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REVIEW ARTICLE

Wastewater treatment to enhance the economic viability of microalgae culture

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Abstract Microalgae culture is still not economically viable and it presents some negative environmental impacts, concerning water, nutrient and energy requirements. In this context, this study aims to review the recent advances on microalgal cultures in wastewaters to enhance their economic viability. We focused on three different culture concepts: (1) suspended cell systems, (2) cell immobilization, and (3) microalgae consortia. Cultures with suspended cells are the most studied. The nutrient removal efficiencies are usually high for wastewaters of different sources. However, biomass harvesting is difficult and a costly process due to the small cell size and lower culture density. On the other hand, the cell immobilization systems showed to be the solution for this problem, having as main limitation the nutrient diffusion from bulk to cells, which results in a reduced nutrient removal efficiency. The consortium between microalgae and bacteria enhances the growth of both microorganisms. This culture concept showed to be a promising technology to improve wastewater treatment, regarding not only nutrient removal but also biomass harvesting by bioflocculation. The aggregation mechanism must be studied in depth to find the process parameters that would lead to an effective and cheap harvesting process.

Keywords Cell immobilization · Microalgal consortia · Nutrient removal · Suspended cell cultures · Wastewater treatment

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Introduction

Microalgae are photosynthetic microorganisms that can be found in both marine and freshwater environments (Becker 1994). Their photosynthetic process is similar to that of terrestrial plants. However, they are more efficient in the utilization of the solar energy to produce biomass (being responsible for a significant fraction of the world oxygen production) due to the following characteristics: (1) simple cellular structure and (2) growing in aqueous environment, which enables the efficient access to water and nutrients (Demirbas and Demirbas 2010; Oswald and Golueke 1960; Benemann 1997).

Some microalgal strains presents high growth rates (biomass concentration can double within hours), which attributes to microalgae an undeniable economical potential (Grima et al. 2003; Norsker et al. 2011; Spolaore et al. 2006). Considering their characteristics, microalgae are associated with several potential environmental applications that have been intensively studied in the recent years: (1) CO_2 capture from industrial flue gases, (2) bioenergy production, and (3) nutrient removal from wastewaters (Sturm and Lamer 2011; Bhatnagar et al. 2011; Rawat et al. 2011; Pires et al. 2012). However, none of the referred applications is economically viable, mainly due to the requirements of water, nutrients and energy. Moreover, one of the costly processes is the microalgae harvesting, which represents about 30 % of the total costs. Consequently, several studies were performed to reduce the overall cost of microalgae production, also taking into account its environmental impact (water usage and greenhouse gas emissions). Several authors have studied the integration of wastewater treatment and CO_2 capture to generate energy (Park and Craggs 2011; Park et al. 2011; Pittman et al. 2011; Craggs et al. 2011). Some wastewaters are rich in nutrients that enhance microalgal growth. Their use as culture medium will reduce the requirement of fresh water and nutrients and, at the end of the process, a clean effluent may be achieved to discharge in a watercourse. Thus, the evaluation of microalgae growth in

different wastewaters is important to assess the technical and economical feasibility of their environmental applications when wastewater is used as culture medium. Thus, the aim of this study was to review the recent advances on microalgal cultures in wastewaters concerning the environmental applications of microalgae such as CO_2 mitigation, bioenergy production, and wastewater treatment potential. We focused on three different culture concepts: (1) suspended cell systems, (2) cell immobilization, and (3) microalgae consortia.

Environmental applications of microalgae

CO_2 capture from flue gases

CO_2 is one of the most important greenhouse gases (GHGs), and its atmospheric concentration has been increasing in the last decade due to anthropogenic emissions. The European Union has proposed a long-term climate goal of limiting global mean surface warming to 2 °C (above pre-industrial level), which corresponds to an atmospheric CO_2 concentration of 450 ppm (Singh and Ahluwalia 2012; Pielke 2009; Torvanger et al. 2012). The achievement of this target would require changing of the global energy system in the next decades, which represents a challenge with several obstacles. The adoption of bioenergy and carbon capture and storage (CCS) will be essential (Gough and Upham 2011; Pires et al. 2013). The current CCS technology comprises different processes such as the physicochemical processes for CO_2 capture (absorption, adsorption, gas separation membranes and cryogenic distillation), its transport (by ship or pipelines) and storage in geological formations (Pires et al. 2011). CCS is a costly procedure (mainly due to the CO_2 capture), and it presents several concerns regarding CO_2 storage. Thus, it is considered as a short-term solution for CO_2 abatement policies.

CO_2 capture can also be performed by enhancing the natural sinks of this gas: (1) forestation, (2) ocean fertilization, and (3) microalgal cultures (Berberoglu et al. 2009). With higher photosynthetic efficiency than terrestrial plants, microalgae have been proposed as a sustainable CO_2 removal option (Murakami and Ikenouchi 1997; Mikkelsen et al. 2010; Wang et al. 2008; Milledge 2011; Spolaore et al. 2006; Bilanovic et al. 2009). An important research initiative was performed by the Japanese government (Research Institute of Innovative Technology for the Earth) during the period from 1990 to 2000. The main objective was to investigate an efficient use of light energy in microalgal cultures (Waltz 2009). Despite the great efforts spent on this project, no commercial applications were developed due to the high production cost of microalgae. Land use is also an important limitation for bioconversion of CO_2 by microalgae. Their culture requires large areas due to the light needed by the cells to perform photosynthesis. However, nowadays, with increased concerns about global warming, renewed attention has been focused to reduce the costs of microalgal cultures, aiming to achieve an economically viable process for CO_2 mitigation that can replace the CCS technology.

Bioenergy production

The continuing depletion of fossil fuel resources (followed by the increase of their prices) leads to the intensive research focusing on alternative energy sources. Food crops were considered for production of biodiesel and bio-ethanol (aiming to replace the conventional diesel and gasoline, respectively); however, the production yields are unable to meet the world energy demand (Singh et al. 2011; Gallagher 2011; Gonçalves et al. 2013). Avoiding the competition with human food market, microalgae represent one promising alternative resource to fossil fuel. One of the most research projects in this area was financed by United States Department of Energy from 1978 to 1996 (costing $25 million) in its aquatic species program at the National Renewable Energy Laboratory, which significantly improved the scientific knowledge about this topic (Sheehan et al. 1998). The research focused on cultivation conditions, bioreactor design, light distribution, cell harvesting and oil extraction. Some microalgal strains were found with very high lipid content. These microorganisms can grow on places that are unsuitable for agriculture, not competing with land for food production; however, the main disadvantage of biodiesel production from microalgae is the difficult and costly harvesting process (Savage 2011; Rawat et al. 2011). In addition, the culture requires high amounts of fresh water and nutrients that contributes to a significant environmental impact and to the increase of the process cost (the price of nutrients almost doubled in the last decade) (Vasudevan et al. 2012; Menetrez 2012). The use of wastewater as culture medium reduces the requirements of nutrients and freshwater. Thus, microalgal culture should couple wastewater treatment and CO_2 capture to offer an economically viable and environment-friendly process.

Nutrient removal from wastewaters

Human activities led to the increase of chemical and biological contaminants in water systems. The reduction of anthropogenic nutrient inputs (from agricultural practices, urban wastewater and industries) in the aquatic ecosystems is required to protect drinking water supplies and to reduce eutrophication (Schindle 1974; Kong et al. 2010; Conley et al. 2009).

Microalgae enhance the removal of inorganic nutrients, organic contaminants and heavy metals from wastewaters. Regarding nitrogen, the ammonia present in some wastewaters can be removed due to cell assimilation and ammonia volatilization. The last phenomenon results from the increase of pH value due to microalgal growth (Riano et al. 2011;

Garcia et al. 2000). As microalgae consume CO_2 due to their photosynthetic activity and, if the replacement in medium is not performed via absorption from atmosphere and bacterial oxidation of organic matter, the pH of the culture starts to increase (Larsdotter et al. 2007). On the other hand, microalgae assimilates phosphorus for their growth and they are able to store this nutrient as polyphosphate (Powell et al. 2009; Larsdotter 2006; Rao et al. 2011; Powell et al. 2008, 2011). This storage is then used in future starvation periods (Eixler et al. 2006). Phosphorus can also be removed by chemical reactions that occur in cultures. The pH increase (consequence of microalgae photosynthetic activity) leads to phosphorus precipitation by complexation with metal ions (calcium, magnesium and iron) in solution, reducing the concentration of this nutrient in the medium.

For both nutrients, the removal kinetics may be described as function of external concentration using the Monod model (Vymazal 1995; Li et al. 2010). Their removal efficiencies depend on several factors: (1) microalgal culture concepts, (2) initial concentrations, (3) nitrogen to phosphorus (N/P) ratio, (4) microalgal strain, (5) growth conditions, (6) nutrients source, and (7) wastewater characteristics. In the following sections, the research studies are separately discussed according to culture concept.

Microalgal culture concepts

Suspended cells

The conventional microalgal culture concept is the suspended growth system. This biological process has been normally applied as a tertiary treatment of wastewater. One of the important parameters for the success of wastewater treatment by microalgae is the initial cell concentration. For lower values, the wastewater treatment takes more time; otherwise, for higher values, light limitation on microalgal growth can occur due to self-shading. Lau et al. (1995) tested four different inoculum sizes of *Chlorella vulgaris* on nutrient removal from primary settled sewage (average nutrient concentrations: 35.5 mg l^{-1} of NH_4^+; 0.40 mg l^{-1} of NO_3^-; 3.89 mg l^{-1} of PO_4^{3-} between other compounds; photobioreactor [PBR] with working volume [V]=300 ml; air flux 0.5 vvm; temperature [T]=24±1°C; pH=7; light intensity [LI]=4300±300 lx; light/dark ratio [LDR]=16:8; culture time [CT]=10 days). It was concluded that most concentrated cultures (for initial cell concentration equal to 1×10^7 cells ml^{-1}) achieved high removal efficiencies in less time (7 days) than the other cultures, which means that the self-shading may not limit the microalgal growth in that experiments.

Microalgae require nitrogen and phosphorus in a determined ratio. In the presence of low concentrations of one of these nutrients, the growth rate is limited even if the concentration of other nutrient is high. Thus, regarding wastewater treatment, it is important to know in advance its chemical composition to infer the feasibility of microalgal cultures to achieve high removal efficiencies. In this context, Wang and Lan (2011) evaluated the effect of N/P ratio on nutrient removal by *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent (experimental conditions presented in Table 1). With excess of phosphorus (concentration of 108 mg l^{-1}), the maximum removal rate of nitrate (43.7 mg l^{-1} day^{-1}) was achieved with the concentration of this nutrient at 144 mg l^{-1}, corresponding to an N/P ratio of 1.33. Microalgae presented high nitrate removal efficiency; however, high concentrations of phosphorus should be presented in the treated wastewater. Fixing the nitrate concentration at 140 mg l^{-1}, the highest removal rates were 27.5 mg l^{-1} day^{-1} (N) and 9.4 mg l^{-1} day^{-1} (P) when phosphorus initial concentration was 47 mg l^{-1} (N/P ratio of 3). In these conditions, high removal efficiencies were achieved for both nutrients.

Culture conditions influences the microalgae growth and consequently the nutrient removal from medium. Lodi et al. (2003) studied the effect of temperature on nutrient removal efficiencies of *Spirulina platensis*. Cultures of 0.5 L were performed under limited light conditions (LI=40 μmol m^{-2} s^{-1}). Different temperatures were tested in the range between 23 °C and 40 °C. The highest removal rates were achieved with 30 °C; however, the removal efficiencies were not satisfactory.

Table 1 shows the nitrogen and phosphorus removal efficiencies from wastewater from several sources by microalgae. The most relevant microalgal culture conditions used in the studies are presented. Several microalgal strains were tested in wastewater treatment and high removal rates were achieved. However, the suspended cell cultures have a major drawback regarding the biomass harvesting. An effective and cheaper process should be determined to safely discharge the treated water.

Immobilized cells

Trying to solve the biomass harvesting problem of suspended cell cultures, the immobilization of the microalgae was proposed. In wastewater treatment, it aims to keep the living cells within a gel matrix metabolically active having very limited mobility (de-Bashan and Bashan 2010). This process can be natural ("passive", using the natural tendency of microorganisms to attach surfaces) or artificial ("active", using a gel matrix). Several synthetic and natural polymers were tested for microalgae immobilization. Regarding the wastewater treatment, the natural polymers are less stable than synthetic ones. Nevertheless, the most common applied polymers are the natural polymers alginate and carrageenan (Lau et al. 1997).

Table 1 Nitrogen and phosphorus removal efficiencies from wastewater with suspended microalgal cultures

Microalgae	Wastewater	Removal (g m^{-3} h^{-1} or %)			Experimental set-up	Ref.
		NH_4^+-N	NO_3-N	TP		
Botryococcus braunii	Domestic wastewater		79.63 %	100 %	BioFlo Fermenter; V=9 l; T=25 °C; LI=3,500 lx; LDR=12 h:12 h; CT=14 days	(Sydney et al. 2011)
Botryococcus braunii	Secondarily treated piggery wastewater		80 %		Column bioreactors; V=500 ml; T=25 °C; LI=100 μE m^{-2} s^{-1}; CT=12 days	(Hernandez-Caraballo and Marco-Parra 2003)
Chlorella kessleri	Synthetic wastewater		19 %		Conic bioreactors; V=100 ml; T=30 °C; LI=45 μmol m^{-2} s^{-1}; LDR=12 h:12 h; CT=72 h	(Lee and Lee 2001)
Chlorella pyrenoidosa	Soybean processing wastewater	89.1 %		70.3 %	Conic bioreactors; V=500 ml; T=27±1 °C; LI=40.5 μmol m^{-2} s^{-1}; LDR=14 h:10 h; CT=5 days	(Su et al. 2011)
Chlorella sp.	Municipal wastewater	93.9 %		80.9 %	Coil bioreactor; V=25 l; *T*=25±2 °C; LI=50 μmol m^{-2} s^{-1}; CT=14 days	(Li et al. 2011)
Chlorella vulgaris	Agro-industrial wastewater	95 %		95 %	Cylindrical bioreactors; V=2 l; T=20±2 °C; LI=60 μmol m^{-2} s^{-1}; CT=9 days	(Gonzalez et al. 1997)
Chlorella vulgaris	Steel-making plant wastewater	0.92			Bioreactor not defined; T=27 °C; LI=110 μE m^{-2} s^{-1}	(Yun et al. 1997)
Chlorella vulgaris	Synthetic wastewater	97 %		96 %	Column bioreactors; V=2 l; *T*=30 °C; LI=3,000 lx; CT=14 days	(Peng et al. 2011)
Haematococcus pluvialis	Primary-treated sewage wastewater		100 %	100 %	Conic bioreactors; V=130 ml; T=23 °C; LI=50 μmol m^{-2} s^{-1}; LDR= 12 h:12 h; CT=5 days	(Kang et al. 2006)
Neochloris oleoabundans	Synthetic wastewater		99 %	100 %	Cylindrical bioreactors; V=400 ml; T=30 °C; LI=1,280 lumens; CT=7 days	(Wang and Lan 2011)
Phormidium bohneri	Domestic wastewater	0.83		0.58	Triangular bioreactors; V=24 l; outdoor conditions	(Laliberte et al. 1997)
Phormidium bohneri	Fish farm wastewater	82 %		85 %	Photobioreactors; V=70 l; outdoor conditions; CT=30 days	(Dumas et al. 1998)
Scenedesmus dimorphus	Agro-industrial wastewater	95 %			Cylindrical bioreactors; V=2 l; T=20±2 °C; LI=60 μmol m^{-2} s^{-1}; CT=9 days	(Gonzalez et al. 1997)
Spirulina	Pig wastewater	84–96 %		72–87 %	Raceway ponds of 6 and 24 m^2; outdoor conditions; CT=7 days	(Olguin et al. 2003)

V volume, *T* temperature, *LI* light intensity, *LDR* light/dark ratio, *CT* culture time

Assuming that biomass retention in immobilization matrix is near 100 %, the harvesting process is not required before the discharge of the effluent (Lau et al. 1997; Boelee et al. 2011; Fierro et al. 2008). However, some fragments with cells can be separated from the matrix due to operation variables (i.e., hydrodynamics) of this matrix. These agglomerations of cells are then easily harvested (more than suspended cell cultures) due to their size. Regarding biomass production, the microalgal cultures in immobilization matrices are characterized by having a longer lag period when compared with suspended cell systems (Moreno-Garrido 2008; Mallick 2002). After this phase, the specific growth rates of microalgae in both culture concepts are very similar.

As discussed above, the culture concept and the microalgal strain may have an important role on the nutrient removal efficiency from wastewaters. Ruiz-Marin et al. (2010) evaluated the ability of *Scenedesmus obliquus* and *Chlorella vulgaris* for nitrogen and phosphorus removal from urban wastewater. This study compared the removal efficiency of the immobilized and suspended microorganisms (culture conditions presented in Table 2). *Scenedesmus obliquus* presented a greater adaptation to urban wastewater than *Chlorella vulgaris*, as it had a shorter lag phase in suspended cell cultures. However, when both species grew immobilized, they did not show the lag phase. *Scenedesmus obliquus* was more efficient in nitrogen removal than *Chlorella vulgaris* after 48 h of culture. Comparing the two growing procedures, the suspended cells generally achieved higher nutrient removal rates. This phenomenon may be justified by the additional resistance in mass transfer of the nutrients caused by immobilization matrix. Fierro et al. (2008) also studied the conventional free cell system and the immobilized one for nutrient

Table 2 Nitrogen and phosphorus removal efficiencies from wastewater with immobilized microalgal cultures

Microalgae	Wastewater	Removal (g m^{-3} h^{-1} or %)			Experimental set-up	Ref.
		NH_4^+-N	NO_3-N	TP		
Chlorella vulgaris	Simulated domestic wastewater	100 %		95 %	Column bioreactors; V=5 l; T=23±2 °C; LI=100 μE m^{-2} s^{-1}; CT=2 days; IM=calcium alginate	(Tam and Wong 2000)
Chlorella vulgaris	Synthetic wastewater		0.22	0.05	Conical bioreactors; V=120 ml; T=25 °C; LI=100 μE m^{-2} s^{-1}; LDR=18 h:6 h; IM=carrageenan	(Lau et al. 1998)
Dunaliella salina	Synthetic wastewater	42.2 %	62.0 %	64.7 %	Conical bioreactors; V=100 ml; T=26 °C; LI=14 W m^{-2}; LDR=14 h:10 h; CT=36 h; IM=sodium alginate	(Thakur and Kumar 1999)
Scenedesmus obliquus	Urban wastewater	96.6 %		55.2 %	Column bioreactors; V=2.5 l; T=25±1 °C; LI=135 μE m^{-2} s^{-1}; CT=2 days; IM=sodium alginate	(Ruiz-Marin et al. 2010)
Scenedesmus rubescens	Synthetic wastewater	96 %	95 %	90 %	Column bioreactors; V=2 l; T=30 °C; LI=20–120 μmol m^{-2} s^{-1}; CT=9 days; twin-layer system	(Shi et al. 2007)
Scenedesmus sp.	Domestic secondary wastewater	100 %		100 %	Parallel-plate bioreactor; V=350 ml; T=20±2 °C; LI=5,000±300 lx; LDR=13 h:11 h; CT=15 min; IM=calcium alginate	(Zhang et al. 2008)
Scenedesmus sp.	Effluent from a secondary wastewater treatment plant	43 %		40–80 %	Sedimentation bioreactor; V=96 l; T=20–22 °C; LI=2,800 lx; LDR=6 h:6 h; CT=72 h; algae-immobilized fiber-bundle carrier	(He and Xue 2010)
Scenedesmus sp.	Synthetic wastewater		70 %	94 %	Conical bioreactors; V=250 ml; T=32±1 °C; LI=43 μmol m^{-2} s^{-1}; CT=12 h; IM=chitosan	(Fierro et al. 2008)

V volume, *T* temperature, *LI* light intensity, *LDR* light/dark ratio, *CT* culture time, *IM* immobilization matrix

removal, but with only one microalga (*Scenedesmus* sp.). Chitosan was tested as immobilization agent and cells viability and growth were analysed. The microalgae presented similar growth rates in both cell systems, showing that chitosan is suitable for cell immobilization. Using synthetic wastewater, nitrogen and phosphorus removal efficiencies were higher with immobilized cells (70 % and 94 %, respectively) than with suspended cells (20 % and 30 %, respectively). Jiménez-Pérez et al. (2004) compared the suspended and immobilized cultures of *Scenedesmus intermedius* Chod. and *Nannochloris* sp. for nutrient removal from wastewater. The used species were isolated from pig manure. The achieved removal rates were higher than those obtained for commercial species. This observation was justified by the better adaptation of the used species to the nutrient concentrations profile of the wastewater.

The immobilization of microalgae can be performed in plane surfaces or in beads. If beads are chosen, their concentration should be optimized. Thus, Tam and Wong (2000) immobilized *Chlorella vulgaris* with calcium alginate as microalgae beads to remove nutrients from simulated settled domestic wastewater. Different microalgae bead concentrations (from 4 to 20 beads ml^{-1}) were tested and the optimal value was 12 beads ml^{-1}, able to remove practically all nitrogen and 95 % of phosphorus. Higher and lower bead concentration values led to the achievement of lower nutrient removal efficiencies.

Nowack et al. (2005) developed a new system for microalgae grow that consists in an immobilization technique with twin-layer system. Using this immobilization system, Shi et al. (2007) studied the nutrient removal from wastewater by two microalgae: *Chlorella vulgaris* and *Scenedesmus rubescens*. The authors observed that microalgae were effectively separated from the bulk. The experiments showed that both microalgae species grew well in synthetic wastewater without leakage of cells into wastewater. No significant resistance in nutrient diffusion (from the bulk to the cells) by the twin-layer was detected, presenting high nitrogen and phosphorus removal rates from synthetic wastewater. Taking into account the European Union legislation, the total phosphorus and nitrogen concentrations discharged by wastewater treatment plants should be less than 2 and 15 mg l^{-1}, respectively. These values were achieved after 2 days of treatment with microalgal culture for both species, which means that the microalgae immobilization on twin-layers is an effective procedure to remove these nutrients from wastewaters.

Table 2 shows the nitrogen and phosphorus removal efficiencies using several immobilized microalgal cells. The results of several combinations of strains, wastewaters and immobilization matrixes are presented. Although high nutrient removal efficiencies were achieved, more experiments in pilot scale must be performed to verify the reproducibility of the results.

Microalgal consortia

The consortium between microalgae and bacteria has been studied due to the potential benefits for both microorganisms (symbiosis). When cultivated in wastewaters, microalgae can provide oxygen (by photosynthesis) that can be used by bacteria for degradation of organic matter, reducing the need for external aeration (Munoz and Guieysse 2006). The oxidation of organic matter by bacteria produces carbon dioxide that supports the photoautotrophic growth of microalgae (Subashchandrabose et al. 2011; Park et al. 2008). Moreover, the degradation of N-containing organic compounds is more efficient with microalgal consortium and bacteria than with bacteria alone because microalgae can assimilate the released NH_4^+ (one of the main nutrients for microalgae). Thus, both microorganisms may have higher growth rates in mixed cultures when compared with monocultures (transgressive overyielding) (Weis et al. 2008). Additionally, mixed cultures can perform tasks that are difficult for individual species (Brenner et al. 2008). They are less influenced by environmental fluctuations. The species in consortium are able to share metabolites during periods of nutrient limitations and offer resistance to invasion by other species. Another advantage of this consortium is related with biomass harvesting (one of the costly processes in microalgal production). The bioflocculation can help in the separation of biomass by gravity sedimentation, which avoids the use of harvesting conventional methods. These phenomena were already studied for high rate algal pounds (Craggs et al. 2011; Park et al. 2011). This type of bioreactors was considered the only economically viable way to produce microalgae for bioenergy production with minimum environmental impact.

Bacteria strains that promote microalgal growth are designed using microalgal growth-promoting bacteria. One of the most used bacterium is *Azospirillum* sp., which is already applied to enhance the growth and yield of many terrestrial crop plants (de-Bashan et al. 2008a). The bacteria induces the production of phytohormones that changes the cell metabolism, allowing better mineral and water absorpotion and the achievement of high culture densities.

de-Bashan et al. (2008b) isolated a microalgae (*Chlorella sorokiniana*) from wastewater stabilization ponds under extremely hot desert conditions. This microalgae presented high growth rates in synthetic wastewater at temperatures above 40°C and light intensity of 2,500 μmol m^{-2} s^{-1}. The ammonium removal was higher in these environmental conditions than with lower temperature (28 °C) and lower light intensity (60 μmol m^{-2} s^{-1}). Inoculation with bacteria *Azospirillum brasilense* enhanced the microalgal growth and the ammonium removal rate. The consortium was able to uptake this nutrient from the wastewater (concentration of 10 mg l^{-1}) to undetectable levels in 4 days under very extreme environmental conditions.

de-Bashan et al. (2004) reported nitrogen and phosphorus removal from municipal wastewater by microalgae-bacterium consortiums (*Chlorella vulgaris*/*Azospirillum brasilense* and *Chlorella sorokiniana*/*Azospirillum brasilense*). These microorganisms were immobilized in alginate beads. The consortium was able to remove up to 100 % ammonium, 15 % nitrate, and 36 % phosphorus within 6 days. On the other hand, microalgae alone removed up to 75 % ammonium, 6 % nitrate, and 19 % phosphorus, which means that the consortium with bacteria improves the nutrient removal efficiency of microalgae.

Hernandez et al. (2006) studied the effect of the starvation period of two microalgal species (*Chlorella vulgaris* and *Chlorella sorokiniana* in saline solution) on their growth and phosphorus removal efficiency. These microalgae were immobilized with *Azospirillum brasilense* in alginate beads. The starvation period of 3 days favoured phosphorus absorption by both microalgae. Moreover, it was observed that the negative effects of starvation on microalgal growth were mitigated by *Azospirillum brasilense*. The proposed biological process showed to be a good alternative to remove phosphorus from wastewaters. Hernandez et al. (2009) immobilized *Chlorella vulgaris* and *Bacillus pumilus* ES4 (a plant growth-promoting bacterium) in alginate beads to remove nitrogen and phosphorus from wastewater. The authors also tested this microalgal consortium in a synthetic medium without nitrogen. Under these conditions, the bacterium was able to fix nitrogen, promoting the accumulation of ammonium in the medium. The nutrient removal efficiency of *Chlorella vulgaris* was not enhanced with the presence of *Bacillus pumilus*. However, this microalgal consortium may be applied to remove nutrients from wastewaters with lower concentrations of nitrogen, as the nutrient needed for microalgal growth can be provided by the bacterium.

The consortium between different microalgal strains was also tested. With this consortium, an eventual loss of population of one strain due to the culture conditions may be compensated by the other, which constitutes its main advantage in wastewater treatment. Bhatnagar et al. (2011) evaluated the biomass production with mixotrophic microalgae (*Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga*) using several carbon sources and wastewaters. Cultures with two and three microalgal strains were performed in poultry litter extract and untreated carpet industry wastewaters and their biomass concentrations were determined. The results showed that the consortia *Chlamydomonas*

globosa–Chlorella minutissima and *Scenedesmus bijuga–Chlorella minutissima* presented the best results for poultry litter extract and untreated wastewater, respectively, while the consortium with the three strains was suitable for both wastewaters.

Table 3 shows the nitrogen and phosphorus removal efficiencies from wastewaters using microalgal consortia. Some of them presented lower removal efficiencies. The experiments were performed at a small scale only, and the results were not satisfactory. The interaction between microalgae and bacteria should be better studied to prevent competition between them for the nutrients in wastewater and to understand the bioflocculation mechanism.

Energy and economic aspects

Bioenergy production from microalgae may be economically and energetically feasible when wastewater is used as the culture medium (Pittman et al. 2011). Sturm and Lamer (2011) performed an energy assessment of coupling nutrient removal from wastewaters with microalgae biomass production. Without an energy credit for nutrient removal, the biofuel production was considered energetically favourable in open ponds. Nutrient removal and recovery of CO_2 and heat from biogas combustion can improve the energy balance and reduce the environmental footprint of the wastewater treatment plants.

Regarding the microalgae culture concept, the immobilized living cells present some advantages when compared with suspended cells. As the cells are entrapped, the cell harvesting is not required before the discharge of the treated wastewater, which represents a significant improvement in the energy balance of the process. Moreover, some harvesting processes require the use of chemicals, which represents a significant cost in the whole process (Cai et al. 2013). However, the immobilization matrix is costly, prohibiting its use for biofuel production (Christenson and Sims 2011). In this context, surface-attached microalgal biofilms can offer the same advantages of the matrix-immobilized cultures at a lower cost. When compared with suspended cultures, microalgal biofilms can better integrate production, harvesting, and dewatering operations with reduced downstream processing costs.

Research needs

Microalgae have shown high potential for CO_2 capture and bioenergy production. However, their culture is still not economically viable for these applications. The integration of one or both applications with wastewater treatment should be tested at a commercial scale to eliminate the need for fresh water and nutrients, reducing the culture costs and its environment impact.

Research should be focused on the optimization of microalgal culture and harvesting, maintaining the discharged

Table 3 Nitrogen and phosphorus removal efficiencies from wastewater with microalgal consortia (with two different strains or with bacteria)

Microalgal consortium	Wastewater	Removal			Experimental set-up	Ref.
		NH_4^+-N	NO_3-N	TP		
Chlorella vulgaris/ *Azospirillum brasilense*	Municipal wastewater	100 %	15 %	36 %	Conical bioreactors; V=600 ml; T=26±2 °C; LI=31.8 W m^{-2}; CT=6 days	(de-Bashan et al. 2004)
Chlorella vulgaris/ *Azospirillum brasilense*	Synthetic wastewater	100 %		83 %	Chemostat; V=500 ml; T=28±2 °C; LI=30 μmol m^{-2} s^{-1}; CT=6 days	(de-Bashan et al. 2002)
Chlorella vulgaris/ *Azospirillum brasilense*	Synthetic wastewater	22 %		31.5 %	Inverted conical bioreactor; V=750 ml; T=28±1 °C; LI=90 μmol m^{-2} s^{-1}; LDR=12 h:12 h; CT=5 days	(Perez-Garcia et al. 2010)
Chlorella vulgaris/ *Planktothrix isothrix*	Municipal wastewater	80 %		100 %	Erlenmeyer flasks; V=250 ml; T=28 °C; LI=60 μmol m^{-2} s^{-1}; CT=9 days	(Silva-Benavides and Torzillo 2012)
Chlorella sorokiniana/ Activated sludge bacteria	Piggery wastewater	21 %		54 %	Jacketed glass tank photobioreactor; V=3.5 l; T=25 °C; LI=10 klx; CT=4.4 days	(de Godos et al. 2010)
Euglena viridis/Activated sludge bacteria	Piggery wastewater	34 %		53 %	Jacketed glass tank photobioreactor; V=3.5 l; T=25 °C; LI=10 klx; CT=4.4 days	(de Godos et al. 2010)
Limnothrix sp., *Phormidium* sp., *Anabaena* sp., *Spirogyra* sp., *Fischerella* sp., *Westiellopsis* sp.	Primary treated sewage water		90 %	97.8 %	Beakers; V=1 l; T=between 17±2 °C and 36±3 °C; LI= between 420±100 and 1,760±400 μmol m^{-2} s^{-1}; CT=6 days	(Renuka et al. 2013)

V volume, *T* temperature, *LI* light intensity, *LDR* light/dark ratio, *CT* culture time

effluent with parameters below the standard limits. Regarding microalgal culture, studies should be performed to evaluate the microalgal growth in a wide range and extreme environmental conditions, such as light, pH and pollutant concentrations (qualitative and quantitative profiles). Using wastewater as culture medium, the study of this last parameter is extremely important for the selection of microalgal strain.

Research studies about interactions between microalgae and other microbial species should be performed. The studies presented in this review concluded that microalgal consortia achieved higher growth rates than monocultures. Furthermore, this type of microbial association can be beneficial for biomass harvesting. The small size and low density of microalgal cells are the main aspects that increase the difficulty and cost of harvesting using conventional methods (centrifugation, gravity sedimentation or filtration). On the other hand, natural aggregation and bioflocculation promote simple gravity settling, simplifying the separation of cells from the medium. For some species, the aggregation may be achieved by nitrogen limitation and CO_2 addition. In this context, the physiological characteristics of the colonial microalgae and the aggregation mechanism should be studied further to find culture conditions that promote the preferred harvesting processes.

Conclusions

This study presents a review of the recent studies reporting on nutrient removal from wastewaters by microalgae, aiming at the enhancement of economical viability of CO_2 capture and bioenergy production by these microorganisms. The main research achievements with three culture concepts were presented. Using the conventional concept, microalgal cultures presented high nutrient removal efficiencies, but biomass harvesting remains a difficult process. The immobilization of microalgae showed to be the solution for this issue. Moreover, a microalgal consortium with other microorganisms can favour their growth. The study of microbial interactions should be studied in depth to optimize their growth and, at same time, to find the culture conditions that promote their aggregation, helping the biomass harvesting process.

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