

Chapter 10

Microalgal Consortia: From Wastewater Treatment to Bioenergy Production



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Abstract Cultivation of microalgae has been the focus of several research studies worldwide, due to the huge potential of these photosynthetic microorganisms in a wide range of applications, namely environmental and biotechnological ones. Regarding environmental applications, these microorganisms can play an important role in CO₂ uptake and wastewater treatment processes and can be used as raw materials for bioenergy production. However, cultivation of these microorganisms for these applications still faces some problems: (1) it is very difficult to maintain pure cultures of these microorganisms in wastewater treatment processes and (2) bioenergy production process using these microorganisms is still not economically viable. To face these challenges, several studies have reported the use of microalgal consortia. When using microalgal consortia, cooperative interactions can occur, enhancing biomass productivities and therefore nutrients uptake and lipids content. Additionally, these systems tend to be more resistant to environmental conditions' oscillations, facilitating the overall production process. In this study, an overview on the use of microalgal consortia for CO₂ capture, wastewater treatment and bioenergy production is provided, focusing on the interactions that can occur between these microorganisms and how they can improve these environmental applications.

10.1 Introduction

Anthropogenic activities, such as agricultural practices, urbanization and industrialization, as well as fossil fuel-based economies, have contributed to the degradation of air and water quality and to the depletion of fossil fuel resources (O'Neill and Oppenheimer 2002; Aslan and Kapdan 2006; Demirbas 2011). These activities have been responsible for the increase of atmospheric CO₂ concentration in the last

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decades, which has resulted in negative environmental impacts, such as the greenhouse effect and oceans' acidification (O'Neill and Oppenheimer 2002). Additionally, population increase has contributed to an augmented disposal of wastes into natural water resources, resulting in their contamination with nitrogen and phosphorus and in the scarcity of freshwater (Aslan and Kapdan 2006; Rawat et al. 2011; Renuka et al. 2013). Finally, the continuous increase in the world energy demand and the use of non-renewable energy resources, such as fossil fuels, has resulted in the depletion of this energy resource (Ranjan et al. 2010; Demirbas 2011).

In opposition to the negative impacts mainly caused by anthropogenic activities, it becomes urgent for world economies to: (1) reduce CO₂ emissions and provide new strategies to reduce CO₂ concentration in the atmosphere and in flue gas emissions (Pielke 2009); (2) provide new methods to reduce nitrogen and phosphorus concentrations present in discharged effluents and avoid eutrophication (Renuka et al. 2013; Ruiz et al. 2013); and (3) search for different energy supplies that are renewable and environmentally friendly (carbon neutral) (Chisti 2007; Demirbas 2011).

Due to the huge potential of microalgae and cyanobacteria in several applications, especially in environmental ones, such as nutrients uptake and bioenergy production, cultivation of these photosynthetic microorganisms for these purposes has attracted researchers worldwide. Through autotrophy, microalgae also fix CO₂ from the atmosphere or from flue gas emissions, reducing the concentrations of this greenhouse gas in the atmosphere (Allen et al. 2009; Ho et al. 2011; Tang et al. 2011). Since microalgal growth depends on the presence of inorganic forms of nitrogen and phosphorus (microalgal biomass is mainly composed of carbon, nitrogen and phosphorus—macronutrients), these microorganisms can be grown in nitrogen- and phosphorus-rich wastewaters, assimilating these nutrients and reducing their concentration in these effluents (Rawat et al. 2011; Silva-Benavides and Torzillo 2012). In addition to the remediation potential described for these microorganisms, depending on the culturing conditions and on the culture medium used, microalgal biomass can be further applied to human food and animal feed and in the production of drugs, cosmetics, functional food, biofuels and fertilizers (Allen et al. 2009; Brennan and Owende 2010; Parmar et al. 2011; Odjadjare et al. 2015). Besides the wide variety of applications described for microalgae, cultivation of these microorganisms presents other advantages: (1) they present higher growth rates, higher biomass and lipid productivities and higher nutritional values (on a per unit area basis) than other photosynthetic organisms, such as terrestrial crops; (2) they can be grown in non-arable land and require far less land than terrestrial crops, thus not competing with land required for agricultural practices and food production; and (3) they can also grow in a wide variety of environmental conditions and in low-quality waters, reducing the requirements for freshwater and nutrients (Pulz and Gross 2004; Chisti 2007). However, cultivation of these microorganisms still presents some challenges regarding the achievement of high biomass and lipid productivities and high nutrients removal efficiencies at reduced costs.

One possibility to face these challenges includes the use of microalgal consortia (microalgal and microalgal–bacterial), in order to establish an effective system in

terms of CO₂ capture, nutrients removal and bioenergy production. Recently, several studies have reported the potential of these consortia in different applications, including biomass and lipids production, CO₂ capture and nutrients removal (Muñoz and Guieysse 2006; Rawat et al. 2011; Subashchandrabose et al. 2011; Olguín 2012; Unnithan et al. 2014; Ramanan et al. 2016). This study presents an updated review on the use of microalgal consortia for biotechnological applications, such as CO₂ capture, nutrients removal from wastewaters and lipids production for biofuels.

10.2 Applications of Microalgae

Due to the huge taxonomic diversity of microalgae and to their extensive environmental distribution, these photosynthetic microorganisms have numerous applications in diversified areas, such as environment (CO₂ removal and wastewater treatment), energy (biofuels production), pharmaceutical and cosmetics industries, aquaculture, animal feed and human food (Spolaore et al. 2006; Allen et al. 2009; Brennan and Owende 2010; Show et al. 2017; Khan et al. 2018). Through photosynthesis, microalgae are able to assimilate CO₂ from the atmosphere, as well as from flue gas emissions. Thus, microalgae can be applied to mitigate the increasing tendency of atmospheric CO₂ concentration that has been observed since Industrial Revolution (Allen et al. 2009; Ho et al. 2011; Tang et al. 2011; Show et al. 2017; Khan et al. 2018). Additionally, these photosynthetic microorganisms assimilate other compounds, such as nitrogen and phosphorus. These nutrients are frequently found in wastewaters, meaning that microalgae can be a promising alternative in wastewater treatment processes (Rawat et al. 2011; Silva-Benavides and Torzillo 2012; Show et al. 2017; Khan et al. 2018). For human food and animal feed, algal biomass is suitable because they are an important source of natural vitamins, minerals and fatty acids. They can be used to feed different animals, such as cats, dogs, aquarium fish, birds, horses and cows (Hu 2004; Spolaore et al. 2006). Several compounds, such as pigments, antioxidants, β -carotenes, proteins, polysaccharides, triglycerides, fatty acids and vitamins, can be extracted and used as raw materials for the production of cosmetics, drugs and functional food (Hu 2004; Singh et al. 2005; Bhalamurugan et al. 2018). Finally, the fatty acids produced by microalgae can be extracted and used for biodiesel production and residual biomass can be fermented to produce ethanol or methane and fertilizers (Brennan and Owende 2010; John et al. 2011; Parmar et al. 2011). Figure 10.1 presents a schematic representation of the main applications described for microalgae. Although microalgae can be used in all these applications, only a few of them are currently applied at a commercial scale (Table 10.1). This is a result of the high costs associated with microalgal biomass production, which limits microalgal biomass applications to the commercialization of high-valued products.

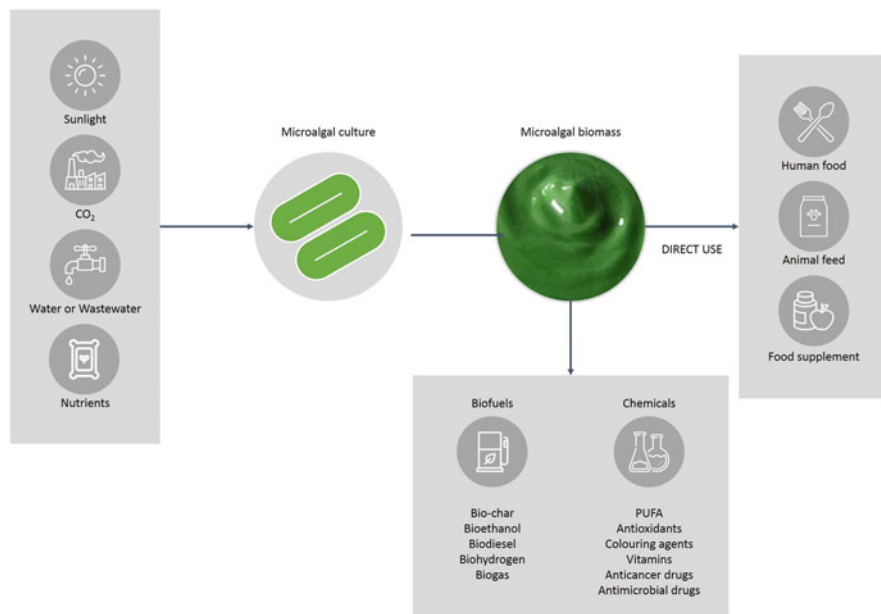


Fig. 10.1 Main applications described for microalgae [adapted from Khan et al. (2018)]

Among the applications described for microalgae, this study focuses on the use of microalgae and microalgal consortia for CO₂ capture, nutrients removal from wastewaters and bioenergy production.

10.2.1 CO₂ Capture

Carbon is the most important element for microalgal growth, followed by nitrogen and phosphorus (microalgal biomass contains approximately 50% w/w of carbon, which is all derived from CO₂). Accordingly, the production of 1 g of microalgal biomass corresponds to a CO₂ fixation of approximately 1.83 g, which means that these microorganisms can be effectively applied in CO₂ capture (Cheah et al. 2015).

Microalgae can fix CO₂ from both the atmosphere or flue gas emissions. Table 10.2 presents CO₂ uptake rates determined for different microalgae using both CO₂ sources. The use of atmospheric CO₂ allows higher flexibility when selecting the location of the microalgal facility, since it does not need to be located close to a CO₂ emission source and it does not require CO₂ transporting systems (Cheah et al. 2015; Moreira and Pires 2016). Several studies have reported the use of microalgae in CO₂ capture from the atmosphere (Cheng et al. 2006; López et al. 2009; Hulatt and Thomas 2011; Arbib et al. 2014). In the study performed by López et al. (2009), the growth of the cyanobacterium *Anabaena* sp. in bubble-column

Table 10.1 Current status of large-scale applications of microalgae

Microalgae	Applications	Country	Status
<i>Aphanizomenon flos-aquae</i>	Human nutrition	USA	Commercial
<i>Chaetoceros muelleri</i>	Aquaculture feed	Global	Commercial
<i>Chlorella</i> spp.	Human nutrition, aquaculture feed, cosmetics	Japan, Taiwan, Czech Republic, Germany	Commercial
<i>Chlorella vulgaris</i>	Human nutrition, aquaculture feed, animal feed, cosmetics	Portugal, USA, Japan, Taiwan, Czech Republic, Germany	Commercial
<i>Cryptocodinium cohnii</i>	Docosahexaenoic acid	USA	Commercial
<i>Dunaliella salina</i>	Human nutrition, aquaculture feed, β -carotene	Australia, Israel, China, India, USA	Commercial
<i>Dunaliella tertiolecta</i>	Aquaculture feed	Global	Commercial
<i>Haematococcus pluvialis</i>	Aquaculture feed, astaxanthin	USA, Sweden, Israel	Commercial
<i>Isochrysis</i> spp.	Aquaculture feed	Global	Commercial
<i>Monochrysis lutheri</i>	Aquaculture feed	Global	Commercial
<i>Nannochloropsis oculata</i>	Lipids, PUFA	Portugal, USA, Spain, Germany, Belgium, Italy	Commercial
<i>Nannochloropsis</i> spp.	Aquaculture feed	Global	Commercial
<i>Pavlova</i> spp.	Aquaculture feed	Global	Commercial
<i>Shizochytrium</i> sp.	Docosahexaenoic acid	USA	Commercial
<i>Skeletonema</i> spp.	Aquaculture feed	Global	Commercial
<i>Spirulina platensis</i>	Human and animal nutrition, cosmetics, phycobiliproteins	Thailand, USA, China, India, Vietnam, Japan	Commercial
<i>Tetraselmis suecica</i>	Aquaculture feed	Global	Commercial
<i>Thalassiosira pseudonana</i>	Aquaculture feed	Global	Commercial

Adapted from Spolaore et al. (2006), Paul et al. (2013), Zhu (2015)

PBRs (working volume of 1.8 L), using atmospheric air, has resulted in a CO₂ fixation rate of 1.45×10^3 mg CO₂ L⁻¹ day⁻¹. Arbib et al. (2014) have evaluated the potential of three microalgal species (*Chlorella kessleri*, *Chlorella vulgaris* and *Scenedesmus obliquus*) in CO₂ capture from the atmosphere using 2-L flasks as PBRs. After 10 days of culturing, CO₂ fixation rates determined for the studied microorganisms were 320, 297 and 418 mg CO₂ L⁻¹ day⁻¹, respectively. However, atmospheric CO₂ concentration (approximately 0.04% v/v) can be limiting to microalgal growth, due to low mass transfer rate of CO₂ from the gaseous stream to the liquid medium. According to McGinn et al. (2011), CO₂ diffuses into the liquid medium 10⁴ times slower than through the gaseous medium. Accordingly,

Table 10.2 Application of microalgae in CO₂ capture from the atmosphere and flue gases and respective fixation rates

Microorganisms	CO ₂ source	% CO ₂ (v/v)	System and operation mode	Time (days)	CO ₂ fixation rate (mg CO ₂ L ⁻¹ day ⁻¹)	References
<i>Anabaena</i> sp.	Atmospheric air	≈0.04	Closed suspended system, continuous mode; V = 1.8 L	–	1.45 × 10 ³	López et al. (2009)
<i>Anabaena</i> sp.	Atmospheric air + pure CO ₂	10	Closed suspended system, batch mode; V = 5 L	4	1.01 × 10 ³	Chiang et al. (2011)
<i>Botryococcus braunii</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V = 8 L	15	497	Sydney et al. (2010)
<i>Chlorella</i> sp.	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V = 0.6 L	7	583	Ryu et al. (2009)
<i>Chlorella kessleri</i>	Atmospheric air	≈0.04	Closed suspended system, batch mode; V = 2 L	10	320	Arbib et al. (2014)
<i>Chlorella sorokiniana</i>	Flue gas	15.6	Closed suspended system, batch mode; V = 1.4 L	8	124	Kumar et al. (2014)
<i>Chlorella vulgaris</i>	Atmospheric air	≈0.04	Closed suspended system, batch mode; V = 2 L	10	297	Arbib et al. (2014)
<i>Chlorella vulgaris</i>	Atmospheric air + pure CO ₂	2	Closed suspended system, batch mode; V = 1 L	10	430	Yeh and Chang (2011)
<i>Chlorella vulgaris</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V = 8 L	15	252	Sydney et al. (2010)
<i>Chlorella vulgaris</i>	Atmospheric air + pure CO ₂	6.5	Closed suspended system, batch mode; V = 0.09 L	7	2.22 × 10 ³	Anjos et al. (2013)
<i>Dunaliella tertiolecta</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V = 8 L	15	272	Sydney et al. (2010)
<i>Scenedesmus obliquus</i>	Atmospheric air	≈0.04	Closed suspended system, batch mode; V = 2 L	10	437	Arbib et al. (2014)

<i>Scenedesmus obliquus</i>	Atmospheric air + pure CO ₂	10	Closed suspended system, batch mode, V = 1 L	12	550	Ho et al. (2010)
<i>Spirulina platensis</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V = 8 L	15	318	Sydney et al. (2010)
Microalgal consortium obtained from a river	Atmospheric air	≈0.04	Closed suspended system, batch mode, V = 2 L	10	418	Arbib et al. (2014)

V Working volume

costly CO₂ sparging might be required to increase the retention time of CO₂ in the culture medium. As an alternative to the use of pure CO₂ to feed microalgal cultures, several authors have reported the use of flue gases. CO₂ concentration in flue gases typically ranges between 6 and 15% (v/v). For this reason, several authors have evaluated the effect of different CO₂ concentrations on microalgal growth and CO₂ uptake. For example, in the study performed by Morais and Costa (2007a), the effect of CO₂ concentrations of 0, 6 and 12% (v/v) on biomass productivities and CO₂ fixation rates of *Spirulina* sp. and *S. obliquus* was evaluated. In this study, the authors have reported higher biomass productivities, specific growth rates and CO₂ fixation rates in cultures performed at 6 and 12% (v/v) of CO₂, with maximum values obtained at 6% (v/v). As a result, current microalgal production plants are being projected near large CO₂ emission sources, such as power plants and refineries (Moreira and Pires 2016). However, flue gases usually contain large amounts of nitrogen oxides (NO_x) and sulphur oxides (SO_x), which can significantly reduce the pH of the culture medium, thus inhibiting microalgal growth (Pires et al. 2011; Cheah et al. 2015; Moreira and Pires 2016). In the case of NO_x, these compounds can be used by microalgae as nitrogen source, thus not presenting such a negative impact for microalgal growth. On the other hand, high SO₂ concentrations (between 100 and 250 mg L⁻¹) can be harmful to microalgae, due to the formation of bisulphite (HSO₃⁻), sulphite (SO₃²⁻) and sulphate (SO₄²⁻), which drastically decrease the pH of the culture medium to values between 2.5 and 3.5 (Lam et al. 2012; Cheah et al. 2015). Additionally, high CO₂ concentrations (between 10 and 20% v/v) also contribute to a decrease in pH to approximately 5.5. Although some microalgal species are able to perform photosynthesis in these conditions, thus counterbalancing the pH decrease due to high CO₂ levels, other species are unable to withstand this acidic environment (Cheah et al. 2015). The presence of particulate matter and high temperatures are two additional characteristics of flue gases that should be taken into account when using these gases for microalgal growth. Particulate material resulting from combustion processes presents a quite variable composition, depending on the emission sources. These materials can be divided in aerosols, fly ash and soot, and their effect on microalgal growth was already assessed by few authors (Costa et al. 2017). For example, in the study performed by Vaz et al. (2016), the effect of flue gas ashes on the growth of *Spirulina* sp. LEB 18 and *Chlorella fusca* LEB 111 was evaluated. In this study, the authors have concluded that addition of 40 ppm ashes from coal burning has not inhibited the growth of these microorganisms and that this particulate matter can act as a source of trace elements for microalgal metabolism. Regarding temperature, flue gases from power plants can reach temperatures up to 120 °C, which limits its application for microalgal growth to thermophilic microalgal strains or requires a previous cooling step, which is energy-demanding and costly (Costa et al. 2017).

Due to these constraints, only a few studies have reported the use of real flue gases for microalgal growth. When growing *Chlorella* sp. in an outdoor open thin-layer PBR using a flue gas containing 6–8% (v/v) of CO₂, Doucha et al. (2005) have reported CO₂ removal efficiencies between 10 and 50%. A CO₂ removal efficiency

of 40.2% was obtained by Li et al. (2011) when growing *S. obliquus* in a flue gas containing 6–18% (v/v) of CO₂. Furthermore, some authors have referred that native species isolated near thermal power plants are the best candidates for CO₂ capture from these gaseous streams (Morais and Costa 2007b; Radmann et al. 2011).

10.2.2 *Nutrients Removal from Wastewaters*

As mentioned above, the main nutrients required for microalgal growth are carbon, nitrogen and phosphorus. Regarding carbon uptake, although microalgae are mainly autotrophic, some microalgae are heterotrophic, using only organic carbon (e.g. acetate, glucose, glycerol and ethanol) as carbon source, whereas others are mixotrophic, using facultatively an organic carbon source in addition to CO₂. In the case of nitrogen, eukaryotic microalgae are able to assimilate fixed nitrogen, such as ammonium-nitrogen (NH₄-N), nitrate-nitrogen (NO₃-N) and nitrite-nitrogen (NO₂-N). Finally, phosphorus is assimilated by microalgae in the forms of H₂PO₄⁻ and HPO₄²⁻ (Gonçalves et al. 2017).

Due to the high concentrations of these nutrients in wastewaters from different sources and to the need of reducing microalgal production costs and environmental impact (reduce the requirements for freshwater), several studies have reported the use of wastewaters for microalgal growth. In addition, the use of microalgae to remove nitrogen and phosphorus from wastewaters is seen as a viable alternative to overcome the drawbacks associated with currently used methods. Nitrogen and phosphorus present in wastewaters are mainly removed in the tertiary treatment phase and the most commonly used methods include biological processes, such as anaerobic digestion followed by nitrification and denitrification (Queiroz et al. 2007; Renuka et al. 2013). However, these methods require several tanks and internal recycles of activated sludge, resulting in an overall increase of process costs, complexity and energy input (Foess et al. 1998; Jeyanayagam 2005; Larsdotter 2006; Singh and Thomas 2012). Alternatively, nitrogen and phosphorus removal may be achieved by chemical methods, such as precipitation using aluminium and iron salts. However, these methods are costly and produce large amounts of sludge contaminated with chemical compounds that require further treatment (Wang et al. 2006). The use of microalgae for nutrients removal presents several advantages over those commonly applied in the tertiary treatment step because: (1) nitrogen and phosphorus assimilated by microalgae can be recycled by the production of fertilizers from microalgal biomass; (2) the resulting biomass can be used for the production of bioenergy, food, animal feed and pharmaceuticals; and (3) an oxygenated effluent is discharged into the water bodies (Aslan and Kapdan 2006; Rawat et al. 2011; Renuka et al. 2013).

Domestic, leachate, agricultural, refinery and industrial wastewaters are examples of wastewater sources that have already been used for microalgal growth (Chojnacka

et al. 2004; Safonova et al. 2004; Mustafa et al. 2012; Hernández et al. 2013; Posadas et al. 2013). Table 10.3 presents nitrogen and phosphorus removal rates determined when culturing microalgae in different wastewaters.

Although the majority of the studies refer to the use of suspended-growth systems, Shi et al. (2007) have assessed nitrogen and phosphorus removal from a municipal wastewater collected in Cologne (Germany) using an immobilization method—the twin-layer system. In this method, the microalgae *C. vulgaris* and *Scenedesmus rubescens* were immobilized through self-adhesion on a substrate layer and another layer provided the growth medium required for microalgal growth. Using this system, microalgae remained 100% immobilized, being able to completely remove $\text{NO}_3\text{-N}$ (initial concentration between 3.7 and 6.2 mg N L^{-1}) after an exposure period of 4 days. More recently, Gouveia et al. (2016) have cultured *C. vulgaris*, *S. obliquus* and a native consortium in a municipal wastewater collected from Figueira da Foz (Portugal), aiming to determine the best candidate in terms of wastewater remediation, biomass productivity and quality for further uses, such as biofuels, biofertilizers and bioplastics production. The studied cultures have effectively removed nitrogen and phosphorus from the wastewater, reaching nitrogen removal efficiencies of 84–98% and phosphorus removal efficiencies of 95–100%. Taking into account these results, the authors have proposed the native consortium as the best option for nutrients removal and biomass production.

Valderrama et al. (2002) have cultured *C. vulgaris* in an industrial effluent resulting from ethanol and citric acid production, achieving $\text{NH}_4\text{-N}$ and phosphate-phosphorus ($\text{PO}_4\text{-P}$) removal efficiencies of 71.6 and 28%, respectively (initial $\text{NH}_4\text{-N}$ concentration in this effluent ranged between 3 and 8 mg N L^{-1} , whereas initial $\text{PO}_4\text{-P}$ concentration ranged between 0 and 0.36 mg P L^{-1}). Similarly, Lim et al. (2010) have grown *C. vulgaris* in high rate algal ponds (HRAPs) fed with a textile industry wastewater to evaluate the potential of this microalga in nitrogen and phosphorus removal. Although *C. vulgaris* was able to grow in the textile wastewater ($\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ initial concentrations of 6.50 mg L^{-1} and 7.14 mg L^{-1} , respectively), nitrogen and phosphorus removal efficiencies achieved were not very high: 44.4–45.1% and 33.1–33.3%, respectively.

In the study performed by Hernández et al. (2013), *Chlorella sorokiniana* was grown in a potato-processing wastewater presenting an initial $\text{NH}_4\text{-N}$ concentration of 12.1 mg N L^{-1} and $\text{PO}_4\text{-P}$ concentration of 3.4 mg P L^{-1} . After a cultivation period of 10 days, nitrogen and phosphorus removal efficiencies achieved were 95 and 80.7%, respectively. Liu et al. (2016) have determined the remediation potential of the filamentous microalgae *Klebsormidium* sp. and *Stigeoclonium* spp. grown in an outdoor Algal Turf Scrubber using horticultural wastewater as culture medium ($\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ initial concentrations of 47.2 mg L^{-1} and 11.6 mg L^{-1} , respectively). With this study, the authors have demonstrated that these microalgae can effectively remove nitrogen and phosphorus from this wastewater, since nitrogen removal efficiencies achieved oscillated between 88 and 99% and phosphorus removal efficiencies were higher than 99%.

The use of microalgae for the remediation of different anaerobically digested effluents has also been reported in the literature. For example, Wilkie and Mulbry

Table 10.3 Application of microalgae in nitrogen and phosphorus removal from different wastewaters and respective removal efficiencies

Microorganisms	Waste stream	System and operation mode	Removal time (days)	Nitrogen		Phosphorus		References
				S_i (mg N L ⁻¹)	R (%)	S_i (mg P L ⁻¹)	R (%)	
<i>Chlorella vulgaris</i> / <i>Scenedesmus rubescens</i>	Municipal wastewater	Immobilized system, continuous mode, V = 2 L	4	3.7–6.2 NO ₃ -N	≈100	0.4–0.7 TP	n.a.	Shi et al. (2007)
<i>Chlamydomonas reinhardtii</i>	Municipal wastewater	Closed suspended system, batch mode, V = 2 L	10	128.6 TN	33–43	120.6 TP	10–13	Kong et al. (2009)
<i>Chlorella vulgaris</i> / <i>Scenedesmus obliquus</i>	Municipal wastewater	Closed suspended system, semi-continuous mode, V = 150 L	13	119.3–346.6 NH ₄ -N	84–95	4.6–8.3 TP	92–95	Gouveia et al. (2016)
<i>Chlorella vulgaris</i>	Ethanol and citric acid industry wastewater	Closed suspended system, batch mode, V = 16.5 L	6	3–8 NH ₄ -N	71.6	1.5–3.5 PO ₄ -P	28	Valderrama et al. (2002)
<i>Chlorella vulgaris</i>	Textile industry wastewater	Open suspended system, batch mode, V = 36 L	10	6.50 NH ₄ -N	44.4–45.1	7.14 PO ₄ -P	33.1–33.3	Lim et al. (2010)
<i>Chlorella sorokiniana</i>	Potato-processing wastewater	Closed suspended system, batch mode, V = 5 L	30	12.1 NH ₄ -N	>95	3.4 PO ₄ -P	80.7	Hernández et al. (2013)
<i>Klebsormidium</i> sp./ <i>Stigeoclonium</i> spp.	Horticultural wastewater	Immobilized system, semi-continuous mode, V = 65 L	9	47.2 NO ₃ -N	≈100	11.6 PO ₄ -P	70–84	Liu et al. (2016)
<i>Chlorella zofingensis</i>	Piggery wastewater	Closed suspended system, batch 23 mode, V = 1.37 L	10	148.0 TN	78.72	156.0 TP	85.00	Zhu et al. (2013)
Native microalgae	Anaerobically digested dairy industry wastewater	Immobilized system, semi-continuous mode, V = 75 L	23	78 TN	62	7 TP	70	Wilkie and Mulbry (2002)
<i>Spirulina</i> sp.	Anaerobically digested piggery wastewater	Open suspended system, semi-continuous mode	6–7	1209–1481 NH ₄ -N	84–96	164–620 PO ₄ -P	72–87	Olguín et al. (2003)

Note: n.a. not applicable, NH₄-N ammonium-nitrogen, NO₃-N nitrate-nitrogen, PO₄-P phosphate-phosphorus, R removal efficiency (in %), S_i initial concentration (in mg L⁻¹), TN total nitrogen, TP total phosphorus, V working volume

(2002) have evaluated nutrients recovery in an anaerobically digested dairy industry effluent using native microalgae, reporting total nitrogen (TN) and total phosphorus (TP) removal efficiencies of 62 and 70%, respectively (initial TN and TP concentrations were 78 mg L⁻¹ and 7 mg L⁻¹, respectively). On the other hand, Olguín et al. (2003) and Ledda et al. (2015) have focused on nutrients removal and biomass production in anaerobically digested piggery wastewaters. In the study performed by Olguín et al. (2003), cultivation of *Spirulina* sp. in outdoor conditions and semi-continuous mode has resulted in biomass productivities between 11.8 and 15.1 g m⁻² day⁻¹. In the same conditions, NH₄-N removal efficiencies ranged between 84 and 96% and PO₄-P removal efficiencies ranged between 72 and 87% (initial NH₄-N and PO₄-P concentrations were about 1209–1481 mg L⁻¹ and 164–620 mg L⁻¹, respectively). Similarly, Ledda et al. (2015) have grown *Chlorella* sp. in an anaerobically digested piggery wastewater (with NH₄-N and PO₄-P initial concentrations of 60 mg L⁻¹ and 18 mg L⁻¹, respectively), reporting biomass productivities of 0.10 g L⁻¹ day⁻¹ and nitrogen and phosphorus removal efficiencies of 95 and 85%, respectively.

Although microalgae have been successfully applied in nutrients removal from different wastewaters, its application on an industrial scale presents some challenges, especially regarding contaminations control. For this, the following options must be taken into account: (1) selection of fast-growing and highly resistant microalgae, such as *Chlorella* or *Scenedesmus* and (2) manipulation of the operational conditions, such as hydraulic residence times and recirculation of biomass to sustain specific microalgal populations (Benemann et al. 1980; Wood 1987; Muñoz and Guieysse 2006).

10.2.3 Bioenergy Production

Microalgae have been proposed as a potential renewable source of fuel, replacing the first- and second-generation feedstocks (e.g. food, oil crops and lignocellulosic residues). In an industrial scale, the production of biofuels using microalgae is particularly interesting because: (1) it does not compete for land with agricultural crops (thus not interfering with food production); (2) it does not require arable or fertile land for cultivation; (3) microalgae present high biomass productivities and high growth rates; and (4) microalgae have higher lipid contents and productivities compared to terrestrial crops (Rawat et al. 2013; Odjadjare et al. 2015; Khan et al. 2018; Mathimani et al. 2019). In fact, microalgae can provide feedstock for several different types of biofuels including biodiesel, bio-oil, biosyngas and biohydrogen. Nowadays, the most attractive application and the one with higher research efforts is biodiesel, since microalgae have the potential to completely displace fossil diesel (Chisti 2007). The average oil content in microalgae varies from 20 to 50% (Table 10.4), but 85% can be achieved with some species (Chisti 2007; Rawat et al. 2013). Different microalgal species produce many different types of lipids and, although the majority are suitable for biodiesel production, others are not

Table 10.4 Oil content of different microalgae

Microalgae	Oil content (% dry wt.)	References
<i>Botryococcus braunii</i>	25–86	Chisti (2007), Dayananda et al. (2007)
<i>Chlorella protothecoides</i>	55	Xu et al. (2006)
<i>Chlorella</i> sp.	28–35	Chisti (2007), Andruleviciute et al. (2014)
<i>Cryptocodinium cohnii</i>	20	Chisti (2007)
<i>Cylindrotheca</i> sp.	16–37	Chisti (2007)
<i>Dunaliella primolecta</i>	23	Chisti (2007)
<i>Haematococcus</i> sp.	23	Andruleviciute et al. (2014)
<i>Isochrysis</i> sp.	25–33	Chisti (2007)
<i>Nannochloris</i> sp.	20–35	Chisti (2007)
<i>Nannochloropsis</i> sp.	31–68	Chisti (2007)
<i>Neochloris oleoabundans</i>	29–65	Maity et al. (2014)
<i>Nitzschia</i> sp.	45–47	Chisti (2007)
<i>Phaeodactylum tricornutum</i>	20–30	Chisti (2007)
<i>Scenedesmus</i> sp.	67	Andruleviciute et al. (2014)
<i>Schizochytrium</i> sp.	50–77	Chisti (2007)
<i>Tetraselmis suecica</i>	15–23	Chisti (2007)

satisfactory. *Chlorella* sp., *Chlorococcum* sp., *Haematococcus pluvialis* and *Neochloris oleoabundans* were considered a good option for biodiesel production (Maity et al. 2014).

The production of biodiesel requires the extraction of the lipids and fatty acids from the microalgal biomass. This step is usually done in lyophilized biomass using a solvent, such as hexane, ethanol (96%), or a mixture of the previous ones. Then, the algal oil is submitted to a process known as transesterification (Ogdjare et al. 2015; Show et al. 2017; Bhalamurugan et al. 2018). This process is a multiple step reaction where the triglycerides react with alcohol (usually methanol) in the presence of a catalyst, producing fatty acid methyl esters (FAME) or biodiesel and glycerol (Chisti 2007). The resulting biodiesel has very similar characteristics to the conventional diesel fuel (e.g. flash point, kinematic viscosity and higher heating value—HHV) (Meher et al. 2006; Raheem et al. 2018). Miao and Wu (2006) evaluated the effect of the catalyst concentration, the molar ratio methanol:oil and temperature on the quality of the biodiesel produced from the microalga *Chlorella protothecoides*. Microalgal cultivation was performed at 26 °C under a continuous illumination intensity of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The aeration was provided through the injection of air bubbles. *C. protothecoides* was cultivated under autotrophic and heterotrophic conditions, with the latter using glucose as a substrate. The experiments used sulphuric acid (H_2SO_4) as a catalyst and different concentrations were tested (25, 50, 60 and 100%). Additionally, different methanol:oil molar ratios (25:1, 30:1, 45:1, 56:1, 70:1 and 84:1) and different temperatures (30, 50 and 90 °C) were tested. The authors concluded that heterotrophic growth of *C. protothecoides* resulted in higher lipid content (55.2%) and that higher biodiesel quality can be obtained in the presence of

100% catalyst at the highest evaluated temperature (90 °C). On the economic point of view, the authors concluded that the best factor combination was a catalyst concentration of 100% with molar ratio of 56:1 at 30 °C. Xu et al. (2006) evaluated the potential to produce high-quality biodiesel from the microalga *C. protothecoides*. The microalga was heterotrophically cultivated in a 5-L fermenter tank where corn powder hydrolysate was used as substrate. The cultivation temperature was controlled at 28 °C and the aeration rate and the agitation speed were set at 0.5 volume per volume liquid per minute (vvm) and 300 rotations per minute (rpm), respectively. The biodiesel was obtained through acidic transesterification with 56:1 molar ratio of methanol:oil at 30 °C. The authors obtained a lipid content of 46.1% and concluded that the properties of the biodiesel from microalgal oil were comparable to the ones of diesel fuel, with a HHV of 41 MJ kg⁻¹, a density of 0.864 kg L⁻¹ and a viscosity of 5.2 × 10⁻⁴ Pa s (at 40 °C). Johnson and Wen (2009) evaluated the potential of producing a high-quality biodiesel from the microalga *Schizochytrium limacinum*. The biodiesel was produced through two methods: (1) oil solvent extraction followed by transesterification; and (2) direct transesterification. For the transesterification, a mixture of methanol (3.4 mL), H₂SO₄ (0.6 mL) and a solvent (chloroform, hexane or petroleum ether—4.0 mL) was heated at 90 °C for 40 min. For each process, wet and dry biomass was used as feedstock. The authors concluded that when the dry biomass was used, the two-stage process led to a biodiesel yield of 57% and a FAME content of 66.4%. The one-stage process resulted in higher biodiesel yield; however, higher FAME content (63.5%) was only obtained when chloroform was used as a solvent. The authors concluded that the direct transesterification using dry biomass of the microalga *S. limanicum* is suitable to produce good-quality biodiesel.

10.3 Interactions and Benefits of Using Microalgal Consortia

Although microalgae have been successfully applied in several environmental applications, maintaining microalgal monocultures can be a hard task to achieve (Padmaperuma et al. 2018). To overcome this problem, several studies have exploited the potential of microalgal consortia in these applications, reporting several advantages over single-species cultures (Wilkie and Mulbry 2002; Muñoz and Guieysse 2006; González-Fernández and Ballesteros 2012; Subashchandrabose et al. 2011; He et al. 2013). These cultures can result in the development of robust systems able to resist to stress conditions, thus promoting effective degradation processes and improved biomass and bioenergy productivities (Paerl and Pinckney 1996; Subashchandrabose et al. 2011; Gonçalves et al. 2017; Nath et al. 2017a).

Microalgal consortia can naturally occur in the environment or can be artificially engineered/ designed for a specific application (Jagmann and Philipp 2014; Padmaperuma et al. 2018). These consortia can be constituted exclusively by photosynthetic microorganisms (microalgal consortia) or by photosynthetic microorganisms and heterotrophic bacteria (microalgal–bacterial consortia) (Gonçalves et al.

2017). The following sections describe the main interactions established in both types of consortia (microalgal and microalgal–bacterial), with emphasis on how these interactions can improve CO₂ capture, nutrients removal and bioenergy production.

10.3.1 *Microalgal Consortia*

Interactions between photosynthetic microorganisms are not well documented in the literature (Qin et al. 2016). However, it is thought that both cooperative and competitive interactions can occur in these consortia (Nath et al. 2017a). Regarding cooperative interactions, metabolites' exchange between the microorganisms integrating the consortium is the most common and can be very advantageous in biomass production and hence, nutrients uptake and bioenergy production (Mendes and Vermelho 2013). As competitive interactions, several studies have referred the excretion of metabolites, also known as allelochemicals, that exhibit a negative effect towards the co-cultivated microorganisms (Cembella 2003; Gross 2003; Mendes and Vermelho 2013). For example, when growing a microalgal consortium composed of *C. vulgaris* and *Pseudokirchneriella subcapitata*, Fergola et al. (2007) have reported that *C. vulgaris* excreted a fatty acids mixture (also known as chlorellin), which was responsible for the inhibition of *P. subcapitata* growth. Allelochemicals production can be influenced by both abiotic and biotic factors. Regarding abiotic factors, nutrients starvation, low light intensities and temperatures and high pH values promote the excretion of these secondary metabolites. The influence of biotic factors is related to the concentration/predominance of microalgal species that produce these toxic compounds in the consortium (Mendes and Vermelho 2013).

In CO₂ capture, wastewater treatment and bioenergy production processes, interactions between photosynthetic microorganisms can have the following advantages: (1) enhancement of the overall biomass productivities and lipids production; (2) enhancement of the overall nutrients uptake, providing that sufficient nutrients are supplied; (3) resistance to contaminants and predators through the induction of allelochemicals production; and (4) the development of a settleable system (by combining single-cell microorganisms with flocculating ones), thus avoiding the requirements for a harvesting method and reducing biomass recovery costs (Gonçalves et al. 2017; Nath et al. 2017a, b). Additionally, the use of microalgal consortia for these purposes ensures the viability of a remediation process because the loss of one microorganism can be compensated by the other microorganisms integrating the consortia (Renuka et al. 2013).

10.3.2 *Microalgal–Bacterial Consortia*

Regarding microalgal–bacterial consortia, several authors have reported that both cooperative and competitive interactions can occur (Muñoz and Guieysse 2006;

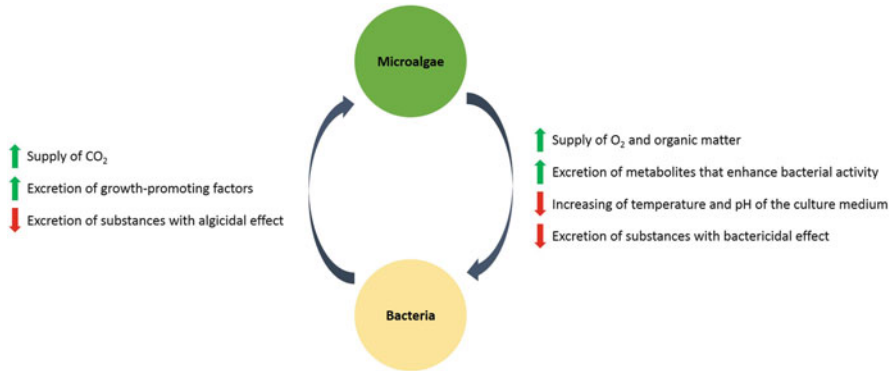


Fig. 10.2 Schematic representation of the cooperative and competitive interactions that can occur in microalgal–bacterial consortia [adapted from Gonçalves et al. (2017)]

Natrah et al. 2014; Unnithan et al. 2014; Solimeno and García 2017). Figure 10.2 presents the main cooperative and competitive interactions already described for microalgal–bacterial consortia.

In competitive interactions, both microalgae and bacteria can have adverse effects on each other: microalgae can excrete secondary metabolites presenting bactericidal effect (Pratt et al. 1944; Kellam and Walker 1989; Najdenski et al. 2013; Natrah et al. 2014) and bacteria, in turn, can excrete metabolites with algicidal activity (Natrah et al. 2014). For example, chlorellin produced by *Chlorella* presents bactericidal activity against Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Pratt et al. 1944). Besides metabolites' excretion with antibacterial activity, the increase in pH due to photosynthetic growth inhibits bacterial growth (Muñoz and Guieysse 2006; Unnithan et al. 2014; Gonçalves et al. 2017).

As cooperative interactions between microalgae and bacteria, it is possible to refer nutrients' exchange between these microorganisms: (1) microalgae supply bacteria with organic compounds that can be used as carbon and energy source and with O₂, which is required for the oxidation of organic matter; and (2) bacteria provide microalgae with the CO₂ required for photosynthetic activity (Paerl and Pinckney 1996; Bordel et al. 2009; Godos et al. 2009; Fouilland 2012; Nath et al. 2017a). Besides cooperative interactions through nutrients' exchange, other symbiotic relationships can occur between these microorganisms. Microalgae can enhance bacterial growth through the release of extracellular metabolites, such as extracellular polymeric substances, and can serve as a habitat for bacteria, protecting them from adverse environmental conditions (Unnithan et al. 2014). Mandal et al. (2011) have demonstrated that extracellular polymeric substances produced by the microalga *Amphidinium carterae* have stimulated the growth of the bacterium *Bacillus pumilus*. On the other hand, bacteria can excrete growth-promoting factors, such as vitamins and siderophores (chelating agents for microalgal growth under iron-limiting conditions), which can promote microalgal growth (Subashchandrabose et al. 2011;

Gonçalves et al. 2017). For example, in the study performed by De-Bashan et al. (2004), co-cultivation of *Azospirillum brasilense* with *C. vulgaris* and *C. sorokiniana* in alginate beads enhanced microalgal growth and improved nitrogen and phosphorus removal from a municipal wastewater used as culture medium.

Apart from being effective in nutrients removal, these systems can further improve current wastewater treatment processes because (1) the costs associated with the oxygenation of activated sludge tanks can be significantly reduced and (2) the greenhouse effects associated with wastewater treatment plants can be considered negligible, since the CO₂ released by bacteria is converted into organic matter by microalgae (Godos et al. 2009; Quijano et al. 2017). Due to the symbiotic interactions that can occur and consequent increase in biomass productivities, the use of these consortia can also improve fatty acids productivities and, hence, bioenergy production.

10.4 Applications of Microalgal Consortia

10.4.1 CO₂ Capture

Although microalgal consortia have been mostly applied in nutrients removal processes, some authors have also reported their application in CO₂ capture. Boonma et al. (2014) have cultivated a microalgal consortium (composed of 65.7% *Scenedesmus* spp., 25.4% *Micractinium* sp., 3.6% *Dictyosphaerium* sp., 2.7% *Pseudanabaena* sp., 0.8% *Monoraphidium* sp., 1% *Chlamydomonas* sp., 0.4% *Chlorella* sp. and 0.4% *Euglena* sp.) using different CO₂ concentrations (0.04, 10 and 30% v/v), achieving the highest CO₂ fixation rates in the cultures supplemented with 30% (v/v) CO₂: 0.0271 g CO₂ L⁻¹ day⁻¹. When growing a *Chlorella* sp./*Scenedesmus* sp. consortium in a primary-treated municipal wastewater, Koreivienė et al. (2014) have demonstrated an accumulation of CO₂ in microalgal biomass ranging between 0.65 and 1.37 g CO₂ L⁻¹ day⁻¹. Bhakta et al. (2015) aimed to isolate a highly CO₂-tolerant microalgal consortium and evaluate its potential on CO₂ fixation. The isolated consortium was mainly composed of *Chlorella* sp., *Scenedesmus* sp., *Sphaerocystis* sp. and *Spirulina* sp. and it was able to grow in CO₂ concentrations up to 50% (v/v). CO₂ removal efficiencies of the isolated consortium oscillated between 53 and 100%, which corresponds to 150–291 mg g⁻¹. Besides being effective in CO₂ uptake, this consortium was also effective in nutrients (nitrogen and phosphorus) removal and lipids production. Nath et al. (2017b) evaluated biomass productivities and carbonic anhydrase activity (the enzyme responsible for the active transport of CO₂ into microalgal cells) of two microalgal consortia (A and B) grown in different culture media: BG-11 medium and Zarrouk medium. Consortium A was composed of *Synechococcus* PCC 7942, *Chlorella* sp., *Nostoc muscorum*, *Oscillatoria* sp. and *Spirulina platensis*, whereas consortium B was composed of *Synechocystis* PCC6803, *Scenedesmus dimorphus*, *Anabaena cylindrica*, *Lynghya* sp. and *S. platensis*. When comparing microalgal

biomass productivities obtained in these consortia with biomass productivities obtained for monocultures of each microorganism present in the consortia, the authors have concluded that microalgal growth in the consortia was higher. Additionally, the authors have determined higher carbonic anhydrase activity in microalgal consortia, which results in a better CO₂ fixation in these cultures.

10.4.2 Nutrients Removal (Wastewater Polishing)

The use of microalgal consortia for wastewater polishing (nitrogen and phosphorus removal) using wastewaters from different sources is well documented in the literature. When using a native microalgal consortium from a carpet mill industry effluent, Chinnasamy et al. (2010) have reported an almost complete removal of NO₃-N and PO₄-P, with removal efficiencies ranging between 96.6 and 99.8%. In the study performed by Koreivienė et al. (2014), a non-native consortium composed of *Chlorella* sp. and *Scenedesmus* sp. was effectively applied in nitrogen and phosphorus removal from a primary-treated municipal wastewater. In this study, the authors have reported TN and TP removal efficiencies ranging between 88.6–96.4% and 99.7–99.9%, respectively. Gonçalves et al. (2016a) have evaluated nitrogen and phosphorus removal efficiencies of three artificially designed microalgal consortia: (1) *Synechocystis salina* + *C. vulgaris*; (2) *S. salina* + *P. subcapitata*; and (3) *S. salina* + *Microcystis aeruginosa*. In this study, the authors have grown single and dual-species cultures using a synthetic medium containing initial NO₃-N and PO₄-P concentrations of approximately 45 mg N L⁻¹ and 10 mg P L⁻¹, respectively. Results from this study have revealed that microalgal growth in the consortia have improved both nitrogen and phosphorus removal efficiencies. NO₃-N and PO₄-P removal efficiencies obtained for single cultures were 52–71% and 50–81%, respectively, whereas NO₃-N and PO₄-P removal efficiencies determined for the consortia ranged between 85–78% and 86–97%, respectively.

Microalgal–bacterial consortia have also been successfully applied in nitrogen, phosphorus and organic matter removal from different wastewater sources (Solimeno and García 2017; Quijano et al. 2017). When growing a microalgal–bacterial consortium composed of *C. vulgaris* and primary-treated municipal wastewater native bacteria in tubular PBRs processing a primary-treated municipal wastewater (NH₄-N and TP initial concentrations of 17–207 mg L⁻¹ and 1.4–19.5 mg L⁻¹, respectively), He et al. (2013) have demonstrated removal efficiencies ranging between 30.9 and 100% for nitrogen and between 65 and 98% for phosphorus. Alcántara et al. (2015) have used a closed tank to evaluate the performance of a microalgal consortium from a HRAP treating diluted vinasse and activated sludge native bacteria in nitrogen and organic matter removal from a synthetic wastewater containing 120 mg L⁻¹ of NH₄-N and 200 mg L⁻¹ of total organic carbon (TOC). These authors have reported NH₄-N removal efficiencies ranging between 75 and 96% and TOC removal efficiencies ranging between 86 and 90%. Gonçalves et al. (2016b) have evaluated the potential of dual-species cultures of the microalga *C. vulgaris* and a bacterium isolated from a

municipal wastewater treatment plant (*Enterobacter asburiae*, *Klebsiella* sp. or *Raoultella ornithinolytica*) in biomass production and wastewater polishing from a synthetic medium that mimics a secondary-treated effluent (nitrogen and phosphorus initial loads of about 45 mg N L⁻¹ and 10 mg P L⁻¹, respectively). The authors have concluded that when growing in consortium with *E. asburiae* and *R. ornithinolytica*, *C. vulgaris* growth has significantly increased. Additionally, the three studied consortia have also contributed to higher nutrients removal, since the time required to achieve the limits in discharged effluents (established by European Union legislation) was reduced to at least half of the value determined for the single *C. vulgaris* culture. When comparing ammonium removal from artificial wastewater using a microalgal consortium composed of *Anabena variabilis*, *Chlorella* sp., *Chlorococcus* sp. and *Spirulina* sp. and microalgal–bacterial consortium composed of the same microalgal species and mixed liquor activated sludge from the Harnaschpolder wastewater treatment plant (Delft, The Netherlands), Rada-Ariza et al. (2017) have concluded that the microalgal–bacterial consortium removed ammonium at higher rates (100 mg L⁻¹ day⁻¹) than the microalgal consortium (44 mg L⁻¹ day⁻¹). More recently, Foladori et al. (2018) have evaluated and optimized total nitrogen removal from municipal wastewater using a mixed microalgal–bacterial consortium spontaneously acclimatized to real wastewater in a photo-sequencing batch reactor. With this study, the authors have reported high chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN) removal efficiencies: 86 and 97%, respectively. To avoid the requirements for further harvesting of microalgal biomass, some studies have reported the use of these consortia in immobilized growth systems, such as immobilization in solid carriers and biofilms. De-Bashan et al. (2004) have used an immobilized culture of *Chlorella* sp. and *A. brasilense* in alginate beads to treat a municipal wastewater with the following composition (in mg L⁻¹): 0.1–4.3 NH₄-N, 4–5.2 NO₃-N and 4.1 PO₄-P. This study has resulted in an effective removal of nitrogen (both NH₄-N and NO₃-N) and phosphorus, with removal efficiencies ranging between 92 and 100%. When growing *C. sorokiniana* and activated sludge native bacteria in a tubular biofilm PBR treating primary-treated piggery wastewater containing 656 mg L⁻¹ of NH₄-N, 117 mg L⁻¹ of PO₄-P and 1247 mg L⁻¹ of TOC, Godos et al. (2009) have reported the following removal efficiencies: 94–100% for NH₄-N, 70–90% for PO₄-P and 45% for TOC. Posadas et al. (2013) have used a biofilm reactor to promote primary-treated domestic wastewater treatment (TN, PO₄-P and TOC concentrations of 91 mg L⁻¹, 7 mg L⁻¹ and 181 mg L⁻¹, respectively) by a centrate wastewater native microalgal–bacterial consortium. This study has revealed TN, PO₄-P and TOC removal efficiencies of 70%, 85% and 90%, respectively. Miranda et al. (2017) have isolated and characterized natural microalgal biofilms from freshwater, saline lakes and marine habitats around Melbourne, Australia. These consortia were composed of several microorganisms, such as cyanobacteria, microalgae, diatoms, bacteria and fungi. From the studied biofilms, Biofilm #52 (composed of two filamentous cyanobacteria, clustered with *Spirulina* and *Oscillatoria* species, one unicellular microalgae, clustered with some *Chlorella* species, and two diatoms, identified as *Nitzschia* sp., and *Fistulifera* sp.) was considered the most promising in terms of nutrients removal from a selenium-rich synthetic

wastewater and bioenergy production. Regarding nutrients removal, after 3 days of treatment, the selected biofilm was able to uptake 24% of $\text{NH}_4\text{-N}$, 26% of $\text{NO}_3\text{-N}$ and 17% uptake of $\text{PO}_4\text{-P}$ from the synthetic wastewater. To reduce time and costs associated with microalgal harvesting, some authors have also described the use of artificial consortia consisting of flocculating microorganisms. Van Den Hende et al. (2011) have reported the use of microalgal–bacterial flocs, mainly composed of *Chlorella* sp., *Pediastrum* sp., *Phormidium* sp., *Scenedesmus* sp. and activated sludge native bacteria, to treat a primary-treated municipal wastewater. The results obtained in this study have shown that these flocs were able to remove 61.2% of TN and 30.2–56.8% of $\text{PO}_4\text{-P}$. More recently, Arcila and Buitrón (2017) have evaluated the influence of the solar irradiance level on the formation of microalgal–bacterial aggregates, settling velocity and nutrients removal from a municipal wastewater. With this study, the authors have concluded that the highest irradiance level evaluated ($6213 \text{ W h m}^{-2} \text{ day}^{-1}$) has resulted in a poor wastewater treatment performance, with TN and COD removal efficiencies of 36 and 50%, respectively. In contrast, $\text{PO}_4\text{-P}$ removal efficiencies obtained in these conditions were 92%. Additionally, these conditions have resulted in low settling velocities and settleability, associated with a poor rate of aggregates formation. On the other hand, low irradiance levels ($<3800 \text{ W h m}^{-2} \text{ day}^{-1}$) have contributed to the formation of microalgal–bacterial aggregates with high settling velocity and settleability and to the increase of TN and COD removal efficiencies: in these conditions, TN and COD removal efficiencies were 60 and 89%, respectively.

10.4.3 Bioenergy Production

In terms of bioenergy production, the potential of both microalgal and microalgal–bacterial consortia has already been evaluated.

Regarding microalgal consortia, Chinnasamy et al. (2010) have evaluated the potential of a microalgal consortium composed of 15 native species from a carpet industry effluent on bioenergy production. In this study, the authors have reported lipid productivities of 6.82% of dry weight. Additionally, the authors have concluded that about 63.9% of algal oil obtained from the consortium could be converted into biodiesel. When evaluating the potential of a microalgal consortium in CO_2 capture using different CO_2 concentrations in the air stream, Boonma et al. (2014) have also studied lipids productivity. Maximum productivity was achieved for a CO_2 concentration of 30% (v/v) and the value obtained was $4.8 \text{ mg L}^{-1} \text{ day}^{-1}$ (approximately 27.6% of dry weight). In the study performed by Qin et al. (2016), four microalgal consortia and respective single cultures were grown in a dairy industry wastewater to assess their potential in both nutrients removal and biomass and bioenergy production. The consortia evaluated in this study were the following: (1) *Chlorella* sp./*Chlorella zofingiensis* (1:1); (2) *Scenedesmus* spp./*C. zofingiensis* (1:1); (3) *Chlorella* sp./*Scenedesmus* spp. (1:1); and (4) *Chlorella* sp./*Scenedesmus* spp./*C. zofingiensis* (1:1:1). Bioenergy production was assessed in terms of lipids

productivity, which has shown to be higher in the consortia rather than in each single culture (143.7–150.6 mg L⁻¹ day⁻¹). Furthermore, FAME profiles indicated that the lipids produced from microalgal consortia were more suitable for biodiesel production than those produced by single cultured microalgae. More recently, nine native microalgal consortia isolated from rural wastewaters were evaluated in terms of biomass productivities for bioenergy production (Choudhary et al. 2016). Through biochemical characterization of the biomass resulting from the best performing consortium, the authors estimated a lipid content of 31% of dry weight and a theoretical methane potential of 0.79 m³ kg⁻¹ volatile solids, which suggests a good potential of this consortium for biogas generation. In the study performed by Gonçalves et al. (2016a), where single- and dual-species cultures of *S. salina* and *C. vulgaris*, *P. subcapitata* and *M. aeruginosa* were grown in a synthetic medium to evaluate their potential in nutrients removal, fatty acids concentration was also determined. This study has demonstrated higher lipid contents in biomass resulting from microalgal consortia, especially in the consortia *S. salina* + *P. subcapitata* and *S. salina* + *M. aeruginosa*.

Microalgal–bacterial consortia performance in terms of lipids productivity was also studied. For example, in the study performed by Zhao et al. (2014), lipid contents determined in a consortium composed of *Chlorella pyrenoidosa* and native bacteria from a landfill leachate ranged between 14.5 and 20.8% of dry weight, with maximum lipid productivity achieved being 24.1 mg L⁻¹ day⁻¹. In the study performed by Miranda et al. (2017), the authors have studied several microalgal–bacterial biofilms, selecting one of them (Biofilm #52) as the most promising in terms of feedstock for bioenergy production, as it was possible to modify the levels and compositions of saturated, monosaturated and polyunsaturated fatty acids through the promotion of the growth of selected individual photosynthetic inhabitants in the consortium.

High lipid contents were also determined in a mixed culture composed of *C. pyrenoidosa* and a yeast (*Rhodotorula mucilaginosa*), both isolated in Nuevo Leon, Mexico (Reyna-Martínez et al. 2015). In this study, the authors have reported lipids contents of about 20% of dry weight, with 94.6% corresponding to triglycerides composed of fatty acid chains between 16 and 18 carbons.

10.5 Research Needs

Although several studies have successfully applied microalgal and microalgal–bacterial consortia in CO₂ capture, nutrients removal and bioenergy production, further research is required for the optimization of culturing parameters, especially for large-scale applications. Additionally, interactions between photosynthetic microorganisms are not fully understood.

Taking into account large-scale applications and the necessity to clearly understand microorganisms' interactions in the consortia, research should be focused on the following topics (Bordel et al. 2009; Fouilland 2012; Padmaperuma et al. 2018):

(1) study of the influence of different environmental conditions (such as light, nutrients availability, pH and temperature) on the consortia behaviour; (2) large-scale outdoor experiments with real environmental conditions; (3) complete understanding of the interactions established between the microorganisms integrating the consortia; and (4) development of reliable mathematical models that correctly describe the behaviour of these consortia, which may be very helpful in process design and operational conditions determination.

To further improve the effectiveness of microalgal consortia in the environmental applications referred in this study, engineering of microalgal consortia should be taken into account (Padmaperuma et al. 2018). For example, microalgal consortia combining microorganisms with different metabolic capacities, specific metal binding abilities, affinities for different nitrogen sources, different affinities to increased CO₂ concentrations and with different lipid contents should be studied. This step will allow the re/introduction or elimination of microorganisms as needed and the complete monitoring of the tasks developed within the consortia.

10.6 Conclusions

The use of microalgae in environmental applications, such as CO₂ capture, nutrients uptake and bioenergy production, has been extensively reported in the literature, with several success cases. Regarding microalgal consortia, various studies have referred their potential in the same applications, especially due to the symbiotic/cooperative interactions that can occur in these combined systems. However, due to the huge number of possible combinations that can be engineered or can naturally occur, the use of microalgal consortia in the referred applications is still under research. The majority of studies have focused on the use of microalgal consortia in nutrients removal from wastewaters, but only a few studies have evaluated their potential in CO₂ capture and bioenergy production.

This chapter provided an overview of current status of research on the use of microalgal consortia, focusing on the main studies conducted regarding CO₂ capture, nutrients removal and bioenergy production. As main conclusion, it is possible to refer that both microalgal and microalgal–bacterial consortia can be effectively applied in wastewater polishing processes. Although further studies are required, it is also possible to conclude that microalgal consortia may be very promising in CO₂ capture and that microalgal and microalgal–bacterial consortia can be an important source of lipids for bioenergy production.

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