalgae biomass produced (Section 5.1); and iii) in the latter case, to reduce the high energetic demand of the harvesting system (see Table 2) as it can account for 0.2-5 kWh·kg microalgae biomass^{-1} (Fasaei et al., 2018).

5.1 Products from microalgae biomass

The produced and harvested microalgae can be used for energy production. Depending on the transformation process, microalgae biomass can be converted into biogas, biodiesel, bioethanol, biohydrogen, etc. (Goswami et al., 2020; Ubando et al., 2020). If microalgae are anaerobically digested, biogas will be produced. However, microalgae are often hard to degrade due to their robust cell membranes and their low carbon:nitrogen (C:N) ratio which is not optimal for anaerobic digestion. Co-digestion of algae with carbon substrates such as primary sludge thus appears as a suitable option for improving biogas production as long as anaerobic microorganisms are adapted to this co-substrate (Serna-García et al., 2020). Another option is to pre-treat microalgae biomass by sonication or thermal hydrolysis (González-Fernández et al., 2013; Kurokawa et al., 2016). However, this would increase biogas production costs and the environmental impacts of the process. Biodiesel can be produced via transesterification of the lipid fraction of microalgae biomass (Rajesh-Banu et al., 2020). It is widely known that algae can accumulate higher amount of lipids under nutrient-deplete conditions (Shahid et al., 2020). However, maximum performance of microalgae-based wastewater treatment processes is obtained under nutrient-replete conditions (González-Camejo et al., 2020a; 2020b). This hinders the lipid extraction process and its conversion to biodiesel, which remains inefficient to be implemented at large scale (Préat et al., 2020). Microalgae are also able to accumulate significant amounts of carbohydrates that can be utilised to produce bioethanol (Abinandan and Shanthakumar, 2015; Javed et al., 2019). Moreover, microalgae biomass can be used for bio-hydrogen production by water photolysis or dark fermentation (Goswami et al., 2020; Guldhe et al., 2017). Another possibility is the thermochemical conversion of the biomass by gasification, liquefaction or pyrolysis to produce syngas, bio-oil or bio-char (Chai et al., 2021; Nagarajan et al., 2020; Shahid et al., 2020). Nevertheless, these technologies present high production costs that constrain their feasibility (Behera et al., 2018).

It must be noted that the production of valuable compounds such as pigments, omega fatty acids, vitamins, etc. from microalgae biomass produced in wastewater treatment processes is hindered since current legislation forbids the use of microalgae biomass for human-related purposes (Acién et al., 2018). However, it could be used as biofertiliser, biostimulant or biopesticide to improve crop productions and reduce the impacts of the agricultural industry or as a renewable source of bioplastics (Acién et al., 2021; Bhattacharya and Goswami, 2020; Tua et al., 2021). Microalgae have also gained recent attention as potential producers of green metal nanoparticles due to their capacity to accumulate heavy metals during cultivation in wastewater (Goswami et al., 2020; Jacob et al., 2020).

5.2 Biorefinery approach

Current technologies which take advantage of the microalgae biomass obtained in wastewater treatment processes basically rely on extraction and purification technologies that focus on producing primary bioproducts alone (Bhattacharya and Goswami, 2020; Ubando et al., 2020). This usually makes microalgae biomass be underused, resulting in inefficient microalgae-based treatment processes. To improve this, the microalgae biorefinery concept has been recently developed (Goswami et al., 2020; Rajesh-Banu et al., 2020). It mainly consists of optimising the use of microalgae biomass obtained during wastewater treatment, producing a wide range of products (instead of a single one) by converting the wastes generated from other conversion pathways (Préat et al., 2020). In this respect, biodiesel production from lipids results around 65% residues of total microalgae biomass, which is also rich in carbohydrates thatcan be extracted to produce ethanol. The residues produced after these extractions can be anaerobically digested for biogas production, to obtain biofertilisers, biostimulants, biopesticides, nutrients or for other purposes such as hydrogen production or thermochemical transformation (Acién et al., 2021; Shahid et al., 2020). This integrated approach improves the feasibility of the microalgae cultivation process (Zabed et al., 2020).

A microalgae biorefinery is thus a facility wherein microalgae-based wastewater treatment systems (Figure 3) and various conversion methods (thermochemical, chemical, mechanical and biological) are integrated to produce sustainable bio-based products efficiently (Javed et al., 2019; Ubando et al., 2020). The products to be obtained depend on the chemical composition of the microalgae strains employed (Cuevas-Castillo et al., 2020) and on the transformation processes (Figure 4). There are multiple biorefinery routes, depending on the goal products that want to be obtained (Table 4). Microalgae biorefinery therefore appears as the most competitive configuration of microalgae-based wastewater treatment technology. However, it is also the most complex to implement. Some companies (for instance, Algaeon Inc., Algatechnologies, BioReal Inc., BlueBioTech Int. and Cyanotech Coproration) are currently able to obtain valuable products from microalgae biomass at industrial scale (Bhattacharya and Goswami, 2020). Nevertheless, the linking of these production processes with wastewater treatment and the different biorefinery routes is still at an early stage of technological implementation. Biorefineries present other drawbacks. The current production capacity of microalgae by-products is not enough to have a significant impact on the market (Acién et al., 2021). Moreover, the microalgae biomass transformation processes (described in Section 5.1) are sometimes unfeasible in comparison to other alternative resources (Bhattacharya and Goswami, 2020; Préat et al., 2020). Future research should hence focus on implementing the biorefinery concept at industrial scale to make it more competitive.

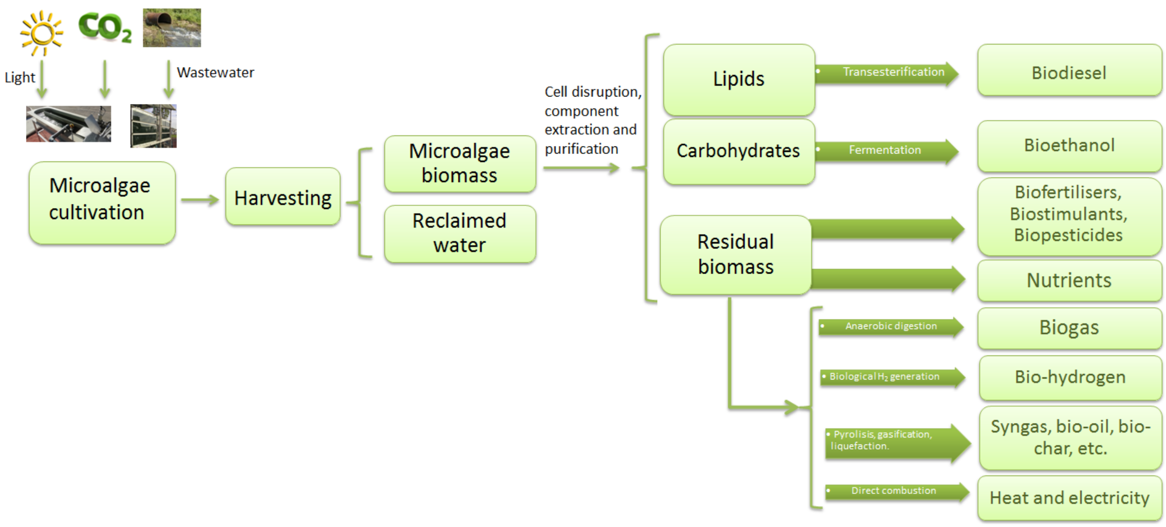


Figure 4. Diagram of integrated microalgae biorefinery.

Table 4. List of different microalgae-based biorefinery configurations to treat urban wastewater.

Species	Reactor (volume)	Evaluation	Products	Downstream processes	Reference
Green microalgae	Raceway ponds	Review proposal	Fatty acids Biodiesel Proteins N-free extract[1]	Solvent extraction Transesterification Protein extraction Molecular sieving[1]	Cuevas-Castillo et al. (2020)
			Pigments Bio-oil Bio-char Heat and power[2]	Solvent extraction Pyrolisis Combustion[2]	
Mix indigenous microalgae	Open pond (60 L)	Experimental (Outdoor cultivation)	Biodiesel Bioethanol	Lipid extraction Transesterification Hydrolisis Fermentation	Hemalatha et al. (2019)
Chlorella vulgaris	-	Life Cycle Assessment and Life Cycle Costing	Lipids Biogas Fertiliser[1]	Lipid extraction Anaerobic digestion[1]	Préat et al. (2020)
			Lipids Biogas Fertiliser[2]	Lipid extraction Anaerobic digestion[2]	
			Lipids Dried biomass[3]	Lipid extraction Drying[3]	
Cyanobacteria + green microalgae	Horizontal Tubular PBR (30 m^3)	Experimental (Outdoor cultivation)	Bioplastics Fertilisers	Anaerobic co-digestion Stabilisation in wetlands	Uggetti et al. (2018)

[1]Route 1; [2]Route 2; [3]Route 3.

CONCLUSIONS

Intensive microalgae-based wastewater treatment is receiving increasing interest due to its environmental benefits in terms of carbon dioxide absorption and nutrient recovery from different wastewater streams, which enables to apply circular economy principles in the wastewater treatment sector. However, large-scale applications are still scarce. When microalgae are cultivated outdoors many factors have to be considered: climatic conditions; type of system (open/closed, horizontal/vertical, suspended/attached); light path; non-photic volume; orientation; cultivation mode; operating conditions; and decide whether or not include culture mixing, pH control, temperature control and artificial lighting.

Outdoor microalgae cultivation has been traditionally carried out in waste stabilisation ponds, and more recently in open ponds or closed PBRs. However, only raceway ponds have appeared as a feasible option to intensively treat wastewater at industrial scale due to their lower capital and operating costs in comparison to closed PBRs and higher performance than WSP. Due to the flexibility of mix microalgae (and bacteria) cultures, microalgae cultivation can be applied to treat different urban wastewater streams such as raw wastewater, primary and secondary effluents, centrates and effluents of anaerobic digestion systems. The feasibility of microalgae cultivation technology depends on the combination with other processes to take advantage of the microalgae biomass and the water effluent. For this reason, the biorefinery concept has been developed. It consists of combining the wastewater treatment process with the production of multiple compounds from the microalgae biomass obtained. Research to implement biorefineries at large scale should be developed in the near future to make microalgae cultivation technology an alternative to conventional wastewater treatment systems.

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