Chapter 3

Process Integration Applied to Microalgal Biofuels Production

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Abstract The rapid development of modern society has resulted in an increased demand for energy and, consequently, an increased use of fossil fuel reserves, compromising the energy sector sustainability. Moreover, the use of this source of energy led to the accumulation of greenhouse gases (GHGs) in atmosphere, which are associated with climate change. In this context, European Union has established new directives regarding GHG emissions and the renewable energy use. Microalgae may have an important role in the achievement of these goals. These photosynthetic microorganisms have a high growth rate, are able to capture CO_2, the biomass can be used to produce biofuels, constituting an undeniable economic potential. Microalgae may also be a source of low carbon fuel, being one of the most studied biofuels feedstock. They are considered a sustainable energy resource, able to reduce significantly the dependence on fossil fuel. They can grow on places that are unsuitable for agriculture, not competing with land for food production. The use of wastewater as microalgal culture medium will reduce the required amount of freshwater and nutrients, achieving simultaneously an effluent with low nutrient concentrations. An important step to increase the competitiveness (promoting simultaneously the environmental sustainability) of microalgal biofuels regarding fossil fuels is the optimization of culture parameters using wastewater as culture medium. Thus, this chapter aims to present the recent studies regarding the integration of wastewater treatment and microalgal cultivation for biomass/biofuel production.

Keywords Biofuel · Microalgae · Process integration · Wastewater treatment
Sustainability

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1 Introduction

The increase of world population is associated with the increase of energy consumption to levels that can compromise the economic growth. Energy is mainly supplied by fossil fuels, which price volatility and sustainable issues (air pollution and climate change) are the main drawbacks. Concerning climate change, the desired balance between CO_2 emissions and sinks (controlling the increase of atmospheric CO_2 concentration) may be achieved through three political strategies (Pires 2017): (i) energy efficiency enhancement; (ii) renewable energy development; and (iii) forest protection. In this context, biofuels have a huge potential to reduce CO_2 emissions to atmosphere (clean energy), as they can substitute fossil fuel energy products without significant technological changes. However, biofuel must be produced from non-edible feedstocks to avoid competition with human food market.

Microalgal culture has attracted the attention of the scientific community due to the high biomass productivity that can be achieved. High growth rates and ability to fix CO_2 are important characteristics to be considered one of the most promising alternatives for biofuel production (Chisti 2007). In addition, they can grow in places that are unsuitable for agriculture, not competing for land with food production practices. However, the cost of microalgal production is still high, being the industrial-scale microalgal culture limited to high-value products. The increase of nutrients (nitrogen and phosphorus) price in the last years is one of the significant contributions to the production cost (Pires et al. 2013). Thus, to obtain microalgal biomass at low cost (to be used for biofuel production), the integration of processes must be performed. To reduce nutrients requirements, microalgae can be cultivated in nutrient-rich wastewater. At the same time, this process integration also reduces the need of freshwater and promotes the treatment of these effluents. Therefore, this chapter aims to present technological issues related to the integration of wastewater treatment and microalgal cultivation for biomass/biofuel production. Recent advances and challenges are also discussed.

2 Microalgae

Microalgae may be classified as prokaryotic or eukaryote organism (Richmond 2004). With respect to the prokaryotic domain, cyanobacteria (also called blue-green algae) are the only ones belonging to this group. On the other hand, in the eukaryotic domain, there are several classes of algae and the most relevant are the following: green algae (*Chlorophyceae*), Golden algae (*Chrysophyceae*) and diatoms (*Bacillariophyceae*). Microalgae can be found more often in the water—freshwater, seawater or brackish water (Lam et al. 2017; Lee 2008). However, they can also be found in all other terrestrial environments, such as snow or hot springs. In most habitats, they act as primary producers in the food chain, synthesizing organic matter from solar energy, carbon dioxide, water and nutrients (e.g. nitrogen and phosphorus) through photosynthesis. In addition, they also produce the oxygen required for metabolism of consumer organisms.

Microalgae are an extremely diverse group of organisms. For example, they contain a significant amount of lipids in the form of fatty acids, which can be extracted for subsequent production of biodiesel (Lam et al. 2017).

2.1 Cellular Metabolism

The algae are able to grow with different cellular metabolisms, focusing on the main forms of nutrition, including autotrophy and heterotrophy (Richmond 2004). Therefore, microalgae may grow based on four types of cell metabolisms: autotrophy, heterotrophy, mixotrophy and photoheterotrophy.

Autotrophic organisms obtain energy and electrons (necessary for CO_2 reduction) through the absorption of solar energy and substrates oxidation (mostly water), respectively (Richmond 2004). On the other hand, the heterotrophic organisms use only organic compounds as carbon and energy source (Abreu et al. 2012). With regard to growth under mixotrophy, it is equivalent to grow under autotrophic and heterotrophic conditions, since both organic compounds and CO_2 can be assimilated by microalgae depending on the growing conditions (Richmond 2004). Thus, mixotrophic microorganisms synthesize compounds characteristic of both types of metabolisms, showing high production rates (Cerón García et al. 2005). Concerning photoheterotrophic metabolism, organisms require light energy as a source of energy and organic compounds as carbon source (Richmond 2004).

2.2 Microalgal Growth Conditions

The growth of the culture as well as the biochemical composition of biomass is not only determined by the microalgae species in cultivation (Rocha 2012). The medium composition, pH, temperature and light intensity are some of the parameters that can influence the growth and biochemical composition of microalgae.

2.2.1 Nutrients

According to Chisti (2007), the molecular formula of the microalgal biomass is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. Thus, the most important nutrients for autotrophic growth (known as macronutrients) are the carbon (C), nitrogen (N) and phosphorus (P) (Richmond 2004).

Carbon is the macronutrient needed in high concentrations, since it is the main constituent of all organic substances synthesized by the cells, such as proteins, carbohydrates, nucleic acids, vitamins and lipids (Richmond 2004). Microalgae have inorganic carbon assimilation processes: diffusion ($5.0 < pH < 7.0$) and active transport ($pH > 7.0$) (Gonçalves et al. 2017). In order to achieve high autotrophic production rates, CO_2 and bicarbonates (HCO_3^-) supply is the most important (Richmond 2004). For certain species of microalgae that grow in mixotrophic conditions, organic compounds (e.g. sugars, acids and alcohols) can be used as carbon source.

Nitrogen has also an important role, since it is a basic element for the formation of proteins, nucleic acids, vitamins and photosynthetic pigments (Richmond 2004). The assimilation mechanism of nitrate and ammonium (NH_4^+) by microalgae is active transport (Gonçalves et al. 2017). Nitrogen is mainly provided in the form of nitrate (NO_3^-), but sometimes ammonium (NH_4^+) and urea can also be used (Richmond 2004). Silva et al. (2015) evaluated the preferred source of nitrogen (NO_3^- and NH_4^+) for two species of microalgae (*Chlorella vulgaris* and *Pseudokirchneriella subcapitata*). The authors concluded that the ammonium was preferred source of nitrogen for microalgae *C. vulgaris*, since its assimilation by the microalgae involves lower energy consumption (Jia et al. 2016). When the microalgae are limited by nitrogen a discoloration of the cells usually occurs (reduction of chlorophylls and carotenoids increase) and a build-up of organic compounds such as polysaccharides and some oils (Becker 1994). Goiris et al. (2015) studied the impact of nutrient limitation in the production of antioxidants in three species of microalgae (*Phaeodactylum tricornutum*, *Tetraselmis suecica* and *C. vulgaris*). The content of chlorophyll *a* in biomass was significantly lower when the microalgae were limited by nitrogen.

Phosphorus is essential nutrient for growth and for many cellular metabolic activities, such as energy transfer, synthesis of nucleic acids, deoxyribonucleic acid (DNA), among others (Richmond 2004). Similarly to nitrogen, phosphorus is also assimilated by the microalgae through active transport (Gonçalves et al. 2017). This chemical element is preferentially added in the form of orthophosphate (PO_4^{3-}), and its absorption is energy dependent (Richmond 2004). The supply of phosphorus also influences the composition of the produced biomass (Borowitzka 1988). The content of lipids and carbohydrates is especially affected by internal and external phosphorus supply. The N:P ratio in the culture medium is also important, as it influences not only the productivity, but also the dominant species in culture (Richmond 2004). In 1934, Alfred C. Redfield estimated the N:P ratio of 16:1 (known as Redfield ratio) through the elemental composition of microalgal cells. However, several studies have tested different ratios (Martin et al. 1987; Minster and Boulahdid 1987; Shaffer et al. 1999; Takahashi et al. 1985). Silva et al. (2015) evaluated the effect of N:P ratio on the growth of microalgae *C. vulgaris* and *P. subcapitata*. The N:P ratios of 8:1, 16:1 and 24:1 were evaluated. The N:P ratio of 8:1 was the one that more favoured the growth of microalgae *C. vulgaris*.

In addition to C, N and P, other nutrients are also important for cell growth, such as the sulphur (S), potassium (K), sodium (Na), iron (Fe), magnesium (Mg) and calcium (Ca) (Richmond 2004). In addition to these, other trace elements (micronutrients) are important, such as boron (B), copper (Cu), manganese (Mn), zinc (Zn), molybdenum (Mo), cobalt (Co), vanadium (V) and selenium (Se).

2.2.2 PH

During the photosynthetic CO_2 fixation, the hydroxide ion (OH^-) accumulates in the growing medium, leading to a gradual increase of pH (Richmond 2004). This shifts the chemical equilibrium of the inorganic carbon present in the medium towards the formation of carbonates (CO_3^{2-}). However, they are not the preferred carbon source for microalgae (Lower 1999). On the other hand, a decrease on the solution pH shifts the chemical equilibrium towards the formation of CO_2, which is one of the preferred carbon sources for microalgae. Nevertheless, this process can lead to the release of CO_2 into the atmosphere, decreasing the concentration of this nutrient extremely important for the cultivation of microalgae.

With regard to nitrogen, when it is provided in the form of ammonium, an increase on the solution pH can result in a decrease on the concentration of nitrogen available for microalgae (Guštin and Marinšek-Logar 2011; Cai et al. 2013). High pH values move the chemical equilibrium of ammonium for the production of ammonia that can be released into the atmosphere due to the aeration of the culture, reducing the availability of nitrogen for microalgae.

The concentration of phosphorus in culture medium can also be influenced by elevated pH, as it can lead to precipitation of phosphate (in the forms of calcium phosphate, iron phosphate and aluminium phosphate) and therefore limit the amount of phosphorus available for microalgae (Wang and Nancollas 2008; Cai et al. 2013).

The pH can directly affect the microalgae, as the pH of microalgal cytoplasm is neutral or slightly alkaline, and enzymes are pH-sensitive and may be inactive in acidic conditions (Chiranjeevi and Mohan 2016). Therefore, extreme pH conditions can cause the disruption of many cellular processes, which may lead to the collapse of culture (Jia et al. 2016).

Tripathi et al. (2015) studied the effect of pH on the growth of *Scenedesmus* sp. microalgae in a range of 7–10 and concluded that the optimal pH for this species was 8. Munir et al. (2015) evaluated the pH effect on the growth of two species of microalgae (*Spirogyra* sp. and *Oedogonium* sp.) in a range of 6.5–9.0, achieving the highest growth at pH 7.5 for both species.

2.2.3 Light Intensity and Temperature

The light energy received by microalgae is a function of the photon flux density that reaches the surface of the culture (Richmond 2004). The cells absorb only a fraction of the photon flux, which is influenced by several factors, such as (i) cell density;

(ii) the optical properties of the cells; (iii) the optical path length of photobioreactor; and (iv) the mixing degree. The photons that are not absorbed by the photosynthetic reaction centres of the cells can be reflected or the associated energy is dissipated in the form of heat. A basic aspect of the interaction of light and temperature is the fact that the optimum temperature for photosynthesis increase with increasing light intensity. Gonçalves et al. (2016) evaluated the effect of light and temperature on the growth of microalgae (*C. vulgaris*, *P. subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*) and nutrients uptake. In the case of *C. vulgaris*, these authors found that the optimum temperature for growth was 25 °C and the optimum daily irradiance was 208 μmol/m^2/s.

Figure 1 presents the variation of photosynthetic rate with the luminous intensity. With the increase in the light intensity, the photosynthetic rate can reach a value corresponding to the saturation (Richmond 2004). A further increase in the light intensity will not result in an increase of growth rate, and it can become unfavourable, manifesting itself by a decrease in growth rate and culminating in photoinhibition and/or the death of culture in extreme cases.

2.3 Microalgal Culture Technology

Currently, microalgal cultivation technologies can be divided in two classes: open systems (raceway ponds, lagoons, among others) and closed systems/photobioreactors (tubular, bubble column and airlift) (Dasgupta et al. 2010; Kochen 2010).

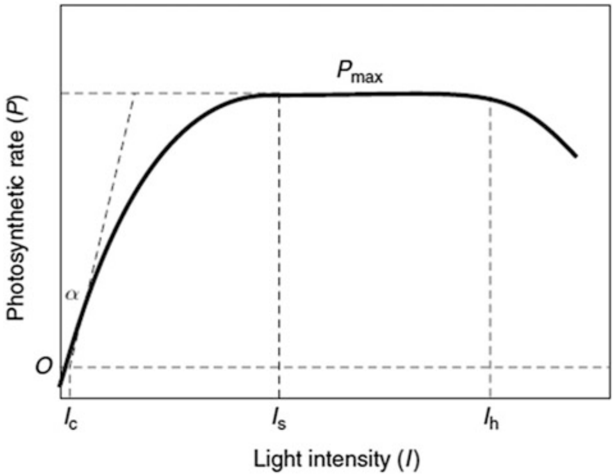

Fig. 1 Variation of photosynthetic rate with light intensity (adapted from Richmond 2004)

2.3.1 Open Systems

Raceway ponds systems consist of a closed-circuit recirculation channel, usually with 0.3 m depth (Benemann and Oswald 1996; Craggs et al. 2012). Mixing and circulation are produced by a paddlewheel, and flow is guided in the curves by deflectors placed in the flow channel, as shown in Fig. 2. During the day, the culture is fed continuously in front of paddlewheel, where run-off begins (Chisti 2007). The paddlewheel operates continuously to prevent sedimentation of biomass.

In open systems, cooling is achieved only by evaporation (Chisti 2007). The temperature varies seasonally and throughout the day, and a significant loss of water by evaporation can be observed. As an open system, the use of CO_2 is less efficient than in closed systems (due to loss of this compound to the atmosphere), also representing a significant cost in the production of microalgae. In addition, the open systems are more susceptible to contamination from other algae or microorganisms. Besides the requirement of large cultivation areas and the limitation of cultivation period (due to contamination problems), these systems are associated to low productivity and inefficient mixture that does not prevent the existence of optically dark areas. However, raceway ponds have a lower production cost, as compared to the closed photobioreactors (Chisti 2007; Harun et al. 2010; Pushparaj et al. 1997). Additionally, the raceway ponds have the advantage of being easily cleaned (removal of biofilm accumulated on the channels walls) (Chisti 2007).

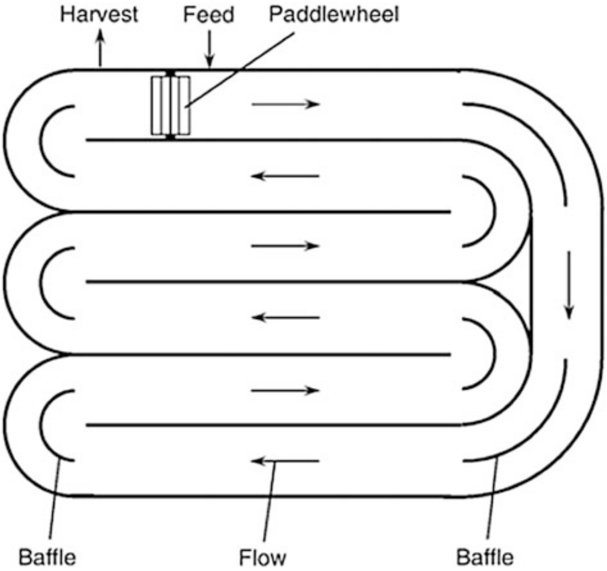

Fig. 2 Cultivation system—raceway pond (adapted from Walter 2011)

2.3.2 Closed Systems

The photobioreactors allow the culture of a single species of microalgae for long periods of cultivation (Molina Grima et al. 1999). Among the various types of photobioreactors for monocultures cultivation, the tubular photobioreactors are the most suitable for large-scale production of microalgae.

The tubular photobioreactors consist of a set of transparent tubes, usually glass or plastic (Chisti 2007). The tubes have typically a diameter less than or equal to 0.1 m. This parameter is limited in order to allow the penetration of light inside the tube, thus guaranteeing light availability to the whole culture. Culture circulates within the tubes, passing by a reservoir (degassing column) and returns again to the tubes, as shown in Fig. 3. There are other variants of photobioreactors; however, they are not usually applied (Carvalho et al. 2006; Molina Grima et al. 1999; Pulz 2001; Tredici 2002).

Some of the advantages of photobioreactors are: (i) the control of cultivation conditions (pH, agitation, concentration of CO_2 and oxygen—O_2) is facilitated; (ii) the reduction of water and CO_2 losses; (iii) the possibility of operating with high cell concentrations and volumetric productivities; and (iv) the reduction of contamination by other microorganisms (Li et al. 2008). However, the photobioreactors have some drawbacks, among them the overheating of the culture, the accumulation of O_2 and the high costs of construction.

2.4 *Microalgal Applications*

The microalgae biomass can be used to generate added-value products. The biomass applicability varies depending on the used microalgae species (Spolaore et al. 2006). Currently, there are numerous commercial applications for microalgae, such

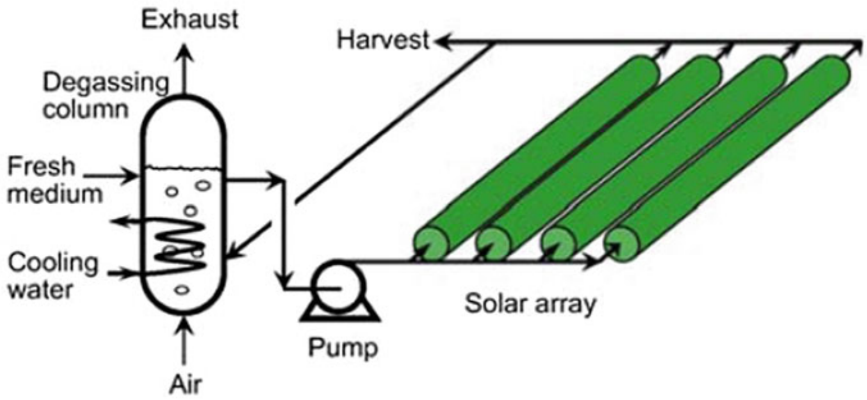

Fig. 3 Tubular photobioreactor (adapted from Chisti 2007)

as: (i) use of microalgae to increase the nutritional value of food and animal feed due to its chemical composition; (ii) extraction of high-value products from microalgae that can be incorporation in cosmetic products; (iii) production of biofuels; (iv) CO_2 capture; and (v) use of microalgae for the depuration of wastewaters.

Gouveia and Empis (2003) concluded that biomass of *C. vulgaris* and *H. pluvialis* was a relatively concentrated natural source of carotenoids, which are natural pigments that exhibit antioxidant capacity. The main carotenoids, found in microalgae, with commercial interest are the β-carotene, lutein and astaxanthin (Mostafa 2012). Besides these compounds, microalgae may be used for the production of biofuels and other bioproducts: proteins, cosmetics, pharmaceutical products, among others.

In the context of environmental applications, microalgae may be used for wastewater treatment in WWTPs (Hoffmann 1998; Oswald 2003). The discharge of wastewaters with high amounts of N and P can cause severe eutrophication of watercourses at downstream (Correll 1998). Thus, the removal of N and P based on microalgae can be quite efficient, cheaper and ecologically safer than physical and chemical treatments currently used (Hoffmann 1998).

Microalgae can also be used in biofixation of atmospheric CO_2 (or from industrial gaseous effluents) through photosynthesis, thus contributing for the reduction of this important greenhouse gas (Nascimento et al. 2015; Sheehan et al. 1998). Microalgae can capture about 1.7–2.4 tons of CO_2 per ton of biomass.

3 Wastewater Treatment by Microalgae

Microalgae can play an important role in the treatment of wastewater, particularly at the level of nutrients removal and reduction of WWTPs operating costs.

3.1 Nutrient Removal

Urban wastewaters are rich in carbon, nitrogen, phosphorus and other minerals, which have to be removed before effluent discharge in water bodies (Cabanelas et al. 2013). An excess of organic carbon and nutrients released into rivers and lakes can lead to decreased dissolved oxygen, toxicity of aquatic life and to eutrophication.

In natural aquatic systems, microalgae assimilate large amounts of nutrients and metals during their growth. Microalgae can digest inorganic sources of nitrogen such as ammonium, nitrite and nitrate (Jia et al. 2016).

The use of microalgae in the wastewater treatment plant was first proposed by Oswald and Gotass (1957) and in recent decades has received a lot of attention. The premise of this approach is that the mixotrophic systems can be designed to reduce the organic carbon, as well as the nutrients in urban wastewater to values lower than discharge limits (McCarty et al. 2011). Microalgae can be a good approach for the tertiary treatment of urban wastewaters because they require large amounts of nitrogen and phosphorus to their growth, including for the synthesis of proteins (40–60% by dry weight), nucleic acids and phospholipids (Silva-Benavides and Torzillo 2011). The wastewater treatment based on microalgae can remove N and P more efficiently than the traditional activated sludge treatment (Lau et al. 1995; Lavoie and De La Noüe 1983; Tam and Wong 1989). In addition to the removal of these nutrients, microalgae have the ability to remove heavy metals from wastewater (Rai et al. 1981). Finally, the microalgae can perform a disinfectant effect in the effluent due to the pH increase inherent to photosynthesis (De La Noue and De Pauw 1988). The mentioned advantages make this system an excellent alternative to the traditional technologies employed for wastewater.

Nutrient removal efficiencies are dependent on the wastewater composition and environmental conditions, such as light intensity, the N:P ratio, the light/dark cycle and microalgal species (Aslan and Kapdan 2006). The most studied microalgal species for the treatment of urban wastewaters are *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* (Chinnasamy et al. 2010; Kong et al. 2010; Olguín 2003; Wang et al. 2010). Taking into account the potential of microalgae for wastewater treatment, Table 1 presents several studies that demonstrate the viability of microalgal cultures in the nutrients removal from different types of wastewater.

3.2 Limitations of Conventional Treatments

The consortium of microorganisms present in activated sludge systems require phosphorus for their growth, which results in partial removal of phosphate during the secondary treatment (Yau 2016). However, to achieve discharge limits of 1 mg P/L normally is required the use of inorganic coagulants (such as lime, aluminium sulphate and iron chloride). Besides the increase on the treatment cost, the addition of these coagulants is less environmentally sustainable than the removal of phosphorus by microalgae.

Another limitation is the main by-product generated in biological treatment: activated sludge waste. To treat 1 million litres of wastewater, the biological treatment produces about 70–100 kg of activated sludge in dry basis (Athanasoulia et al. 2012). Consequently, the treatment and disposal of this waste requires a considerable deposition area and a high-energy expense. In addition, the mechanical aeration (necessary in biological treatment) can cause the release of volatile contaminants into the atmosphere (Jia et al. 2016). The role of microalgae in this step could reduce or even prevent the release of these contaminants, since microalgae would produce oxygen and thus reduce the need for aeration.

Finally, greenhouse gases (such as methane—CH_4, N_2O and CO_2) are released to the atmosphere in the biological treatment (Campos et al. 2016). Conventional

Table 1 Nutrient removal from wastewater with microalgal cultures

Microalgae	Experimental setup	Removal efficiency (%)			References
Wastewater		$[NH_4^+–N]_i$ (mg/L)	$[NO_3^-–N]_i$ (mg/L)	$[TP]_i$ (mg/L)	
B. braunii	Fermenter BioFlo; *V* = 9 L; *T* = 25 °C; LI = 3500 lx; LDR = 12:12; CT = 14 d		80	~100	Sydney et al. (2011)
Domestic			390	385 $[P–PO_4^{3-}]$	
C. vulgaris	Bubble column PBR; *V* = 2 L; *T* = 30 °C; LI = 3000 lx; CT = 14 d	97		96	Feng et al. (2011)
Synthetic		20		4	
Chlorella sp.	Coil bioreactor; *V* = 25 L; *T* = 25 ± 2 °C; LI = 50 μmol/m²/s; CT = 14 d	94		81	Li et al. (2011)
Municipal		83		212	
N. oleoabundans	Bubble column PBR; *V* = 400 mL; *T* = 30 °C; LI = 1280 lm; CT = 7 d		99	100	Wang and Lan (2011)
Synthetic			140	47	

CT cultivation time; *LDR* light:dark ratio; *LI* light intensity; *T* temperature; *V* volume treatments have no capacity to carry out the CO_2 capture and thus prevent its release to the atmosphere.

3.3 Benefits of Microalgae in WWTPs

In the activated sludge treatment, it is estimated that 1 kg of BOD removal consumes about 1 kWh of electricity for aeration (implying 1 kg of CO_2 emissions in the electricity production) and produces about 0.45 kg of biomass residues (Oswald 2003). On the other hand, the removal of 1 kg of BOD by microalgae (photosynthetic pathway), in mixotrophic systems, does not require energy input and it can produce enough biomass to generate methane, which will produce 1 kWh of electricity. The wastewater treatment based on microalgae is an ecological process, without secondary pollution, and it allows the efficient recycling of nutrients (Mulbry et al. 2008; Muñoz and Guieysse 2006; Pizarro et al. 2006).

The microalgae biomass resulting from wastewater treatment systems can give rise to products with commercial interest, such as fertilizer, animal feed, fine chemicals, biofuels, among others, thereby reducing the total cost of the treatment plant (De La Noüe et al. 1992).

Finally, the CO_2 biofixation by microalgae is an environmentally friendly method to remove carbon from the atmosphere (Singh and Yadav 2015). Microalgae have been described as having high capacity to fix CO_2 when compared with land plants (Chen et al. 2013). They may fix the CO_2 released by the activated sludge, avoiding its release to the atmosphere.

The interest in the cultures of microalgae is that conventional treatment processes present some important disadvantages, such as: (i) variable efficiency depending on the nutrient to be removed; (ii) expensive treatment; (iii) chemical processes leading to secondary pollution; and (iv) nutrient loss with possible value (N and P) (De La Noüe et al. 1992).

Thus, the increase in global warming, scarcity of fossil fuels and the need to mitigate emissions of greenhouse gases, the study of the feasibility of biological wastewater treatment based on microalgae (associated with the production of biofuels) is of utmost importance (Rawat et al. 2011).

3.4 Reduction of Operating Costs

The introduction of microalgae in wastewater treatment processes can reduce the costs associated to aeration and coagulation and at the same time can obtain biomass with high commercial value (Christenson and Sims 2011). The microalgae-based treatment system is a less expensive and ecologically safer technology when compared with physical and chemical processes, with the additional benefits of resources recovery and recycling. The biological nitrification/

denitrification system, the most common process used for nitrogen removal, has as end product the nitrogen gas (N_2), while the treatment with microalgae retains the nitrogen compounds on biomass, adding value.

The aerobic photosynthetic pathway is especially interesting, as it allows to reduce the operating costs associated with the aeration of the biological treatment (Borowitzka and Borowitzka 1988), which represents more than 50% of the energy needs in a WWTP (EPA Office of Water 2006). Recent studies have shown that microalgae may also support the aerobic degradation of several hazardous contaminants (Muñoz and Guieysse 2006; Safonova et al. 2004).

4 Microalgal Limitations in Wastewater Treatment

As in all treatment systems, microalgal culture also presents some disadvantages/limitations in wastewater treatment.

4.1 Temperature Variability

The productivity of microalgae increases with increasing temperature up to an optimum value, above which the respiration and photorespiration of microalgae reduce overall productivity (Park et al. 2011). Thus, water temperature too high or too low can have a negative effect on the growth of microalgae and it can cause growth inhibition. The ideal temperature, measured under maximum growth conditions (optimal conditions of nutrients and light), varies from species to species; however, for many species it is between 28 and 35 °C. The optimum temperature changes when the growth is limited by nutrients and/or light. Moreover, microalgal growth decreases when they are subject to sudden changes of temperature (Larsdotter 2006). For example, the exposure of a species at a temperature of 10 °C, when they were adapted to higher values, resulted in a reduction of about 50% of the chlorophyll *a* in 15 h. In addition, a high luminous intensity associated with low temperatures is also another factor that causes growth inhibition.

Taking into account the variability of temperature during the day and the year, it is expected that the efficiency of wastewater treatment based on microalgae can be substantially affected. Therefore, the seasonal temperature variability is one of the main limitations in the introduction of microalgae in WWTPs, since it will be difficult to keep the cultures within a range of acceptable temperatures throughout the year.

4.2 Light/Dark Cycle

The quality, intensity and light period are very important parameters in microalgae production (Cardinale 2011). In outdoor cultivation systems, solar radiation is the only source of light and its availability is therefore dependent on the geographical location, climate and seasonality (Novoveská et al. 2016). Light regimes (to which cultures are submitted) are considered an important factor in the productivity and efficiency of photosynthetic reactions (Sicko-Goad and Andresen 1991; Toro 1989).

Lee and Lee (2001) evaluated the effect of the light/dark cycle in the treatment of wastewater by *Chlorella kessleri*. This study showed that the amount of nitrate removed was 31.6 mg NO_3/L after 3 days under continuous light conditions, and 14.0 mg NO_3/L with the light/dark cycle of 12:12. However, the removal of organic carbon and phosphate was higher for the culture under conditions of light/dark cycle of 12:12. Therefore, *C. kessleri* could grow heterotrophically during the dark periods, once the microalgae are able to metabolize the organic carbon to their growth without photosynthesis. With regard to the species (*C. vulgaris*), Santos et al. (2009) studied the effect of two light/dark cycles: 24:0 and 12:12. This study demonstrated that the cycle of continuous light presented a higher growth rate.

Depending on the types of effluent (primary, secondary or tertiary), the light/dark cycle may have different impacts on the treatment efficiency, since the primary and secondary effluents have high concentrations of organic carbon. On the other hand, the tertiary effluent has low amounts of organic carbon, which can limit the heterotrophic growth of microalgae and, consequently, the treatment efficiency.

4.3 Competition with the Microflora Present in Wastewater

The coexistence of microalgae and bacteria is a biological process that occurs by the interaction of two distinct processes: photosynthesis of microalgae and bacterial

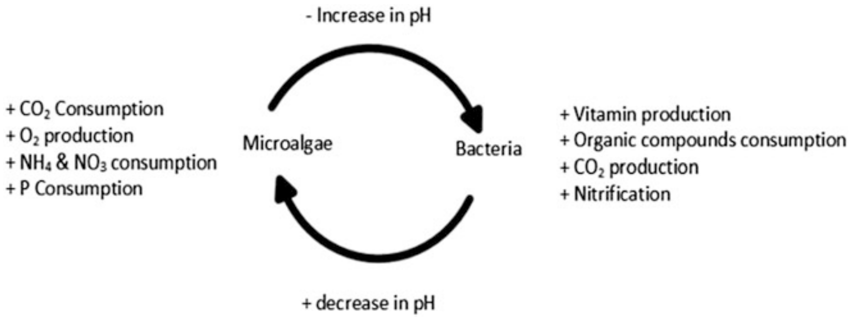

Fig. 4 Nutrient exchange in microalgae-bacteria consortium (adapted from Anbalagan 2016)

respiration (Anbalagan 2016). These two processes occur simultaneously in wastewater with nutrient exchange, as shown in Fig. 4.

Sforza et al. (2014) evaluated the effect of the wastewater native microflora on the growth of microalgae *Chlorella protothecoides*. The obtained results indicated that significant differences were not detected in the growth of microalgae, suggesting that the presence of the native microflora in wastewater does not influence its growth.

4.4 *Biomass Harvesting*

Despite the numerous advantages of wastewater bioremediation by microalgae, there are also some obstacles that limit their application on a large scale, such as harvesting of biomass. Currently, biomass harvesting is quite expensive, since this step is about 20–30% of the biomass production cost (Molina Grima et al. 2003). The microalgae separation from effluent remains the main obstacle for the bioremediation of wastewater in WWTPs, in part due to the small size of the microalgae. The size of eukaryotic unicellular microalgae usually varies between 3 and 30 μm (Molina Grima et al. 2003), and the size of the cyanobacteria varies between 0.2 and 2 μm (Chorus and Bartram 1999). Moreover, the fact that cultures are relatively diluted (200–600 mg/L) (Uduman et al. 2010) with densities close to the water also affects the harvesting process. Finally, the negative charge of microalgae maintains cells in suspension (Danquah et al. 2009a). Until now, there is no method for microalgal harvesting that is economically viable and efficient (Barros et al. 2015). Biomass harvesting techniques applied to microalgae include coagulation/flocculation, auto and bioflocculation, gravitational sedimentation, flotation, electrical processes, filtration and centrifugation. However, none of these techniques meets the ideal conditions for use in large scale (cost vs. efficiency) (Christenson and Sims 2011). Cost reduction of harvesting is therefore considered to be a key factor for the development of sustainable production in large scale of microalgal biomass. An ideal process should be effective for most species of microalgae and it must allow the obtaining of high concentrations of biomass (Danquah et al. 2009b). In addition, the harvesting process must introduce moderate costs of operation, maintenance and energy.

4.5 *Wastewater Characteristics*

The amount and quality of light penetration affect the photosynthetic process of organisms that use sunlight as an energy source (Butler et al. 2017). Thus, as photosynthetic organisms, the colour of wastewater, as well as the amount of particles in suspension should be factors to be used for the cultivation of microalgae (Yaakob and Fakir 2011). The high content of particulates in wastewaters can affect microalgae growth due to shadowing effects. In addition, the microorganisms also contribute to the turbidity of the water, limiting even more the depth of light penetration. Taking into account these factors that limit light penetration, photosynthesis occurs only in the superficial layers of the culture (USEPA 2011), influencing the overall treatment efficiency. In order to maximize the light penetration, the mixing degree inside the photobioreactors is an important factor, as all cells can be exposed to light in a turbulent regime for at least a short period of time, being possible to achieve high productivity (Yaakob and Fakir 2011).

5 Biofuels Production with Microalgae Cultivated in Wastewater: Recent Advances and Challenges

The production of microalgal biofuels has two major challenges: (i) production costs reduction and (ii) identification of the harvesting process. The integration of biomass production and wastewater treatment reduces the requirements of nutrients and freshwater. Studies with real wastewaters should be performed to evaluate the nutrient removal efficiencies (wastewater treatment efficiency) and biomass productivities (possible growth inhibition). With the achieved biomass, the potential for production of different biofuels (biodiesel, bioethanol, biogas, between others) should be assessed. Table 2 shows some recent studies focusing on biofuel production with microalgae cultivated in wastewater. Prandini et al. (2016) evaluated the growth of microalgae *Scenedesmus* sp. in piggery wastewater and bubbled swine wastewater-derived biogas (for biogas filtration). Microalgal culture was able to assimilate $N–NH_3$, $P–PO_4^{3-}$ and CO_2 at a rate of 21 ± 1, 4 ± 3 and 219 ± 5 mg/L/d, respectively. H_2S in biogas (up to 3000 ppm) was not inhibitory and it was completely removed. Hernandez et al. (2016) tested a consortium of microalgae composed by *Chlamydomonas subcaudata*, *Anabaena* sp. and *Nitzschia* sp. for treatment of slaughterhouse wastewater in two high-rate algal ponds—HRAPs (indoor and outdoor) during 115 d. High removal efficiencies of chemical oxygen demand and soluble phosphorus were achieved in both HRAPs. The maximum productivity was 12.7 $g/m^2/d$. High quality of free fatty acids (FFA) was achieved in a ratio of 142 mg FFA/g. Biogas production was also assessed, resulting in 195 mL CH_4/g. Lutzu et al. (2016) evaluated the potential of brewery wastewater as microalgal culture medium. Adjustments in nitrogen and phosphorus concentrations were needed to improve biomass and lipid productivities. The chemical analysis of the fatty acids methyl esters showed that high fractions (67.24%) are unsaturated ones and they are composed mainly by C16–C18. Concerning the wastewater treatment, high removal efficiencies were achieved for nitrogen and phosphorus (>99%) and a significant reduction of chemical oxygen demand was observed (65%). Despite the recent studies reported in the literature, further researches are still needed. Due to the natural variability of wastewater composition, microalgal culture should be tested under environmental stresses in

Table 2 Biofuel production with microalgae cultivated in wastewater

Biofuel/microalgae	Experimental setup	P (mg/L/d)/BFC	Reference
Biogas/*Scenedesmus* sp.	Swine wastewater; $V = 16.9$ L; $T = 22 \pm 2$ °C; LI = 148.5 μmol/m^2/s; LDR = 12:12 and 24:0	$P_x = 142.0$	Prandini et al. (2016)
Biogas and lipids/mix of microalgae	Slaughterhouse wastewater; HRAP; $V = 75$ L; $T = 20$–25 °C; LI = 63–760 μmol/m^2/s; HRT = 10–15 d	$P_x = 1.05$–2.56; $P_L = 13$–15/142 mg FFA/g; 195 mL CH$_4$/g	Hernandez et al. (2016)
Biodiesel/*Chlorella* sp.	Domestic wastewater; HRAP; $T = 20.2 \pm 5.7$ °C; pH = 12; LDR = 8:16	P_x = n.a./SFA = 46–67%; UFA = 10–40%	Drira et al. (2016)
Biodiesel/*Scenedesmus dimorphus*	Brewery wastewater; bubble column PBR; $V = 250$ mL; LI = 100 μmol/m^2/s; LDR = 24:0; CT = 12 d	P_x = n.a./SFA = 31–37%; UFA = 62–68%	Lutzu et al. (2016)
Biodiesel/*Scenedesmus obliquus*	Municipal wastewater; Erlenmeyer flasks; $V = 1$ L; $T = 25 \pm 1$ °C; LI = 100 μmol/m^2/s; LDR = 12:12	$P_x = 4.8$–7.5; $P_L = 0.44$–1.98/ SFA = 27–41%; UFA = 10–26%	Han et al. (2016)
Biogas/*Chlorella vulgaris*	Domestic wastewater; bubble column PBR; $V = 40$ L; pH = 6; LI = 150 μmol/m^2/s; LDR = 24:0; CT = 21 d	P_x = n.a./223–408 mL CH$_4$/g	Calicioglu and Demirer (2016)
Biomass/*Acutodesmus dimorphus*	Industrial wastewater; Erlenmeyer flasks; $V = 1$ L; $T = 35$ °C; LI = 60 μmol/m^2/s; LDR = 12:12; CT = 8 d	$P_x = 210$; LC = 25.05%	Chokshi et al. (2016)
Lipids/*Scenedesmus obliquus*	Domestic wastewater; raceway pond; $V = 533$ L; pH = 8; CT = 5 d	$P_x = 87.3$; LC = 33.6%	Arbib et al. (2017)

BFC biofuel characteristics; *FFA* free fatty acids; *HRAP* high-rate algal ponds; *HRT* hydraulic retention time; *LDR* light–dark ratio; *LC* lipids content; *LI* light intensity; *n.a.* not available; P productivity; P_L lipid productivity; P_X biomass productivity; *SFA* saturated fatty acids; T temperature; *UFA* unsaturated fatty acids; V volume

order to evaluate their tolerance capacity. With a less-controlled environment, the development of an innovative, efficient and cost-effective harvesting process is highly required. In addition, studies with life cycle assessment for economic viability, carbon footprint and sustainability should be performed.

6 Conclusions

The continuous growth of population and energy needs of the industry and transport sectors increased interest in the use of renewable energy sources. Besides not being renewable sources, fossil fuel energy (oil, coal and natural gas) emits considerable amounts of greenhouse gases to the atmosphere leading to increased global warming. Therefore, the production of biofuels from microalgal biomass is considered a source of sustainable energy, since the cultivation of biomass can be integrated with wastewater treatment. The presence of large amounts of C, N and P (macronutrients for microalgal growth) in urban wastewaters allows that this kind of effluents may be used as cultivation media for microalgal culture. Consequently, the cultivation of microalgae in wastewater treatment plants can play a dual role, since it allows the removal of nutrients from effluent and the production of biomass for subsequent production of biofuels. Bioremediation of wastewater is an ecological process and no secondary pollution, since biomass produced is reused and enables the efficient recycling of nutrients. In addition, the cultivation of microalgae using wastewater as culture medium presents numerous advantages, such as: (i) reduced need for aeration; (ii) higher consumption of P than in the biological treatment; and (iii) biofixation capacity of CO_2 by the microalgae. However, there are still some obstacles need to be overcome: the effect of temperature variability, light/dark cycle, competition with the microflora and wastewater chemical composition.

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