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CURRENT ADVANCES IN MICROALGAE-BASED TREATMENT OF HIGH-STRENGTH WASTEWATERS: CHALLENGES AND OPPORTUNITIES TO ENHANCE WASTEWATER TREATMENT PERFORMANCE

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Abstract

Microalgae-based technologies, usually configured as high rate algal ponds (HRAP), are efficient, sustainable, and cost-effective alternatives for wastewater treatment due to their high removal efficiencies at low energy demand, ability to recover nutrients and ease of operation. HRAPs and other photobioreactors have been intensively studied in recent years for the treatment of high-strength wastewaters, which are mainly characterised by high and unbalanced (in terms of microalgae requirements) concentrations of organic carbon and nutrients. This review critically evaluated research papers that used microalgae-based systems for the removal of carbon and nitrogen from high-strength wastewaters. These systems can provide removal efficiencies up to 100% for organic matter and ammonium nitrogen. Relatively large area requirements, high evaporative losses, ammonia inhibition, poor light penetration and scattering, carbon dioxide limitation, and unbalanced nutrient ratios rank among the main current limitations of these technologies. Optimisation strategies, including modifications in bioreactor design and operation, can broaden their full-scale application for the treatment of high strength wastewaters.

Keywords: *high-rate algal ponds, high-strength wastewater, microalgae-based technologies, optimisation strategies.*

Declarations

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Introduction

High-strength wastewaters, such as agro-industrial wastewaters and anaerobic digestates, typically present high concentrations of pollutants, which exceed those in municipal wastewaters. Larger quantities of high-strength effluents will soon be produced due to the demand for resources of a growing world population (Eliasson 2015). Moreover, anaerobic treatment of organic wastes is gaining more attention than final disposal in landfills, thus higher volumes of digestates are expected to be produced (Siddique and Wahid 2018). In addition, the current worldwide interest in low-carbon wastewater treatment processes that can comply with stricter quality standards creates a demand for treatment technologies that are capable of producing high-quality effluents, and allow resource recovery (Bressani-Ribeiro et al. 2019). Different alternatives have been tested, including the direct application of digestates as fertilisers or their treatment for nitrogen recovery through evaporation coupled with physicochemical concentration (Tampio et al. 2016). However, direct application in soil may release nitrogen-related greenhouse gases, and evaporation decreases the volumes of water for reuse, resulting in low environmental sustainability (Rehl and Müller 2011). During the last decades, microalgae-based technologies such as high rate algal ponds (HRAPs) have emerged as a technically feasible and sustainable alternative for the treatment of high-strength wastewaters (Uggetti et al. 2014). Microalgae are photosynthetic eukaryotic and prokaryotic (cyanobacteria) organisms (Posadas et al. 2017; Markou and Georgakakis 2011) that are mainly autotrophic; however, they can also be heterotrophic and mixotrophic (Markou and Georgakakis 2011), which increase their tolerance to the usually stressful conditions in photobioreactors used for wastewater treatment and provide them high metabolic versatility to remove pollutants and incorporate them into biomass. The most commonly applied process for wastewater treatment based on microalgae is biological photo-aeration using suspended microalgal cultures (Muñoz and Guieysse 2006; Posadas et al. 2017). Photo-aeration via microalgal photosynthesis bioconverts CO_2 and H_2O into new cells (valuable organic solids) and O_2 available for heterotrophic bacteria that, in turn, close the cycle by mineralising organic pollutants into H_2O and CO_2 used by microalgae as carbon source (De Godos et al. 2017). Photo-aeration in microalgae-based systems represents a more sustainable alternative for wastewater treatment than processes that rely on mechanical aeration, such as activated sludge, as it achieves high removal efficiencies at lower energy demand (De Godos et al. 2009b; Mata et al. 2010; Acién et al. 2016). Furthermore, the recovered microalgae biomass can be used for several applications, including as a biofertiliser, as a feedstock for biodiesel or biogas production (Greenwell et al. 2010; Mata et al. 2010; Passos et al. 2018), and even as raw material for the manufacture of pharmaceuticals and food supplements (Passos et al. 2014; Jha et al. 2017), helping to shift the wastewater treatment approach from "end of pipe" to a "closed-loop".

Microalgae-based treatment processes apply open or closed photobioreactors. The most common open photobioreactor in full-scale applications are HRAPs (Park et al. 2011a; Sutherland et al. 2017), and conventional stabilisation ponds (Dias et al. 2018). HRAPs and stabilisation ponds are shallow open ponds where microalgae photosynthesis occurs in the presence of sunlight. HRAPs differ from maturation ponds in the use of paddlewheels to keep biomass suspended, and they are usually built with oval shapes and panels and deflectors to enhance hydrodynamic performance and increase microalgae productivity. Closed photobioreactors have been applied in multiple configurations such as tubular, flat, or cascade systems with baffles, large bags, and fermenters (for heterotrophic growth) (Borowitzka 1999). These types of bioreactors support higher biomass productivities and can be a suitable option for growing monocultures that produce high-value products. However, closed photobioreactors are challenging to operate, and life cycle analysis suggests that open ponds are still more feasible for wastewater treatment, considering both economic and environmental impacts (Collet et al. 2011).

Some issues still prevent broader applications of microalgae-based wastewater treatment. In the case of HRAPs, along with high evaporative losses and technical limitations in the separation of microalgal biomass (Tricolici et al. 2014), high loads of pollutants in high-strength wastewaters entail relatively large area requirements, which are derived from the necessity to operate at relatively long hydraulic retention times (HRT) (2-15 d), and with shallow depths for adequate sunlight penetration (Garfí et al. 2017; Kim et al. 2018). Other issues such as shading induced by high solids concentrations, ammonia toxicity, carbon dioxide limitation, and unbalanced nutrient ratios affect microalgae growth in both open and closed systems (Marcilhac et al. 2014; Posadas et al. 2017).

In order to critically review current advances and limitations in microalgae-based treatments of high-strength wastewaters, this paper presents a brief historical perspective, followed by a discussion of the main types and characteristics of high-strength wastewaters that have been treated with microalgae-based processes. This study also discusses the main limitations of the technology and the maximum loading rates of carbon and nitrogen that are applied in microalgal photobioreactors, including factors that enhance or limit the achievement of high removal efficiencies. Finally, this review highlights innovative strategies for enhancing carbon and nitrogen removal efficiencies in microalgae-based treatments of high-strength wastewaters.

Microalgae-based wastewater treatment: a brief historical perspective

The first full-scale application of microalgae in HRAPs for wastewater treatment was carried out by Professor William J. Oswald (University of California, Berkeley). By 1960, Professor Oswald engineered HRAPs for microalgae cultivation, which was further upgraded with preliminary stabilisation ponds for wastewater treatment (Oswald 1990). Scientific publications on wastewater treatment have been significantly increasing and diversifying since the 1990s (Figure 1a). In this period, anaerobic treatment, pond systems, constructed wetlands, and physicochemical treatments (such as reverse osmosis and ammonia stripping) have emerged as consolidated solutions, beside activated sludge, which is the most researched technology. Interestingly, microalgae-based wastewater treatment has emerged in the scientific literature as the most investigated technology during the past decade (Figure 1a).

Similarly, the treatment of high-strength wastewaters, including anaerobic digestates, has gained attention in the past decade (Figure 1b). The term "digestate" (frequently also named centrate in the context of wastewater treatment plants) is relatively new in the literature and is used to describe the liquid byproduct of wet anaerobic digestion of organic wastes. One of the main concerns in anaerobic digestion is the management of the digestate, which contains high concentrations of organic matter (up to 71 g $COD \cdot L^{-1}$) (Wilkie and Mulbry 2002) and nutrients (4.6 g $NH_4\text{-}N \cdot L^{-1}$) (Marcilhac et al. 2014).

High-strength wastewaters in microalgae-based systems

Microalgae-based processes have been applied in the treatment of different high-strength effluents such as industrial wastewaters, including piggery wastewater (Fallowfield et al. 1999; Costa et al. 2009; De Godos et al. 2009a, b), dairy farm wastewaters (Guruvaiah et al.; Craggs et al. 2003; Wang et al. 2010; Prajapati et al. 2014a), agricultural wastewater (Hernández et al. 2016), tannery effluent (Rose et al. 1996), acid mine drainage wastewaters (Rose et al. 1998), poultry litter digestate (Singh et al. 2011), centrate wastewater (Ren et al. 2017; Romero-Villegas et al. 2018), carpet mill effluents (Chinnasamy et al. 2010), landfill leachate (Sniffen et al. 2015), food waste (Hou et al. 2016) and food waste digestates (Shin et al. 2015; Torres Franco et al. 2018; Chuka-ogwude et al. 2020). Table 1 presents a compilation of wastewater characteristics in several relevant studies, for which high treatment performance have been achieved, mainly favoured by high nutrients availability.

Anaerobic digestates

Anaerobic digestates are the most common high-strength effluent treated by microalgae-based processes, and mainly include swine and cow manure, food waste, and agricultural wastes. These anaerobic digestates (Table 1) are typically characterised by low organic carbon to nitrogen ratios (Org-C:N), ranging from 1 to 10. Such relatively low-values result from the anaerobic process, which transforms the organic carbon from organic matter into gaseous methane (CH_4) and carbon dioxide (CO_2), whereas organic nitrogen is converted to dissolved ammonia that remains in the digestate with no further transformations (Mata-Alvarez et al. 2000). Organic carbon concentrations in digestates range from 0.1 to 32.9 $g{\cdot}L^{-1}$ (measured in terms of COD, BOD or TOC, see Table 1) and are typically recalcitrant (*e.g.*, lignin) since most of the readily biodegradable carbon is removed during anaerobic digestion (Vaneeckhaute et al. 2017).

High ammonium concentrations, ranging from 0.1 to 4.6 $gNH_4\text{-}N{\cdot}L^{-1}$ (Table 1), are one of the main concerns regarding the treatment of digestates, which also exhibit high pH, resulting in high ratios of free ammonia (NH_3) to dissolved ammonium (NH_4^+). Total phosphorus (TP) is not transformed during AD, although a fraction of the particulate organic P can become soluble and remains in the digestate (Vaneeckhaute et al. 2017), whereas organic P is accumulated in the solids. TP concentrations in digestates range from 11 to 303 mg TP·L^{-1} (Table 1), which are low values compared to nitrogen concentrations, producing high N:P ratios (>10). Additionally, potassium (K), calcium (Ca), magnesium (Mg), and heavy metals are usually not transformed during AD. However, K, Ca, and Mg can become more soluble (Vaneeckhaute et al. 2017). Concentrations reported (Koszel and Lorencowicz 2015) ranged 0.09-2.3 g K·L^{-1}, 0.21-0.25 g Ca·L^{-1}, 0.04-0.09 g Mg·L^{-1}, 0.43-0.49 mg Cu·L^{-1}, 1.90-2.01 mg Zn·L^{-1}, 1.80-2.20 mg Mn·L^{-1} and 19.7-70.7 mg Fe·L^{-1} for digestate and bovine liquid manure. Heavy metals (Cd, Co, Cr, Cu, Ni, Pb and Zn) in digestates can be removed through microalgae-based treatments. Their concentrations are usually low (< 2 mg·L^{-1}), and below the threshold established by the European legislation on sludge spreading (Muñoz and Guieysse 2006; Koszel and Lorencowicz 2015; Solé-Bundó et al. 2017; Yang et al. 2017). Other constituents of concern in digestates may include volatile organic compounds (VOCs), micropollutants, and pathogens. In this context, silicon-containing compounds are frequently measured in biogas produced from digestates (Rasi et al. 2013). Trace concentrations of micropollutants, such as antibiotics and genetic elements of resistance to drugs and antibiotics, can also be present in animal digestates (Cheng et al. 2018). Total coliforms in digestates range between 5 and 8 logs and microbial analysis of digestates revealed the presence of *Pseudomonas*, *Klebsiella*, *Clostridium*, *Bacillus*, *Bacteroides*, *Penicillium*, *Salmonella*, and *Aspergillus* (Owamah et al. 2014; Torres Franco et al. 2018).

Other types of high-strength wastewaters

Microalgae-based systems have also been applied for the treatment of other types of high-strength wastewater, including co-treatment of food waste digestate and primarily treated wastewater (Shin et al. 2015), swine manure wastewater (Wilkie and Mulbry 2002; De Godos et al. 2009a), slaughterhouse wastewater (Hernández et al. 2016), tannery wastewater (Tadesse et al. 2004) and landfill leachates (Sniffen et al. 2015). In these types of wastewater, organic carbon measured as COD, BOD, or TOC ranges from 0.1 to 71.8 g·L^{-1}; ammonium concentrations range from 0.1 to 7.4 gNH_4-$N \cdot L^{-1}$ and TP concentrations from 0.1 to 0.24 g $TP \cdot L^{-1}$. Carbon to nitrogen (C:N) ratios were typically low (<10), but in some cases, high values were also observed (50-100). N:P ratios typically range from 10 to 40, mainly in swine wastewaters.

Limitations of microalgae-based treatment of high-strength wastewaters

The most important limitations for the treatment of high-strength wastewaters, especially in open systems such as HRAPs, include relatively large area requirements and high evaporation rates (Acién et al. 2016; Garfí et al. 2017; Young et al. 2017). Other issues directly affect the ability of microalgae to grow in high-strength wastewaters, mainly ammonia inhibition (Azov and Goldman 1982), light blockage by solids (Mohammed et al. 2013; Marcilhac et al. 2014) and unbalanced macronutrients ratio (Franchino et al. 2013). These limitations restrict the presence and growth of microalgae to a few genera, depending on their ability to adapt to the wastewater composition and environmental conditions in photobioreactors. The main types of microalgae reported include freshwater chlorophytes, such as *Chlorella*, *Scenedesmus*, and *Neochloris* (*e.g.,* Franchino et al. 2013; Posadas et al. 2015a), cyanobacteria (*e.g. Aphanothece saxicola*, *Pseudanabaena* sp. – Marin et al. 2019, Eland et al. 2019), diatoms such as *Phaeodactylum tricornutum* and *Navicula* sp. (Toledo-Fernandez et al. 2016; Massa et al. 2017; Tiwari and Marella 2019), and euglenophytes (*e.g. Euglena gracilis*, Toyama et al. 2018), almost always occurring as mixed microalgae cultures (Toledo-Fernandez et al. 2016; Marcilhac et al. 2014; Marin et al. 2019). Axenic microalgal cultures do not occur in open ponds or even in closed photobioreactors due to the difficulties to eliminate bacteria from the culture medium and to control the bacterial populations and microalgae diversity during the treatment of wastewaters.

Area requirement and evaporative losses

Land requirement is a major bottleneck of microalgae-based treatments of wastewater (Acién et al. 2016). In HRAPs, area footprints are relatively large since depths of ~0.3 m, and long HRTs (2-5 d) are recommended to guarantee sufficient light penetration and high removal efficiencies. For example, in a recent pilot-scale study (Rodero et al. 2019), a 32 m^2-HRAP efficiently treated centrate wastewater at an organic surface loading rate of 0.05 kg $COD \cdot m^{-2} \cdot d^{-1}$. Anaerobic and trickling filters have been operated at higher surface loading rates –pond systems can be in the order of 0.5-1.0 kg $COD \cdot m^{-2} \cdot d^{-1}$ (Sperling 1996), whereas UASB or activated sludge can treat loading rates in the order of a few $kg \cdot m^{-2} \cdot d^{-1}$. This difference in loading rates means that HRAP may require at least 10-fold more area than UASB reactors or activated sludge. Furthermore, HRAPs have been reported as economically feasible, but land prices were not usually included in the economic assessments and, when considered, may affect the selection of HRAPs over other treatment alternatives (Garfí et al. 2017; Arashiro et al. 2018).

The extensive area necessary to expose the biomass to high sunlight intensities entails high evaporation rates, which vary depending on the local climate but can be up to 15-30% of treated influent or ~0-20 $L \cdot m^{-2} \cdot d^{-1}$ (Posadas et al. 2014; Matamoros et al. 2015; Rodero et al. 2019). High water evaporation results in higher pollutants concentration in the final effluent, and in some cases, the contribution of wastewater is not enough to compensate for evaporative losses. Thus, the addition of "make-up" water may be necessary, which decreases the environmental sustainability of microalgae-based technologies (Guieysse et al. 2013). Additionally, high water evaporation rates entail higher concentrations of solids and algae biomass, with the consequent decrease in light availability in the culture broth. Some of the strategies applied to reduce the impact of evaporative losses and extensive area requirements in HRAPs include the use of deeper reactors and shorter HRTs (Young et al. 2017).

Ammonia inhibition

High concentrations of NH_3 interfere in autotrophic metabolism, either by increasing photosensitivity (which eventually results in oxidative damage to algal membrane and photosystems) or by uncoupling photophosphorylation, reducing the pH gradient required to power the intracellular conversion of ADP to ATP (Gutierrez et al. 2016; Zhao et al. 2019). Evidence suggests that unionised ammonia (NH_3) is the most inhibitory form. The toxicity of NH_4^+ is almost 100-fold less than that of NH_3 in *Nephroselmis pyriformis* (Källqvist and Svenson 2003). The ratio of unionised ammonia to ammonium ion increases by a factor of 10 for each unit increase in pH and by a factor of 2 for each 10 °C rise in temperature over the 0–30 °C range (Collos and Harrison 2014). In this sense, high ammonia content at high pH conditions (>9.5) and temperatures (>20 °C) (Gutierrez et al. 2016) result in higher relative concentrations of NH_3 than NH_4^+ and thus, in a higher risk of microalgae growth inhibition.

Beyond the ammonium form, the resistance of each microalgae strain is also critical to identify threshold concentrations (Azov and Goldman 1982; Collos and Harrison 2014). For instance, Uggetti et al. (2014) reported a reduction in microalgae growth by 77% when concentrations increased from 9 to 34 mg NH_3-N·L^{-1} (corresponding to 185 to 260 mg NH_4-N·L^{-1} for pH at 7-9). Likewise, Gutierrez et al. (2016) also reported inhibition of *Neochloris oleoabundans* and *Dunaliella tertiolecta* at ammonia concentrations of 2.3 and 3.3 mg NH_3-N·L^{-1}, whereas *Chlorella sorokiniana* and *Nannochloropsis oculata* were not affected by concentrations of 16.7 mg NH_3-N L^{-1}. Rossi et al. (2020) detected higher resistance of chlorophytes compared to cyanobacteria, with $E_{50,\ NH3}$ values of 52.6, 60.9, 77.7 and 96.3 mg NH_3·L^{-1} for *S. obliquus*, *C. vulgaris*, *S. quadricauda* and *C. Sorokiniana*, respectively, which were consistently higher than the range detected for cyanobacteria (*i.e. Synechococcus* sp., *Synechocystis* sp., and *Leptolyngbya* sp., 4.3–34.8 mg NH_3·L^{-1}), as also reported by Collos & Harrison (2014).

High concentrations of NH_3 can limit the diversity in microalgal populations to the resistant species, mainly chlorophytes, which exhibit compensatory mechanisms to counteract detrimental effects of ammonia on pigments and take advantage of exogenous phytohormones or accessory pigments to increase nitrogen metabolism-related enzymes that contribute to detoxification of ammonia (Collos and Harrison 2014; Safafar et al. 2015; Zhao et al. 2019). Besides the variation of inhibitory levels of NH_3 for different microalgae strains, another challenge is that microalgae-based systems are dominated by diverse genera of microalgae, introducing broad variation in threshold concentrations. Based on studies reported in Table 2, chlorophytes and mixed cultures of chlorophytes and cyanobacteria presented higher productivities and growth rates than diatoms and cyanobacteria under NH_4^+ concentrations ranging from 0.05 to 4.6 g L^{-1}. In all cases, a decline in microalgae productivities is observed with increases in total and ammonia nitrogen (Table 2). Still, the growth of microalgae occurred since pH was close to neutrality, which significantly decreased the amount of NH_3 even under high NH_4^+-N concentrations. Furthermore, the buffer capacity of digestates and other high-strength wastewaters, or the control of pH to prevent alkaline conditions, must be considered in order to prevent ammonia inhibition (Marcilhac et al. 2014; Xia and Murphy 2016; Ayre et al. 2017).

Light availability and photoinhibition

The availability of photosynthetic active radiation (PAR) is one of the main factors that determine the kinetics of microalgae photosynthesis (Amini Khoeyi et al. 2012), affecting the performance of microalgae-based treatment systems. Under no nutrient limitation, photosynthesis increases with light intensity until a maximum beyond which photoinhibition may occur. The impinging light intensities can be sufficiently high to cause photoinhibition or too low to limit O_2 generation, both resulting in low photosynthetic rates. In outdoor open systems, high or low light intensities occur on a daily and seasonal basis. While PAR can reach maximum values of about 2000 μmol photons$\cdot m^{-2} \cdot s^{-1}$ in summer (Torzillo et al. 2003), these values decrease below 800 μmol$\cdot m^{-2} \cdot s^{-1}$ during winter or rainy seasons (Franco 2011). The tolerance to high light intensities is dependent on the strain and culture conditions such as density, temperature, and nutrients availability (Sorokin and Krauss 1958). For instance, sunlight intensities ranging from 200 to 300 μmol$\cdot m^{-2} \cdot s^{-1}$ were regarded as optimal conditions in terms of biomass production and treatment efficiencies during the outdoor cultivation of *Chlorella* spp. in wastewater (González-Camejo et al. 2019). PAR availability for the culture broth does not depend on only sunlight intensities but is also influenced by excessive blocking and scattering of light by suspended solids present in high-strength wastewaters (Mohammed et al. 2013; Marcilhac et al. 2014). Additionally, high turbidity and colour in most digestates also reduce light availability for autotrophic and mixotrophic growth during microalgae cultivation (Marcilhac et al. 2014). Strategies to control the negative effects of high suspended solid concentrations in influent wastewaters include diluting, screening, settling, filtering, or centrifuging, but there are no quantitative studies on the impact of these digestate pretreatment methods on microalgal growth rates (Xia and Murphy 2016). Furthermore, methods such as wastewater dilution or centrifugation are not economically feasible alternatives in large scale plants since prohibitive volumes of freshwater would be required, and centrifugation is a highly energy-intensive process to be applied during primary treatment.

Unbalanced nutrients ratios

Carbon, nitrogen, and phosphorus are the main macronutrients required for microalgae growth. However, the typical unbalanced ratios (C:N <10; N:P>30, Table 1) of these compounds in most high-strength wastewaters affect removal efficiencies and biomass production in several ways.

Carbon

Assimilation by heterotrophic bacteria supported by photo-aeration is the main pathway for carbon removal in microalgae-based systems (Posadas et al. 2017). In addition, some microalgae can also aerobically assimilate organic carbon under mixotrophic or heterotrophic metabolism. However, autotrophic metabolism predominates in microalgae-based systems (Cai et al. 2012). The primary sources of dissolved inorganic carbon in wastewaters are the dissolution of CO_2 from the atmosphere and the release of inorganic carbon by aerobic respiration or anaerobic digestion of organic matter. Bicarbonate (HCO_3^-) is the ionised form of CO_2 predominating at pH ~7.0 and the primary source of inorganic carbon for most microalgae since only few algae species can directly take up gaseous CO_2 (Srinivasan et al. 2018). The enzyme carbonic anhydrase and transporters shuttle inorganic carbon across the periplasmic membrane, through the cytosol, across the chloroplast membrane, and convert it to CO_2 in the direct vicinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) (Gardner et al. 2012), which catalyses its fixation using NADPH and ATP during Calvin cycle (Yang et al. 2000). CO_2 depletion derived from intense photosynthetic activity increases the pH and could eventually limit photosynthetic processes. Therefore, CO_2 addition is typically used to control pH and supply inorganic carbon source for microalgae growth (Park et al. 2011).

Some microalgae cultivated in wastewaters, including euglenophytes and chlorophytes like *Chlorella*, *Scenedesmus*, *Chlamydomonas*, and *Micractinium* (Park et al. 2012; Smith et al. 2015), can grow autotrophically, heterotrophically or mixotrophically. In this sense, the inorganic dissolved carbon, and the biodegradable organic compounds present in high-strength wastewaters, can be used as carbon sources. The type and biodegradability of organic carbon exert an influence over the metabolic pathway used by microalgae to assimilate this carbon (Lowrey et al. 2015). Eventually, heterotrophic and mixotrophic growth can support a more positive energy balance than autotrophic growth (Yang et al. 2000). Mixotrophic growth occurs mainly under light-limited aerobic environments with low CO_2 concentrations, and cells take advantage of the synergistic effects arising from the combination of autotrophic and heterotrophic metabolisms, which may outweigh the higher metabolic costs to maintain both systems (Soares et al. 2013). Besides the type of carbon source in wastewater, the prevailing metabolic pathway in microalgae-based systems depends on microalgal species present in the system and light and carbon availability (Posadas et al. 2017).

Photosynthesis prevails during the treatment of wastewaters under favourable conditions of light, temperature and CO_2 availability, but heterotrophic or mixotrophic algal metabolisms can occur. The balance among these different metabolisms is influenced by both the degree to which organic carbon assimilation inhibits the production of chlorophyll and the degree to which the presence of light inhibits the production of organic carbon uptake enzymes (Smith et al. 2015). Organic carbon sources supporting mixotrophic growth of microalgae in wastewater treatment systems include carbohydrates, acetate or glycerol (Yang et al. 2000; Park et al. 2012; Smith et al. 2015). Overall, from the perspective of treatment performance in conventional microalgae applications, typical low contents of inorganic carbon results in the requirement of external CO_2 supply (Posadas et al. 2015a), whereas high loads of organic carbon may exceed the photo-aeration capacity of microalgae, limiting heterotrophic bacterial consumption and resulting in low pollutant removal (Acién et al. 2016). Additionally, carbon recalcitrance affects microalgae-based pathways by limiting heterotrophic bacterial consumption and heterotrophic or mixotrophic metabolism in some microalgae strains (Vaneeckhaute et al. 2016; Loftus.and Jhonson 2019).

Nitrogen

Volatilisation (as NH_3 or N_2) and assimilation are the main pathways for N removal in algal-bacterial photobioreactors. NH_3 volatilisation occurs at high pH conditions, whereas removal as N_2 is mediated by nitrification-denitrification processes (Posadas et al. 2017). Microalgae use nitrogen as precursor organic molecules to synthesise proteins, DNA, RNA, chlorophyll, and other secondary metabolites (Baroukh et al. 2014). The primary source of nitrogen for microalgae metabolism is ammonium (NH_4^+). However, free ammonia (NH_3), nitrate (NO_3^-) or even urea may also be used, depending on the microalgae strain. The nitrogen source and its concentration play a major role in the synthesis of chlorophyll and lipids in microalgae cells (Li et al. 2008). For instance, (Converti et al. 2009) reported an increase in lipid content of *N. oculata* from 7.9 to 15.3% and of *C. vulgaris* from 5.9 to 16.4% when nitrogen concentration dropped from 0.3 to 0.075 g $NaNO_3 \cdot L^{-1}$. Moreover, nitrogen assimilation is not restricted by mixotrophic growth (Perez-García et al. 2010; Gao et al. 2019). On the other hand, nitrogen deficiency, high irradiance, or high salinity promoted the accumulation of carotenoids (Lee 2008). Low nitrogen availability limits microalgae productivities (de-Bashan et al. 2004), whereas high free ammonia concentrations can have an inhibitory effect, as previously described. Nitrogen assimilation is limited by low microalgae productivities and high ammonia volatilisation in open photobioreactors (Aslan et al. 2006; Gonzalez-Fernandez et al. 2016). In systems where nitrification-denitrification occurs, nitrification may be limited by low oxygen concentration and competition by CO_2 between nitrifiers and microalgae (Karya et al. 2013; Risgaard-Petersen et al. 2004), whereas denitrification may be limited by low organic carbon availability and absence of anoxic conditions (Foladori et al. 2018; Toledo-Cervantes et al. 2019).

Phosphorus

Assimilation and luxury phosphorus uptake are the main mechanisms of P removal in microalgae-based treatments (Powell et al. 2008). Phosphorus is also an essential macronutrient for microalgae growth. H_2PO_4 and HPO^{2-} are used in the synthesis of many cellular constituents, including adenosine phosphates (AMP, ADP, and ATP), cell membranes, and nucleic-acids (Martínez et al. 1999; Shelly et al. 2005). Phosphorus can also influence carbon metabolism in microalgae since its availability can regulate carbon partitioning between the synthesis pathway of carbohydrates or lipid by influencing the ADP-glucose pyrophosphorylase activity. For instance, a low N:P ratio favoured the synthesis of oleic acid by *Chlorella* sp. (Zhu et al. 2018). P removal efficiencies are usually low in the treatment of effluents containing high P concentrations since the intracellular content of phosphorus in microalgae is low (0.5-1%) when compared with that of carbon (40-50%) and nitrogen (7-10%). However, the concentrations of phosphorus in wastewater influence its assimilation rates, which are also affected by the N:P ratio, intracellular phosphorus content, pH, temperature, and concentrations of the cations Na^+, K^+, and Mg^{2+} (Martínez et al. 1999).

C:N ratio

Both carbon and nitrogen limitations in the cultivation broth result in low photosynthetic efficiencies and, consequently, in a low microalgae growth potential (Zhan et al. 2016). In this context, C and N availabilities are essential not only in terms of absolute concentrations but also in terms of C:N ratios, which influence microalgae growth and wastewater treatment efficiencies. Microalgae production from high strength wastewaters is typically limited by carbon due to the low C:N ratio of these wastewaters, compared to the C:N ratio found in microalgal biomass (6:1) (Benemann 2003). Indeed, experiments using artificial lighting concluded that optimum C:N ratios for *Chlorella* sp. cultivated in wastewater ranged from 5:1 to 10:1 (Yan et al. 2013). Besides, lipid accumulation is significantly enhanced during nitrogen-limited cultures of microalgae at high C:N ratios. Overall, wastewaters with low C:N ratios (<10, organic carbon) are more favourable for microalgae-based treatments by maintaining bacteria in a relatively low abundance, hence in favor of microalgae enrichment and nutrient recovery (Zhu et al. 2019).

N:P ratio

The N:P ratios in the cells and wastewaters influence the rates of uptake of both nutrients. The availability of nitrogen in relation to phosphorus can be indicative of nutrient deficiency based on the composition of microalgae cells, which have an N:P ratio of 6:1 (on a mass basis) or 16:1 (on a molar basis) under balanced growth. In this sense, microalgae growth is limited by phosphorus concentrations in high-strength wastewaters, since they typically present high N:P ratios (>30). N limitation occurs at low N:P ratios (<10), which is an unusual scenario in high-strength wastewaters. In this context, (Choi and Lee 2015) observed that an increase in the N:P ratio up to ~10 continuously increased the biomass production, which remained constant when phosphorus became limiting. Similarly, Whitton et al. (2015) reported an increased uptake rate of nitrogen at low values of N:P ratios.

Other constituents

Several cations (*e.g*. K, Na, Ca, and Mg) are essential for microalgae growth. Results from experiments with saline-alkaline water indicated that K, Na, Ca, and Mg were assimilated by *Scenedesmus obliquus*. However, higher medium salinity reduced pollutant removal efficiencies (Yao et al. 2013). There are no reports of severe inhibition of microalgae growth at any threshold concentrations of these micronutrients or other micropollutants in high-strength wastewater, but biomass yield increased when using Mg amendment for the growth *Scenedesmus* sp. in swine manure digestate (Bjornsson et al. 2013).

Treatment performance and biomass valorisation

COD removal efficiencies in pilot systems

Significant removals of organic matter measured as COD were achieved in several high-strength wastewater matrices (60-100%, Table 1 and Table S1 – Supplementary materials). Figure 2a presents a systematic analysis of COD removal efficiencies reported for microalgae-based processes. Organic loading rates (OLR) varied from 10 to 540 g $COD\cdot m^{-3}\cdot d^{-1}$ while removal efficiencies ranged from 10 to 90%, presenting a trend to an exponential decrease above 100 g $COD\cdot m^{-3}\cdot d^{-1}$ (Figure 2a). Successful experiences were those with removal efficiencies above 80% when OLRs higher than 100 g $COD\cdot m^{-3}\cdot d^{-1}$ were applied, while unsuccessful experiences were those in which efficiencies lower than 80% were obtained when treating OLRs lower than 100 g $COD\cdot m^{-3}\cdot d^{-1}$. Favourable temperatures of ~20 °C were a common factor in successful experiences, *e.g*. De Godos et al. (2009a), who obtained removal efficiencies of 76% at a COD influent load of ~200 $g\cdot m^{-3}\cdot d^{-1}$ treating pretreated piggery slurry in HRAPs, and Passos et al. (2015), who reported removal efficiencies of 80% at an influent OLR of ~300 g $COD\cdot m^{-3}\cdot d^{-1}$ treating primary pretreated municipal wastewater in HRAPs. When compared to conventional technologies for wastewater treatment (*i.e.*, Activated sludge, UASB reactor), microalgae-based systems have a limited capacity to treat high OLR, even considering experiences where relatively high influent COD loading rates were efficiently treated. For instance, Activated Sludge systems can efficiently treat OLRs ranging between 0.6 and 8 kg $COD \cdot m^{-3} \cdot d^{-1}$ with efficiencies of 90-95% (Ireland 1997), whereas anaerobic systems such as UASB reactors can treat up to 20 kg $COD \cdot m^{-3} \cdot d^{-1}$ with removal efficiencies of 80-90% (Seghezzo et al. 1998). These relatively low efficiencies of organic matter removal at high COD loads suggest that microalgae-based treatments, especially conventional HRAPs, are more competitive when coupled to pretreatments that remove organic matter, setting the primary purpose of microalgae in the removal of nutrients from the final effluent and the production of valuable biomass for further applications. Besides influent loading rates, COD removal efficiencies were mainly affected by 1) the degree of carbon biodegradability; 2) environmental temperatures; 3) pH; 4) HRT, and sludge retention time (SRT). High strength wastewaters, such as anaerobic digestates, can exhibit low biodegradable organic carbon content (*i.e.*, only 10-30% of the total organic matter content is BOD_5) (Alburquerque et al. 2012). However, a clear exception for high removal efficiencies was found for systems coupled to pretreatments (Tadesse et al. 2004), where influent carbon was removed in previous stages, resulting in low removal efficiencies (10-20%) due to higher relative quantities of recalcitrant carbon in the microalgae-based treatment. Temperature and solar irradiation also affect COD removal efficiencies (De Godos et al. 2009a; Hernández et al. 2016; Gutiérrez et al. 2016b; Buchanan et al. 2018). Figure 2a shows that temperatures above 20°C seem to support higher COD removal efficiencies (60-100%), especially at lower influent concentrations. Furthermore, high COD removal efficiencies presented in Figure 2a occurred in temperatures between 17 and 25°C (See also Table S1-supplementary materials). Optimal growth temperatures are in the range of 15–35°C for most microalgae species, while growth is severely hindered below 5°C (Singh and Singh 2015; Delgadillo-Mirquez et al. 2016). Cultures grown at low temperatures are much more subject to photo-inhibition. The lower the temperature, the lower the light intensity at which photo-inhibition occurs (Renaud et al. 2002).

Outdoor systems where heterotrophic bacterial activity was also promoted by controlling pH below 8.0 presented higher COD removal efficiencies (Molinuevo-Salces et al. 2010) since symbiotic interactions between microalgae and bacteria enhance carbon and nutrient removal and biomass productivity (García et al. 2000; Craggs et al. 2012; Ferro et al. 2019). The presence of heterotrophic bacteria during wastewater treatment in microalgae-based photobioreactors is relevant because CO_2 from aerobic respiration of organic matter becomes available for autotrophic metabolisms. The main classes of bacteria growing in microalgae-based treatment systems are Flavobacteria, Gammaproteobacteria, Betaproteobacteria, and Bacteroidia (Su et al. 2012; Posadas et al. 2017; Toyama et al. 2018). Furthermore, recent researches showed that certain bacteria syntrophically interact with microalgae and promote the growth of specific strains (Toyama et al. 2018). Co-culture of *Auxenochlorella protothecoides* and *Chlorella sorokiniana* with native wastewater microbial community enhanced the microalgae growth and the removal of COD and nutrients from winery wastewater. Both species stimulated bacterial growth in a strain-specific way, suggesting different responses of bacteria to microalgal photosynthates, whereas microalgae grew auxotrophically, obtaining cofactors from bacteria (Higgins et al. 2018). Similarly, co-cultivation of *Chlorella vulgaris* and *Rhizobium* sp. led to faster assimilation of nutrients under mixotrophic conditions, since a positive synergistic relationship resulted from the in situ O_2/CO_2 exchange between the microorganisms (Ferro et al. 2019).

HRT also plays a key role in COD removal efficiencies. Typical HRT values range between 6 and 10 d (Table S1 – Supplementary materials). HRT values higher than 10 d consistently provided COD removal efficiencies higher than 60%. HRT must be higher than the minimum microalgae duplication time (~ 2 d) to avoid wash-out and process collapse (Larsdotter 2006). On the other hand, long HRTs entail photobioreactor with larger areas and volumes, resulting in higher costs. Some attempts have been carried out in order to operate microalgal photobioreactors with separate HRT and solids retention time (SRT). The presence of heterotrophic bacteria contributes to flocculation and biomass sedimentation (Su et al. 2012), which improves SRT control by recycling settled biomass (De Godos et al. 2014; Rada-Ariza et al. 2017). The increase in SRT also contributes to the formation of stable microalgal-bacteria flocs of rapid settleability (Medina and Neis 2007; Anbalagan et al. 2016; Rada-Ariza et al. 2017).

Nitrogen transformation and removal

Table 1 highlights that microalgae can grow and treat wastewaters with high ammonia concentrations with relatively high removal efficiencies (30-100%, while a few exceptions between 20-30%). Microalgae-based systems have been applied to a range of influent nitrogen loading rates (NLR) varying from 1.5 to 80 $gNH_4\text{-}N{\cdot}m^3{\cdot}d^{-1}$ with removal efficiencies of TN and NH_4-N varying between 20% and 100% (Figure 2b – Table S1, supplementary material). Successful experiences were those with removal efficiencies above 80% when NLRs higher than 20 g $N{\cdot}m^{-3}{\cdot}d^{-1}$ were applied, while unsuccessful experiences were those in which efficiencies lower than 80% were obtained when treating NLRs lower than 100 g $COD{\cdot}m^{-3}{\cdot}d^{-1}$. Ammonia nitrogen removal efficiencies as high as 100% have been achieved for influent loads of 80 g $NH_4\text{-}N{\cdot}m^3{\cdot}d^{-1}$, mainly through ammonia volatilisation (Molinuevo-Salces et al. 2010). However, some environmental concerns are related to ammonia volatilisation since this process represents nitrogen losses, and volatilised NH_3 may act as a greenhouse gas in the atmosphere (Alcantara et al. 2015). Ammonia volatilisation prevailed as the main process for N removal in most cases, supported by high pH and temperatures that favour the conversion of NH_4^+ to NH_3 (Senzia et al. 2002; Toledo-Cervantes et al. 2017; Rodero et al. 2018).

Besides ammonia volatilisation, the main mechanisms of nitrogen removal in microalgae-based systems are the assimilation of nitrogen by microalgae and nitrification-denitrification processes, removing nitrogen in the form of microalgae biomass and N_2, respectively (Molinuevo-Salces et al. 2010; Park et al. 2011; Passos et al. 2015). However, when ammonia volatilisation was not the predominant process, the removal efficiencies of TN or TKN were usually lower than those of NH_4-N, since biological transformations are often limited. The contribution of ammonia assimilation and nitrification-denitrification range from 30% to 70% of the influent nitrogen (Delgadillo-Mirquez et al. 2016; De Godos et al. 2017; Toledo-Cervantes et al. 2017). Nitrogen assimilation depends on the capacity of microalgae and bacteria to grow and metabolise nitrogen compounds. The diversity of microalgae may have a considerable influence on removal efficiencies, since it can guarantee the presence of at least some species that can be resistant to high ammonia concentrations, such as *Chlorella kessleri*, *C.*

vulgaris and other chlorophytes (Caporgno et al. 2015). The assimilation of dissolved nitrogen increases with higher biomass yields as a result of the dual autotrophic and heterotrophic metabolism of microalgae and bacteria prevailing in the system (De Godos et al. 2009a). Carbon limitation hampers nitrogen uptake, thus increasing CO_2 and light supply will improve the yields and the intensity of phototrophic activity in microalgae and boost nitrogen uptake (Sutherland et al. 2015a). Higher temperatures will also favour nitrogen assimilation (Delgadillo-Mirquez et al. 2016).

Regarding nitrification-denitrification, these processes are favoured in microalgae-based systems at neutral to mildly alkaline pH conditions (7.0-8.0), since the growth of nitrifiers is limited at more alkaline conditions. Nitrification-denitrification is mainly affected by DO concentrations. Low DO concentrations favour denitrification and nitrogen sources conversion to ammonium, while high DO concentrations will favour nitrite or nitrate accumulation due to limited denitrification (Marín et al. 2018). Denitrification tends to be higher inside the flocs formed during microalgae treatment, where low dissolved oxygen (DO) conditions may prevail (González-Fernández et al. 2011), especially during the night-time (Park and Craggs 2011). In addition to pH control, longer HRT and SRTs also favoured nitrification (De Godos et al. 2014; Dhaouefi et al. 2018; Rodero et al. 2018), whereas short HRTs contribute to the wash-out of nitrifiers. Decoupling SRT and HRT avoids nitrifiers wash-out and produces higher nitrogen removal efficiencies (Alcántara et al. 2015; Wang et al. 2015; Rada-Ariza et al. 2017, 2019). Low nitrification was also related to CO_2 limitation (de Godos et al. 2014; Dhaouefi et al. 2018).

The most efficient configuration in microalgal-bacterial systems to carry out nitrification-denitrification processes is composed of an anoxic tank coupled to an open-aerobic photobioreactor (De Godos et al. 2014; Alcántara et al. 2015; García et al. 2017a; Dhaouefi et al. 2018). Anoxic-aerobic photobioreactors show high removal efficiencies of total nitrogen (80-90%) by improving nitrification-denitrification (De Godos et al. 2014; Dhaouefi et al. 2018). In addition, the supply of CO_2 for pH control and as a C source for microalgae and nitrifiers, in anoxic-aerobic photobioreactors coupled with biogas upgrading, can result in removal efficiencies of 81% and 97% of Total-N and NH_4-N, respectively (García et al. 2017).

Removal of phosphorus, micropollutants, and pathogens.

The efficiencies of P removal in microalgae-based photobioreactors are lower than those reported for organic C and N (Table 1). Even so, microalgae-based treatment may be an alternative for P recovery from anaerobic digestates, with typical removal efficiencies ranging from 50 to 100% (Table 1). Assimilation and Luxury phosphorus uptake are the main mechanisms of P removal in HRPAs and other microalgae-based systems. Luxury uptake occurs since microalgae may store acid-insoluble polyphosphate that can be used when the external phosphate concentration becomes limiting. Biological P removal depends on both the microalgae concentration and the amount of P accumulated in the biomass, which can be increased from the typical 1% up to 3.2% (Powell et al. 2008, 2009). Microalgae-based technologies can also remove cations and heavy metals with efficiencies of up to 99% (Munoz and Guieysse 2006) (Muñoz & Guieysse, 2006), in some cases up to 6 logs of coliforms and *E. Coli* (Mohammed et al. 2014; Fallowfield et al. 2018; Torres Franco et al. 2018), and several micropollutants (Vassalle et al. 2020b).

Biomass valorisation opportunities during the treatment of high-strength wastewaters

Many applications have been proposed for the valorisation of microalgae biomass. Currently, biodiesel production is not economically feasible (Stephens et al. 2010), and other alternatives such as pharmaceutical applications are limited by the difficulty of operating real systems under axenic (Vu et al. 2018). One of the most suitable alternatives is the anaerobic digestion of microalgal biomass cultivated in high-strength wastewaters, especially in digestates, coupled to nutrients recovery in the microalgal biomass and methane production. If the microalgae biomass is recycled to the anaerobic reactor for co-digestion with organic wastes, methane yield can range around 180 to 640 mL/g VS_{added} (Passos et al. 2014; Zhen et al. 2016), which means that microalgae biomass has a potential to increase methane yields in methanization platforms and wastewater treatment stations (Vassalle et al. 2020a). Moreover, since nutrients can be assimilated into biomass, they can be recovered from the digestate at relatively high rates and recover in the anaerobic sludge (e.g., 10.1 and 2.0 mg L^{-1} d^{-1} for N and P, respectively; (Marcilhac et al. 2015), preventing losses of these nutrients from treatment platforms. Direct application of microalgae biomass in soils has shown positive results as slow-release fertilisers for food crops (Coppens et al. 2016; Dineshkumar et al. 2018). Studies that evaluated the economic feasibility of fertilisers derived from microalgae biomass showed positive scenarios for mixtures with inorganic fertilisers (Coppens et al. 2016). Furthermore, *in situ* cultivation and application of microalgae-fertilisers increase the economic feasibility of this alternative (Uysal et al. 2015; Wuang et al. 2016), which coupled to the treatment of agricultural wastewaters or digestates, represents a "closed cycle" alternative for nutrients.

Potential alternatives for enhancing treatment performance

Microalgae-based systems can treat high-strength wastewaters with high C and N removal efficiencies (80-100%) at both laboratory and pilot-scales. In addition, the production of microalgal biomass (which can be valuable as a bioenergy feedstock or as biofertiliser in agricultural applications) brings advantages to these processes over other consolidated wastewater treatment technologies. Further research is necessary on integrated treatment of high-strength wastewater using, *e.g.,* activated sludge systems or UASB reactors coupled to cost-efficient photobioreactors. Significant efforts have been dedicated to improving hydrodynamics in HRAP through the installation of deflectors, islands in the middle wall or turbine based propellers (Hadiyanto et al. 2013; De Godos et al. 2017). However, new design strategies are required to reduce the energy need for microalgae suspension.

Figure 3 presents some alternatives for the design and operation of photobioreactors, retrieved from successful experiences reported in literature. Extensive area requirements and high evaporative losses can be attenuated by reducing HRT in systems where HRT and SRT are decoupled using cost-effective biomass separation and recirculation strategies. Artificial LED-lighting may also be an alternative to increase light availability in deeper HRAPs (Yan et al. 2013, 2016; Mohammed et al. 2014; Schulze et al. 2014; Torres Franco et al. 2018), and could be coupled to a better control of solids in the reactors for the achievement of higher productivity. In addition to light conditions, nutrients control is also important. Carbon limitation derived from unbalanced nutrients ratios can be attenuated with external CO_2 addition, which also lowers the risk of ammonia inhibition at high pH. In this context, biogas upgrading can be coupled to wastewater treatment in systems integrating closed and open photobioreactors. Furthermore, higher nitrogen removal efficiencies can be obtained in anoxic-aerobic photobioreactors (de Godos et al. 2014; Dhaouefi et al. 2018).

Decoupling HRT and SRT

Process operation with separated HRT and SRT has been shown to increase C and N removal efficiencies, especially under high loading conditions. Reactors have been typically operated at HRT of up to 10 d, which entails a demand for larger areas. Process operation at HRT ranging from some hours to 2-4 d and SRT ranging from 6 to 20 d can support high COD and N removal efficiencies, since the growth of both heterotrophic bacteria and nitrifiers may be promoted. Consistent wastewater treatment performance and biomass productivities have been achieved in suspended growth systems with decoupled HRT and SRT, *e.g.* Medina and Neis (2007), Gutiérrez et al. (2016a), Marin et al. (2018), Rada-Ariza et al. (2019), Toledo-Cervantes et al. (2019). Biomass settling and recirculation improves bioflocculation of microalgae, thus enhancing biomass harvesting and wastewater treatment efficiencies (Gutiérrez et al. 2016a). The use of biopolymers and other flocculants may also significantly enhance biomass settling by increasing settling velocities above 6.5 $m \cdot h^{-1}$ (Gutiérrez et al. 2016b), which is about 100-fold the values reported for phytoplanktonic species like *Cryptomonas curvata* and *Staurastrum leptocladum* (Chindia and Figueredo 2018). Particular attention should be given to the selection of the flocculant type and dosages to prevent cell damage of the recycled biomass. Higher hydraulic loading rates and changes to pond depth and HRT in systems with SRT in the order of days may induce a better distribution of solids, enhancing light absorption and photosynthetic performance (Sutherland et al. 2015b). Furthermore, artificial lighting may help to increase the photic zone depth (>30 cm) in photobioreactors (Torres Franco et al. 2018).

The growth of attached microalgae may contribute to increase SRT via biomass immobilisation and improve biomass harvesting. Microalgae immobilisation as biofilm can reduce harvesting costs and improve pollutant removal efficiency, thus enhancing the sustainability of the process (Sukačová et al. 2015).

Some examples of microalgae biofilm-based systems showing high performance include inclined plates (Choudhary et al. 2017; Naaz et al. 2019), rotating algal biofilm reactor (RABR), algal turf scrubber (ATS™), revolving algal bioreactor (RAB) and the Algaewheel ® (Kesaano and Sims 2014). Additionally, the design of hybrid suspended-biofilm reactors could be a feasible alternative to take advantage of the features of both suspended and attached growth photobioreactors. However, scaling-up these photobioreactor configurations appears somehow limited, and there is still a lack of knowledge about light utilisation efficiency, mass transport mechanisms, heterotrophic–autotrophic interactions, the dynamics of algal-bacterial communities and construction and maintenance costs (Kesaano and Sims 2014).

External CO_2 supply coupled to biogas upgrading

CO_2 sparging may be required in order to increase dissolved inorganic carbon availability and to prevent strong alkaline conditions, which may lead to ammonia inhibition and volatilization. Since low NH_3 concentrations (*e.g.*, 2.3 and 3.3 mg NH_3-N$\cdot$L^{-1}) may be inhibitory for some microalgae species (Gutierrez et al. 2016), pH conditions above 8.0 should be avoided. pH control at 7-8 is also important to maintain inorganic carbon availability and boost heterotrophic bacterial activity, which in turn can produce more CO_2 for photoautotrophic microalgae growth. Additionally, the availability of inorganic carbon and buffer capacity should always be assessed in relation to wastewater characteristics, in order to take advantage of their chemical composition. An enhanced alternative when CO_2 supply is required can be the coupling of microalgae-based systems with biogas upgrading, which represents a cost-competitive alternative capable of removing CO_2 and H_2S from biogas in a single stage at low environmental impacts and simultaneously treating wastewaters (Marin et al. 2019; Rodero et al. 2019). Biogas upgrading has been performed by installing separate biogas absorption columns, which support both a higher CO_2 gas-liquid mass transport and a lower O_2 stripping compared to direct scrubbing of biogas in the typically shallow photobioreactors (García et al. 2017a). For instance, a successful pilot-scale experience of digestate treatment in a HRAP coupled to an absorption column for biogas upgrading validated the environmental and economic sustainability of this technology (Rodero et al. 2019). In addition to biogas upgrading, the biomass produced during the treatment of digestates or anaerobically pretreated domestic wastewaters, can be recycled to the anaerobic reactor for co-digestion with the raw waste or wastewater. The co-digestion of microalgae biomass can enhance biogas production and increase the sustainability of anaerobic digestion and microalgae-based treatments, since they can be operated as a single closed-loop process (Prajapati et al. 2014a; Prajapati et al. 2014b; Vassalle et al. 2020a).

Hybrid photobioreactors

Hybrid photobioreactors may incorporate the advantages of different conventional alternatives, combining suspended and attached growth, open and closed vessels or sunlight and artificial light. Semi-closed photobioreactors, coupled to biogas-upgrading are a promising alternative for high-strength wastewater reuse and added-value product generation based on their higher photosynthetic efficiencies at lower operating costs (Uggetti et al. 2018). Similarly, flat-panels photobioreactors have been successfully tested at pilot scales with relatively high treatment efficiencies for carbon and nitrogen (80-90% and 70-85%, respectively) (Choudhary et al. 2017; Romero-Villegas et al. 2018; Naaz et al. 2019; Sun et al. 2019). At a demonstration scale, tubular photobioreactors coupled to open tanks showed high performance to treat a mixture of agriculture run-off and municipal wastewater (García et al. 2018). Other alternatives recently explored include the use of biofilm carriers submerged in suspended cultures for favouring nitrifiers growth in microalgae based-systems (*e.g.* Church et al. 2018) and in capillary driven photo-biofilm reactors (Xu et al. 2018). Finally, reactors using a combination of sunlight and monochromatic LEDs seem to be an economically viable technology for microalgae cultivation (Abomohra et al. 2019).

Anoxic-aerobic algal-bacterial photobioreactors

Nitrogen removal in wastewater exhibiting low C/N ratios can be boosted by implementing an anoxic stage before the HRAP. The configuration relies on the use of an anoxic reactor (engineered as a dark vessel) receiving the influent wastewater, followed by a photobioreactor, from which biomass and a nitrate laden stream are returned to the anoxic reactor. This return of biomass and nitrate to the anoxic reactor allows the denitrification of nitrates produced in the photobioreactor together with the consumption of a high fraction of influent organic matter (Alcántara et al. 2015). Anoxic-aerobic microalgae-based systems can support carbon removal efficiencies over 90%, and nitrogen removal efficiencies over 80% through nitrification-denitrification, during the treatment of high-strength wastewaters and can be coupled to biogas upgrading (de Godos et al. 2014; Alcántara et al. 2015; García et al. 2017a; Dhaouefi et al. 2018; Toledo-Cervantes et al. 2019). Furthermore, the cost-effective removal of ibuprofen, naproxen, salicylic acid, triclosan and propylparaben, from urban wastewater was also demonstrated in anoxic-aerobic algal-bacterial photobioreactor (López-Serna et al. 2019).

Conclusions

Microalgae-based processes can be efficient, sustainable, and cost-effective alternatives for the treatment of high-strength wastewaters. Current literature suggests that influent loading rates of 200 $gCOD \cdot m^{-3} \cdot d^{-1}$ and 20 $gTN \cdot m^{-3} \cdot d^{-1}$ can be efficiently treated with high microalgae biomass yields. The alternatives for the valorisation of microalgae biomass increase the environmental sustainability of microalgae-based systems when compared to conventional treatment systems. The main constraints derived from current photobioreactors design and operation and high-strength wastewater characteristics are relatively large area requirements, high evaporative losses, ammonia inhibition, light-blocking by solids, and unbalanced nutrients ratios. The engineering of novel photobioreactor configurations and operational strategies, including decoupling of HRT and SRT, and closed and semiclosed photobioreactors coupled to biogas upgrading, can help to overcome the above-mentioned limitations.

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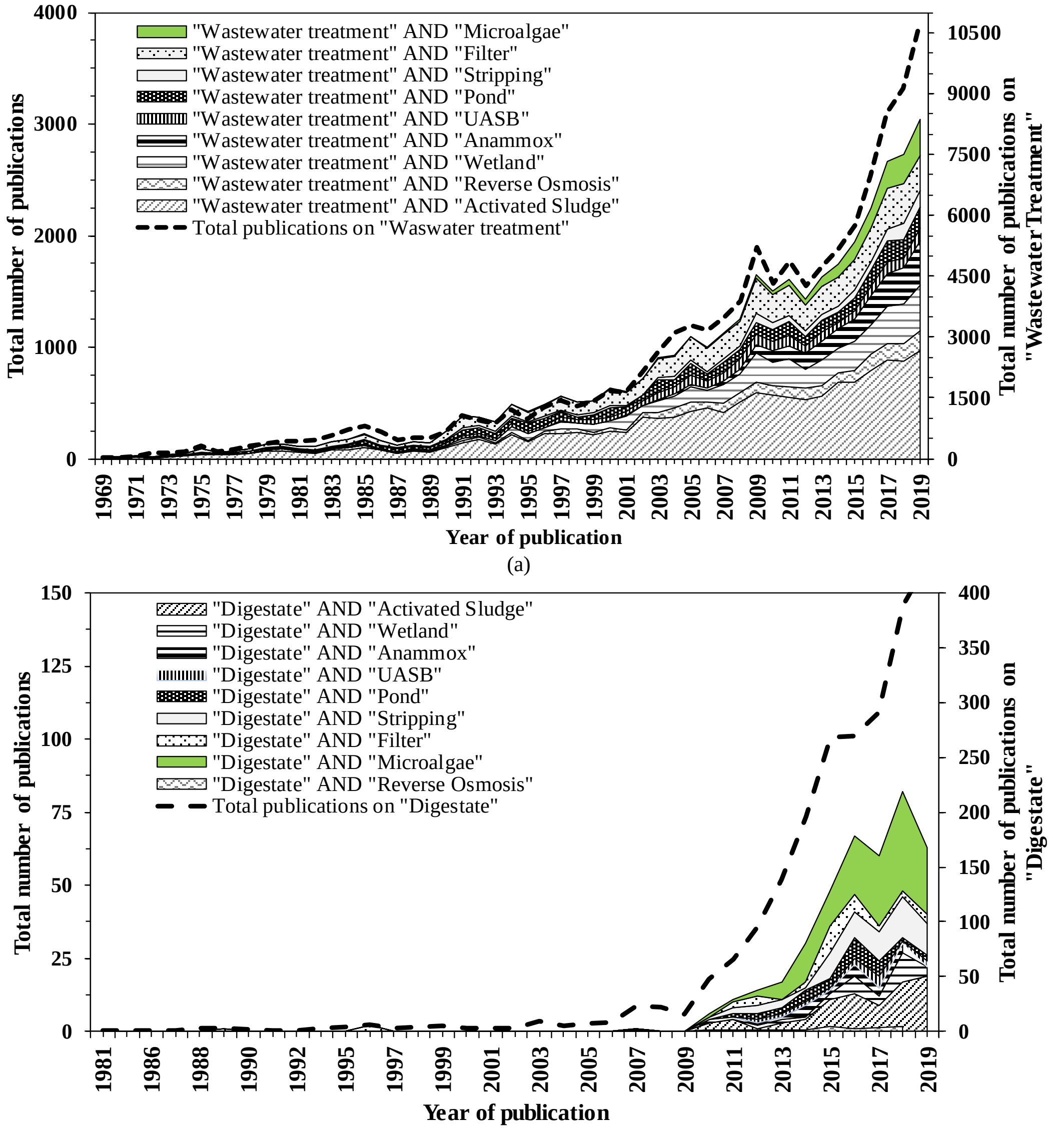
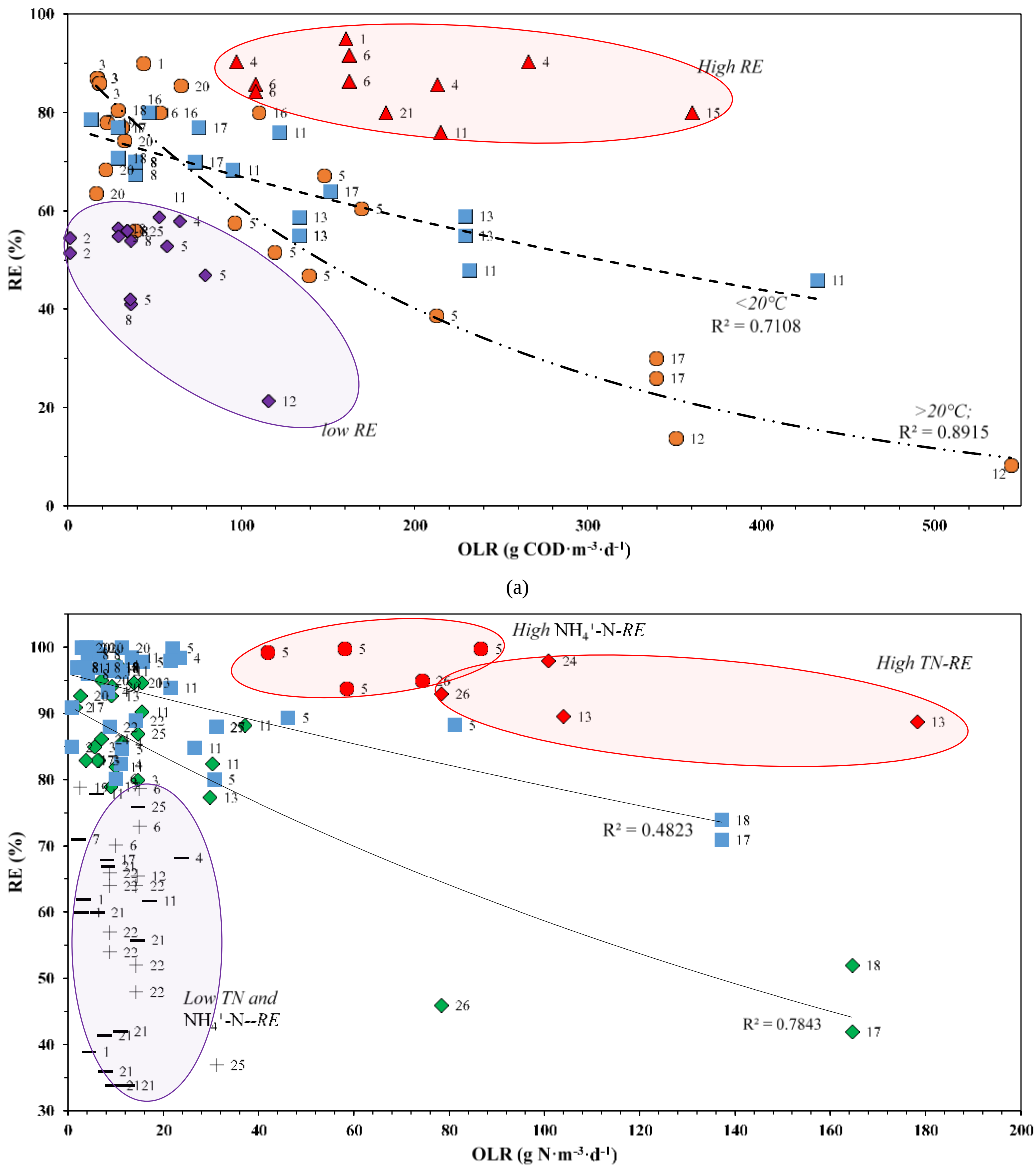


Fig. 1 Literature production concerning wastewater and digestate treatment during recent decades. a) Numbers of records of the term “wastewater treatment” and leading treatment technologies in scientific databases (Scopus). b) Numbers of records of the term “digestate” and leading treatment technologies in scientific databases (Scopus) http://www.scopus.com/scopus/search/form.urli at sept2020



(b)

Fig. 2 (a) Influence of COD Loads on organic matter removal efficiencies in pilot or full-scale algal systems. (●) systems operated at >20°C, (■) systems operated at <20°C; (▲) Experiences with high RE and (♦) low RE, (b) Removal efficiencies for different NH_4-N (■) and TKN (♦) loading rates in microalgal-based systems (NLR), including “outliers” of high removal efficiencies of NH_4-N (●) and TKN (♦) and low removal efficiencies of NH_4-N (+) and TKN (-). – labels correspond to the reference number in Table S1 (supplementary material)

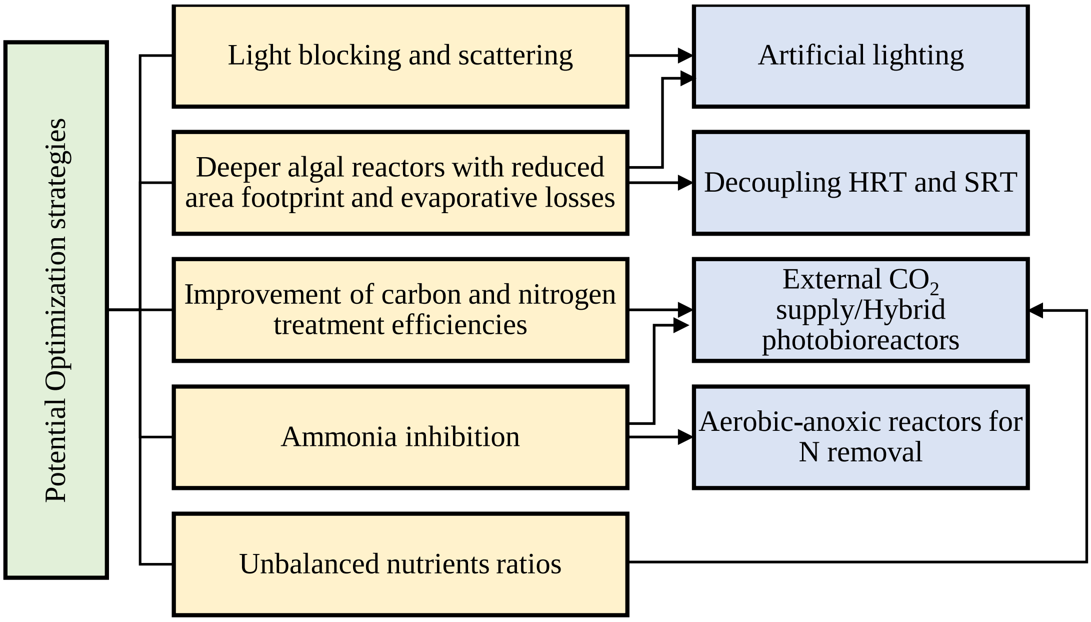


Fig. 3 Potential optimization strategies of microalgae-based photobioreactors treating high-strength wastewaters

Table 1. High-strength wastewater (HSWW) characteristics and pollutant removal efficiencies in microalgal-based treatments

Wastewater	Reference	Org-C, (g L^{-1})	Nitrogen (g L^{-1})	Phosphorus (mg L^{-1})	pH	Org-C:N	N:P	Ptr	TR/O	Microalgae strain	DC (%v/v)	Removal (%)		
												Org-C	N	P
Manure Digestates	Ülgüdür et al. (2019)	-	NH_4-N: 1.6	TP: 42.7	8.84	-	38	S+D	B, PBR	Mixed	16	-	92-93	98
											13	-	63-74	97
											10	-	60-47	96
											8	-	50	96
	Uggetti et al. (2014)	COD: 0.2	NH_4-N: 0.9	PO_4-P: 415	*7-10	0.2	2	N/A	B	Mixed	*5-33	-	-	-
	Singh et al. (2011)	TOC: 0.9	NH_4-N: 1.1	TP:15.4	N/A	0.8	74		B	Mixed	6	-	60	80
Swine Digestate	Jiang et al. (2018)		NH_4-N: 0.5	TP:208.9	8.3	N/A	2	N/A	SBR	*MBC*	100	-	31	50
										C. vulgaris	100			
	Ayre et al. (2017)	TOC: 0.1-0.2	NH_4-N: 0.2-1.6	TP: 33-43	8	-	-	-	HRAP	Mixed	100	-	-	-
	Molinuevo-Salces et al. (2010)	COD: 0.2-0.7	NH_4-N: 0.1-0.7	PO_4-P: 4-34	6.7-9	*2-3	20-25	C	CTPB	Mixed	100	85-90	80-99	73-84
		COD: 0.3-1.8	NH_4-N: 0.1-0.7	PO_4-P: 5–30	8.6- 9.5	*2-3	19-25	C	OTPB	Mixed	100	37-90	88-99	54-80
Agricultural Digestate	Franchino et al. (2013)	BOD:32.9	NH_4-N: 1.6	PO_4-P: 76	7.5	16	48	C	B	*C. vulgaris*	10	-	99	96
										S. obliquus	10	-	84	96.1
										N. oleoabundans	10	-	99	97
	Marcilhac et al. (2014)	-	NH_4-N: 2.4-4.6	PO_4-P: 26-121	-	-	~100-	DCS	B	*C. vulgaris*	10	-	-	-
Food Waste Digestate	Cheng et al. (2016)	COD: 3.1	NH_4-N: 2.1	TP:44	-	1	48		B	*C. pyrenoidosa*	100	68	95	99
	Torres Franco et al. (2018)	COD: 1.8	TN: 0.8	TP: 10.9	7.8	1	73	S	B	*Mixed*	100	84-95	53-90	-
Other Digestates	Shin et al. (2015)	COD: 5.9	TN: 2.3	TP:47.8	-	2	50	C+F	*B*	*S. bijuga.*	10	-	62	73
											5	-	87	90
											3.3	-	91	85
	Ren et al. (2017)[b]	BOD: 1.0-4.6	TN: 0.1-0.2	TP:68-142	5.8-6.7	N/A	N/A	N/A	PBR-G	Mixed	100	84	74	83
									PBR-NG	Mixed	100	84	62	82
	Yang et al. (2018)	BOD:5.7	NH_4-N: 0.3	TP:14.2	7.6	11	36	F	B	*C. vulgaris*	20	53	98	100
									B	*C. vulgaris*	50	38	42	100
									B	*C. vulgaris*	80	59	57	100
Swine WW	García et al. (2018)	TOC: 9.2	TN: 2.6	TP:63	~8.0	3	42	C	OPB	*C. minutissima*	15	86	80	90
										Acutodesmus obliquus	15	87	83	91
										Oscillatoria sp	15	86	83	92
	Fallowfield et al. (1999)	COD: 2.5-27.4	TN: 1.6-7.4	-	8.4-10.5	2	6	S	HRAP, B	Mixed	100	67-99	41-91	48-60
	De Godos et al. (2009b)	TOC: 1.2	NH_4-N: 0.7	PO_4-P: 117	8.4-10.5	2	6	RS+S	CTPB	Mixed	40	61	100	80
											20	60	100	80
											10	44	94	50
Dairy manure WW	Wilkie and Mulbry (2002)	COD: 71.8	TN: 1.2	TP:303	6.9	59	4	NP	GC	Mixed	100	95	60	93
		COD: 32.7	TN: 2.4	TP:240	7.8	14	10	NP	GC	Mixed	100	90	62	70
		COD: 1.6	TN: 0.2	TP:24.7	7.6	7	9	NP	GC	Mixed	100	77	39	51
	Prajapati et al (2014a)	COD: 2.96	NH_4-N: 0.16	PO_4-P: 202	7-9	19	1	NP	B	Mixed	100	80	98	26
Slaughterhouse WW	Hernández et al. (2016)	TOC: 1.6	TN: 0.1	TP: 1.4	7.3	176	7	D	HRAP-I	Mixed	100	86-92	73-80	-
					N/A	1-17	2- 11	D	HRAP -G	Mixed	100	84-86	71-78	-
Tannery WW	Tadesse et al. (2004)	COD: 0.4	NH_4-N: 0.05	PO_4-P: 7	N/A	8	8	FP+SFP	MP	Mixed	100	21	66	0
		COD: 0.9	NH_4-N: 0.1	PO_4-P: 1	N/A	9	133	FP+SFP	MP	Mixed	100	14	21	0
		COD: 0.9	NH_4-N: 0.1	PO_4-P: 1	N/A	7	1	FP+SFP	MP	Mixed	100	8	26	17
Urban WW	Passos et al. (2015)	COD: 0.1-1.0	NH_4-N: 0.1	N/A	N/A	-	-	S	HRAP	Mixed	100	60-92	94-99	-

WW: wastewater, Org-C: Organic carbon, Ptr: pretreatment, TR: Type of reactor, DC:Digestate Concentration. **Pretreatment abbreviations**: S+D: settling+dilution; C: centrifugation; C+F: Centrifugation+filtration, F: Filtered, DCS: decanter centrifuge separation with polymer addition and screw press; NP: not pretreated; : RS+S: rotary screen + Settling; **FP+SFP:** Facultative Pond + Secundary Facultative Pond. **Type of reactor/operation abbreviations:** B: Batch; PBR: photobioreactor; SBR: Sequencing batch reactor, PBR-G: PBR with Glycerol addition; PBR-NG: PBR-No Glycerol addition; OPB: Open Photobioreactor, GC: Growth Chamber, AP: Anaerobic Pond, OTPB: Open tubular Photobioreactor, CTPB: closed tubular Photobioreactor, MP: Maturation Pond; HRAP-I: HRAP-Indoors; HRAP-G: HRAP-Greenhouse. aCW+G: Centrate wastewater + Glycerol; c:PHWWD: Post-hydrothermal liquefaction wastewater digestate

Supplementary material

Table S1. Type of reactor, wastewater, hydraulic retention time (HRT), organic and nitrogen (TKN, NH_4-N) loadings rates (OLR, NLR) and removal efficiencies (RE) used in studies analyzed in Figure 2a and 2b.

No.	Reactor	WW	T (ºC)	HRT (d)	pH	OLR ($g·m^{-3}d$)	COD-RE (%)	NLR -TKN ($g·m^{-3}d$)	NTK-RE (%)	NLR- NH_4-N (g/m3d)	NH4-N-RE (%)	Obs
1	Photobioreactor – BAGC	DMWW	22	7.0	7.0-7.5	160	95	3	60	N.I	N.I	High COD-RE/ Low TKN RE
			22	7.0	7.0-7.5	44	90	3	62	N.I	N.I	Low TKN-RE
			22	7.0	7.0-7.5	31	77	4	39	N.I	N.I	
2	HRAP+MP	DWW	18.5	27.5	8.2	1.2	55	1.2	17	0.7	91	Low COD-RE
			19.2	27.5	8.8	1.2	52	1.2	19	0.7	85	
3	LED Photobireactor	DPW	30	27	8.0	17	87	15	80	N.I	N.I	-
			30	27	8.0	17	87	6	83	N.I	N.I	
			30	27	8.0	18	86	6	83	N.I	N.I	
			30	27	8.0	18	86	6	85	N.I	N.I	
4	HRAP	DWW	23	2.7	7-9	213	86	23.7	68	23.3	98	High COD-RE
			14	7	8.0	97	90	11.2	86	11.0	82	High COD-RE
			22	6.0	7.3-8.4	266	90	N.I	N.I	8.3	93	High COD-RE
			13	6	8.3-8.5	72	58	10.4	97	9.9	97	-
5	Open Photobioreactor	PD	30	8.50	8.6	36	42	N.I	N.I	16	98	Low COD-RE
			30	8.50	9.5	57	53	N.I	N.I	22	100	High NH_4-N RE
			30	8.50	7.9	96	58	N.I	N.I	42	99	High NH_4-N RE
			30	8.50	6.6	139	47	N.I	N.I	58	94	-
			30	8.50	7.6	213	39	N.I	N.I	81	88	
	Closed Photobioreactor	PD	38	8.50	9.0	29	55	N.I	N.I	11	85	-
			38	8.50	7.5	79	47	N.I	N.I	31	80	
			38	8.50	6.7	119	52	N.I	N.I	46	89	
			38	8.50	8.0	148	67	N.I	N.I	58	100	High NH4-N RE
			38	8.50	8.0	169	61	N.I	N.I	87	100	High NH4-N RE
6	HRAP (indoors)	DSWW	25	15.00	7.0-8.5	108	86	N.I	N.I	10	80	High COD-RE
6	HRAP (outdoors)	DSWW	25	10.00	7.0-8.5	162	92	N.I	N.I	15	73	High COD-RE/ Low NH_4-N RE
			20	15.00	7.0-8.5	108	84	N.I	N.I	10	70	High COD-RE/ Low NH_4-N RE
			20	10.00	7.0-8.5	162	86	N.I	N.I	15	79	High COD-RE/ Low NH_4-N RE
7	HRAP	ATPS	15	7-9	7.9	13	79	2.1	71.1	N.I	N.I	Low TKN-RE
8	HRAP	PWW	17	10	8.8	36	41	N.I	N.I	6.6	97	Low COD-RE
			17	10	7.6	36	54	N.I	N.I	N.I	N.I	
			11	10	9.8	34	56	N.I	N.I	1.9	97	
			11	10	9.8	34	56	N.I	N.I	1.9	97	
			13	10	8.2	39	67	N.I	N.I	4.1	96	
			13	10	9.8	39	70	N.I	N.I	4.1	99	
			13	10	9.8	39	70	N.I	N.I	6.6	99	
11	HRAP	PWW	17	10	8.5	215	76	30.2	82	21.4	94	High COD-RE
			7	10	8.3	53	59	5.9	78	3.3	97	-
			7	10	8.5	95	68	10	82	6	93	-
			17	10	8.3	122	76	15.4	90	11.2	96	-
			15	10	8.3	232	48	17	62	13.3	98	Low TKN-RE
			15	10	8.5	433	46	37	88	26.4	85	
12	Opend Pond	TWW	25	4.0	8.0	116	21	N.I	N.I	15	66	Low COD-RE/ Low TKN-RE
			25	3.0	8.0	351	14	N.I	N.I	40	21	Low NH4-N RE
			25	2.0	8.0	544	8	N.I	N.I	74	26	Low NH4-N RE

No.	Reactor	WW	T (ºC)	HRT (d)	pH	OLR ($g \cdot m^{-3}d$)	COD-RE (%)	NLR -TKN ($g \cdot m^{-3}d$)	NTK-RE (%)	NLR- NH_4-N (g/m3d)	NH4-N-RE (%)	Obs
13	HRAP	N.I	18	4	8.3	229	59	178	89	N.I	N.I	
			18	7	8.3	229	55	15	95	N.I	N.I	
			18	4	8.3	134	59	104	90	N.I	N.I	
			18	7	8.3	134	55	9	93	N.I	N.I	
			18	7	8.3	134	55	30	77	N.I	N.I	
15	HRAP	PTDWW	19	8	7.5	360	80	N.I	N.I	9	96.5	High COD-RE
16	HRAP	PTDWW	16	8.1		47	80	N.I	N.I	4	95	
			13	7.8		47	80	N.I	N.I	3	99	
			23	4.2	N.I	110	80	N.I	N.I	8	95	
			24	6	N.I	53	80	N.I	N.I	6	99	
17	HRAP	FFWW+DWW	9	20	8.6	29	77	1	91	N.I	N.I	
			15	10	8.7	75	77	4	83	N.I	N.I	
			19	5	8.3	151	64	8	68	N.I	N.I	Low TKN-RE
			13	7	8.7	73	70	9	79	N.I	N.I	
18	HRAP	DWW	24	4	7.7	339	26	165	42	137	71	
			24	4	8.1	339	30	165	52	137	74	
19	HRAP	DWW	31	19	9.4	22	78			2	79	
20	HRAP	DWW	26	2	10.5	65	85	2.5	93	11.3	100	
			26	4	10.5	33	74	13.8	95	5.6	100	
			26	6	10.5	22	68	9.2	94	3.8	100	
			26	8	10.5	16	64	6.9	95	2.8	100	
21	HRAP	DWW	22	7	9.2	N.I	N.I	8	67.0	N.I	N.I	Low TKN-RE
			22	4	9.0	N.I	N.I	15	55.8	N.I	N.I	Low TKN-RE
			12	10	8.8	N.I	N.I	6	60.0	N.I	N.I	Low TKN-RE
			12	8	8.6	N.I	N.I	8	41.5	N.I	N.I	Low TKN-RE
			23	7	9.4	N.I	N.I	8	36.0	N.I	N.I	Low TKN-RE
			23	5	9.2	N.I	N.I	11	42.1	N.I	N.I	Low TKN-RE
			27	4	9.0	N.I	N.I	9	34.0	N.I	N.I	Low TKN-RE
			27	3	9.1	N.I	N.I	12	34.0	N.I	N.I	Low TKN-RE
22	HRAP	SDWW	23	4	9.3	N.I	N.I	N.I	N.I	14	89	Low NH_4-N RE
			23	4	8.0	N.I	N.I	N.I	N.I	14	52	Low NH_4-N RE
			23	4	7.5	N.I	N.I	N.I	N.I	14	48	Low NH_4-N RE
			23	4	7.0	N.I	N.I	N.I	N.I	14	6	Low NH_4-N RE
			23	4	6.5	N.I	N.I	N.I	N.I	14	64	Low NH_4-N RE
			26	4	9.5	N.I	N.I	N.I	N.I	9	88	Low NH_4-N RE
			26	4	8.0	N.I	N.I	N.I	N.I	9	54	Low NH_4-N RE
			25	4	7.5	N.I	N.I	N.I	N.I	9	57	Low NH_4-N RE
			26	4	7.0	N.I	N.I	N.I	N.I	9	64	Low NH_4-N RE
			26	4	6.5	N.I	N.I	N.I	N.I	9	66	Low NH_4-N RE
23	HRAP	PWW	18	5	N.I	N.I	N.I	12	86	9	86	Low NH4-N RE
24	Anoxic-Aerobic	PWW	25	2	8.9	N.I	N.I	N.I	N.I	100.8	97	High TN RE
25	Anoxic-Aerobic	TxWW	N.I	18	N.I	N.I	N.I	N.I	N.I	15	87	High NH_4-N RE
26	PSBR	PWW	N.I	4	N.I	N.I	N.I	74	85	78	46	
27	Anoxic-Aerobic	SyWW	N.I	4.5	7.2-8.4	N.I	N.I	31	88	31	87	
28	Algal-Biofilm	SyWW	25	12	7.00	183	80.00	21	98.00	-	-	

BAGC: Benthic algae growth chambers; DMWW Dairy manure wastewater; DWW: Domestic wastewater; DPWW: Diluted piggery wastewater; PD: Piggery digestate; DSWW: Diluted Slaughterhouse wastewater; ATPS: Aerobically treated piggery slurry; PWW: Piggery wastewater; TWW: Tannery wastewater PTDWW: Primary treated domestic wastewater; FFWW: Fish Farm wastewater; SDWW: Settled domestic wastewater; TxWW: Textile wastewater; SyWW: Synthetic wastewater,

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