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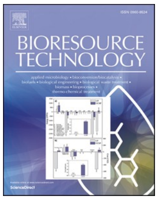

Microalgae bioreactor for nutrient removal and resource recovery from wastewater in the paradigm of circular economy

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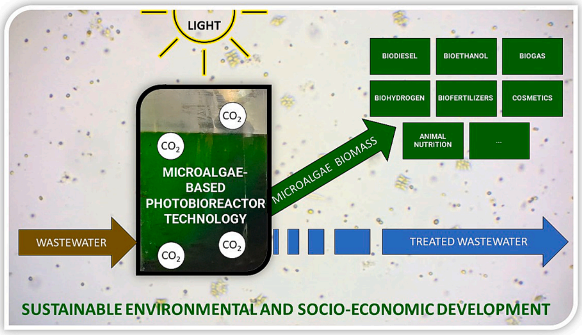
HIGHLIGHTS

- A new compound anti-stripping additive called DMA-NSDD was developed.
- The asphalt and its mixture performance was investigated by experiments.
- A comparison was made of the performance of seven asphalts and their mixtures.
- The performance of asphalt with DMA-NSDD and its mixtures was markedly improved.

GRAPHICAL ABSTRACT



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ABSTRACT

Every day, large quantities of wastewater are discharged from various sources that could be reused. Wastewater contains nutrients such as nitrogen or phosphorus, which can be recovered. Microalgae-based technologies have attracted attention in this sector, as they are able to bioremediate wastewater, harnessing its nutrients and generating algal biomass useful for different downstream uses, as well as having other advantages. There are multiple species of microalgae capable of growing in wastewater, achieving nutrient removal efficiencies surpassing 70%. On the other hand, microalgae contain lipids that can be extracted for energy recovery in biodiesel. Currently, there are several methods of lipid extraction from microalgae. Other biofuels can also be obtained from microalgae biomass, such as bioethanol, biohydrogen or biogas. This review also provides information on bioenergy products and products in the agri-food industry as well as in the field of human health based on microalgae biomass within the concept of circular bioeconomy.

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1. Introduction

The increase of population, industrialization and agricultural activities require high amounts of freshwater, generating 380 trillion·L/year of wastewater worldwide (Qadir et al., 2020). As a consequence, it is necessary a shift in wastewater treatment sector from its vision based on extensive technologies which focus on reducing pollutants to a new paradigm based on intensive technologies which frame within the circular economy principles (González-Camejo et al., 2021).

The current increasing popularity of circular economy tries to realign the linear take-make-use-dispose model of production and consumption with a circular model where waste can be regenerated and re-fed back into the production system (European Commission, 2015).

In this context, wastewater treatment sector could contribute to the transition towards the circular economy due to the large amounts of resources contained in wastewater, which could be recovered and have a secondary use in the economy (IWA, 2016; Sfez et al., 2019). Thus, wastewater would be no longer considered as waste but as a source of reclaimed water, energy and nutrients, such as nitrogen and phosphorus (Robles et al., 2020). Therefore, wastewater treatment plants (WWTPs) emerge as indispensable part of the circular economy due to the integration of resource recovery and clean water production (Leyva-Díaz et al., 2020).

Most of available methods for wastewater treatment are not completely sustainable from an environmental point of view. Currently, increasing attention has been paid to the recovery of different valuable compounds from wastewater. In this context, biological processes for wastewater treatment constitute a promising approach for this recovery. Among these processes, microalgae-based treatments have the capacity of integrating wastewater treatment and resource recovery by producing valuable biomass that presents a wide variety of applications. The use of microalgae for wastewater treatment is advantageous since it enables to remove the inorganic nutrients, such as nitrogen and phosphorus, from wastewater by fixing the carbon dioxide (CO_2) from the atmosphere in presence of light (Arora et al., 2021; Goswami et al., 2020; Goswami et al., 2021a), thus avoiding eutrophication and fostering water reclamation. Apart from nutrient assimilation, microalgae-based treatments are very beneficial due to significant bioaccumulation efficiency and high biomass productivity rate (Rawat et al., 2013).

After the wastewater treatment, microalgae biomass can be used as a

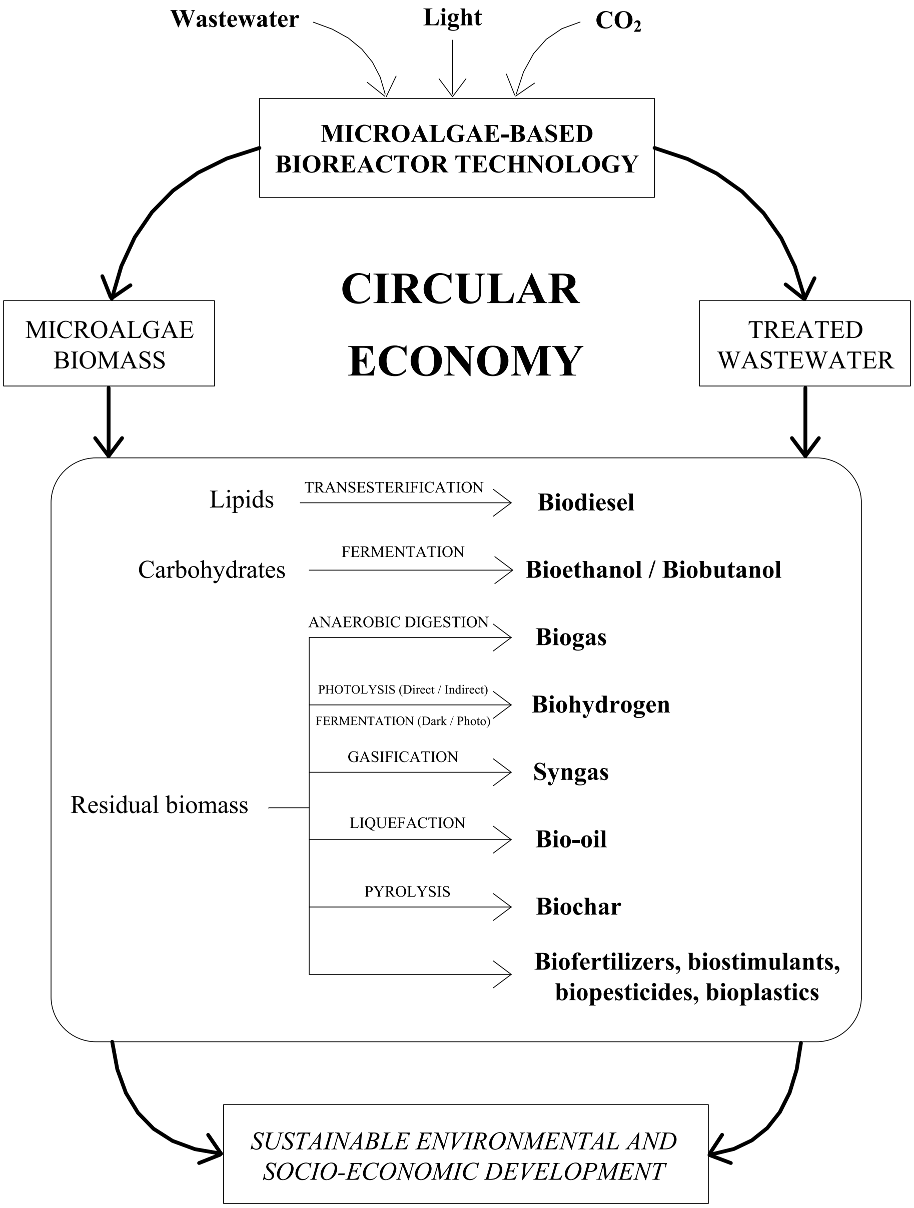

Fig. 1. Implementation of a circular economy model in wastewater treatment for resource recovery through microalgae-based bioreactor systems.

feedstock for the generation of bioenergy and value-added bioproducts (Goswami et al., 2021b; Ubando et al., 2020). Fig. 1 includes the possible applications of microalgae biomass as well as the production of treated wastewater.

Microalgae biomass obtained during wastewater treatment is rich in lipids (10–50 % in microalgae cells) and carbohydrates, which are used for production of different types of biofuels that have application in industries and transportation as energy source (Bhattacharya and Goswami, 2020; Da Silva et al., 2014), providing 42 % of current biofuels (European Union, 2018). In light of this, biodiesel can be obtained via transesterification of the lipid fraction, and bioethanol and biobutanol can be produced via fermentation of carbohydrates (glucose, starch and cellulose) (Javed et al., 2019; A. Kumar, 2021; Rajesh Banu et al., 2020; Sindhu et al., 2019). In general, the residual microalgae biomass produced after these extractions enables to obtain biogas from anaerobic digestion, biohydrogen from direct and indirect bio-photolysis or dark and photo fermentation processes, syngas from gasification, bio-oil from liquefaction and biochar from pyrolysis (Goswami, Mehariya, Obulisamy, et al., 2021; Nagarajan et al., 2020; Shahid et al., 2020). Finally, the residues from microalgae biomass could be also used as biofertilizers, biostimulants, biopesticides or renewable source of bioplastics (Acién Fernández et al., 2021; Arias et al., 2020; Tua et al., 2021).

In consequence, microalgae technology could encourage a shift from the traditional WWTPs to novel water resource recovery facilities (Seco et al., 2018). This constitutes an effective strategy for a sustainable environmental and socio-economic development by implementing the circular economy model (Mishra et al., 2019), which allows to recover value added resources from wastewater by using microalgae as well as treating wastewater pollutants. This could allow producing water to reuse and face the increasing resource demand, shortage of fossil resources in the near future and environmental concerns due to the generation of greenhouse gas such as carbon dioxide (Catone et al., 2021; Goswami et al., 2021c; Hussain et al., 2021). In this way, wastewater should be considered as an opportunity of achieving the 2030 Agenda for Sustainable Development within the circular economy paradigm (United Nations, 2017).

Thus, the present review focuses on the application of microalgae bioreactors for wastewater treatment and resources recovery in the circular economy model. This work includes the most relevant aspects of microalgae in wastewater sector. A special attention is given to the cultivation and adaptation of microalgae. Finally, the most significant applications of microalgae biomass are analysed.

2. Microalgae in wastewater

Wastewater versus freshwater, marine water or culture media have differences in chemical composition and physical properties. Even so, microalgae are able to grow and efficiently utilise the nutrients present in wastewater, as well as natural solar energy and carbon dioxide as a carbon source (Li et al., 2019). Wastewater treatment using microalgae can recover nutrients with focus on circular bioeconomy (Aditya et al., 2022; Chong et al., 2022).

Domestic wastewater typically includes both inorganic and organic matter. Organic compounds include proteins, amino acids, carbohydrates and fats. On the other hand, inorganic compounds include potassium, nitrogen, chlorine, sodium, calcium, phosphate and sulphur, making it suitable for microalgae growth (Abdel-Raouf et al., 2012; Hu, 2019). Depending on the physical and chemical properties of wastewater, it can be classified into several types. Their pH, nutrients or toxic substances can vary widely, influencing microalgae growth, nutrient removal and productivity (López Barreiro et al., 2015). However, these nutrients rarely match the optimal profile formulated in the culture media for microalgae. In fact, one of the critical issues in wastewater treatment with microalgae is the interaction between the nutrients present in the media and the nutrient profile of the microalgae (Li et al., 2019).

Microalgae cultures are an attractive solution for wastewater treatment as they have the capacity to use inorganic phosphorus and nitrogen for their growth, as well as producing oxygen during photosynthesis, producing a disinfectant effect by increasing the pH (Larsdotter, 2006). On the other hand, they are also used for the elimination of coliforms, as the environmental conditions for microalgae growth are unfavourable for these microorganisms (Abdel-Raouf et al., 2012).

The cultivation of microalgae in wastewater requires monitoring because, although microorganisms that favour the growth of microalgae, such as bacteria, viruses or protozoa, are found in wastewater, there are also pathogenic microorganisms that can be harmful to microalgae, leading to contamination of the wastewater (Abdel-Raouf et al., 2012; Javed et al., 2019; Mat Aron et al., 2021). Similarly, microalgae are able to tolerate very wide conditions of pH, temperature, salinity or carbon dioxide and are suitable for cultivation in wastewater (Mat Aron et al., 2021; Tang et al., 2020). Currently, tertiary biological wastewater treatments are very expensive, so microalgae cultures offer a solution to these treatments by removing inorganic nitrogen and phosphorus, and even some toxic compounds, present in wastewater or by reducing the biochemical and chemical oxygen demand (Abdel-Raouf et al., 2012).

The wastewater provides the nutrients needed for microalgae growth, thereby reducing production costs and greenhouse gas emissions (Li et al., 2019; Mat Aron et al., 2021). The reduction in emissions of these gases is due to the fact that the microalgae will absorb part of the carbon generated in wastewater treatment that would be released as carbon dioxide (Mat Aron et al., 2021).

Previous studies have shown that the symbiotic relationship between bacteria and microalgae allows microalgae to survive in wastewater as the bacteria protect them from toxic compounds (Oswald et al., 1957). In addition to removing excess nutrients and preventing eutrophication, the production of microalgae biomass as a raw material emerged (Fallahi et al., 2021). Some of the possible uses of microalgae biomass would be the production and extraction of lipids for subsequent biofuel production (Gentili, 2014) or its digestion to produce biogas (Passos et al., 2013). They can also be used in the production of antioxidants, animal feed, enzymes, carbohydrates, pigments, skin supplements or other bioproducts such as biopolymers, depending on the chemical composition of the biomass generated (Lutzu et al., 2021).

The use of microalgae for wastewater treatment has great advantages (Li et al., 2019; Whitton et al., 2015):

- Lower wastewater treatment costs.
- They can remove nutrients at very low concentrations, meeting increasingly stringent standards.
- Microalgae can harness sources of inorganic carbon (such as carbon dioxide) from the combustion of fossil fuels.
- The culture can be grown with limited nutrients, i.e., without the need for wastewater supplementation.
- Biomass collected after water treatment can be transformed into value-added products (biofuels, biogas, fertilisers, etc.).

Currently, most studies using microalgae for wastewater bioremediation have been conducted using laboratory-scale photobioreactors. However, it has been shown that thin-layer bioreactors could be used on a large scale, obtaining high biomass yields. However, these bioreactors are very shallow, so the volumes of wastewater they can process are smaller. For this reason, raceways are preferred, which allow a larger volume of wastewater to be treated, as well as having a low installation and operating cost (Morillas-España et al., 2020; Morillas-España et al., 2021).

Due to variations in culture conditions, the composition of a particular microalgae species may be different, although lipids and carbohydrates are usually the major components. There are many differences between microalgae species in terms of composition, components and their proportions (carbohydrates, lipids, proteins and nucleic acids) (Li et al., 2019).

One of the most commonly used microalgae species in wastewater treatment is *Chlorella sp*., which includes strains such as *C. sorokiniana* or *C. vulgaris* (Kesaano and Sims, 2014). Other species used are *Spirulina sp*., *Nannochloropsis sp*., *Dunaliella sp*., *Phaeodactylum sp*. or *Scenedesmus sp*., which includes widely studied strains such as. *S. obliquus* (Cai et al., 2013; Kesaano and Sims, 2014). Table 1 shows the different species depending on the ability to remove nitrogen and phosphorus in wastewater.

Some criteria should be considered when selecting the right microalgae strain to be used in wastewater treatment, such as high nutrient removal rate, high growth rate, high biomass productivity and good adaptation to different waters and climates. In case some of the criteria are not met, the strain with a fast growth rate is preferred, as it is usually related to its adaptability and robustness and nutrient removal capacity. Similarly, a fast growth rate compensates for low lipid content, resulting in a generally better biomass yield, leading to good wastewater treatment and higher lipid productivity (Li et al., 2019).

Other studies claim that the use of wastewater as a nutrient source for microalgae cultivation is currently the only economically viable way to obtain microalgae biomass for biodiesel production (Iasimone et al., 2018; Raheem et al., 2018).

3. Cultivation and adaptation of microalgae

Microalgae are able to adapt to adverse environmental conditions, such as extreme temperatures, wastewater or water with high salinity (Chew et al., 2019). Their growth depends on the combination of several parameters such as light and its photoperiod, the availability of nutrients or the temperature at which they are growing (Iasimone et al., 2018).

Most of the composition of the microalgae biomass will depend on the growth conditions of the culture, so it is important to take into account factors such as temperature, pH, nutrient availability and light intensity (Fattore et al., 2021). Likewise, depending on the species of microalgae we cultivate, these conditions will affect their growth and productivity (Mat Aron et al., 2021). For example, in the case of temperature, previous studies have shown that lipid productivity for *Scenedesmus obliquus* and *Isochysis galbana* is higher at higher temperatures, while the opposite is true for *Chlorella vulgaris* (Metsoviti et al., 2019), for which the optimal growth temperature is 30 °C (Okoro et al., 2019). On the other hand, at high light intensities, the species *Botrycoccus braunii* can produce 55 % of lipid biomass. Ruangsomboon (2012)obtained that the algae *B. braunii* algae is able to produce 57 % lipids at a light intensity of 538 μEm^{-2}s^{-1} with a yield of 0.45 gL^{-1}. It was found that at high light intensities (200 and 538 μEm^{-2}s^{-1}) algal growth was lower than at low light intensities (87.5 μEm^{-2}s^{-1}), but their lipid content and yield were higher. At low light intensities, George et al., (2014) obtained in their studies that the algae *Ankistrodesmus falcatus* produces 67.2 % lipids under a light intensity of 60 μEm^{-2}s^{-1} and a light/dark cycle of 12:12 h, while at a higher light intensity (150 μEm^{-2}s^{-1}) a lipid content of 31.9 % was obtained.

Table 1
Nutrient removal rates in wastewater using different microalgae species.

Microalgae	Nitrogen removal ratio (%)	Phosphorus removal ratio (%)	Reference
Tribonema sp.	89	73	(Cheng et al., 2020)
Synechocystis sp.	76	71	(Cheng et al., 2020)
Scenedesmus obliquus	94	99	(Fan et al., 2020)
Chlorella vulgaris	62	100	(Rinna et al., 2017)
Botryococcus braunii	63	100	(Rinna et al., 2017)
Scenedesmus sp.	98	98	(G. Y. Kim et al., 2017)
C. sorokiniana	42	89	(G. Y. Kim et al., 2017)
C. pyrenodiosa	87 – 89	70	(Hongyang et al., 2011)
Scenedesmus obliquus	57	100	(Álvarez-Díaz et al., 2017)
Botryococcus braunii	86	100	(Álvarez-Díaz et al., 2017)
C. sorokiniana	94	100	(Álvarez-Díaz et al., 2017)
Desmodesmus sp.	96	85	(Mehrabadi et al., 2017)

Next, an analysis of the different factors that most influence the growth of microalgae in natural systems will be made.

3.1. Nutrients

Different species of microalgae may require different nutrients or in different proportions. These conditions will also differ depending on whether it is an autotrophic, heterotrophic or mixotrophic culture, which are differentiated by the carbon source used (Khoo et al., 2020). Autotrophs produce biomass by needing an input of light, in the heterotrophic mode of cultivation, organic carbon is metabolised without the need for light, and finally, in the mixotrophic mode, the two are combined, assimilating both inorganic and organic carbon with light (Li et al., 2019).

A large mixture of inorganic compounds can be found in typical wastewater, including ammonium salts, calcium, sodium, magnesium, potassium, sulphur, chlorine, phosphates, bicarbonates and heavy metals. On the other hand, there is organic carbon, present as fats, carbohydrates, proteins, amino acids and volatile acids (Abdel-Raouf et al., 2012). Nitrogen and phosphorus concentration is considered a fundamental factor for microalgae growth as it directly influences their growth kinetics, as well as being related to lipid accumulation and nutrient removal (Iasimone et al., 2018). Some data on nitrogen and phosphorus content in different types of wastewaters are shown in the Table 2.

Nitrogen is the main nutrient for microalgae growth, accounting for between 1 and more than 10 % of the biomass. Therefore, a deficiency of this nutrient could lead to a reduction in the biomass yield of the microalgae culture (Khoo et al., 2020). Nitrates, ammonium and urea are mainly used as nitrogen sources, although other nitrogen compounds can also be used depending on the microalgae culture. This is the key

Table 2
Nutrients in different types of wastewaters.

Wastewater category	N (mg·L^{-1})	P (mg·L^{-1})	N/P	Reference
Municipal wastewater (secondary effluent)	47.6	8.0	5.9	(Almomani et al., 2019)
Swine wastewater	1180 (NH$_3$-N)	188	6.3	(Cheng et al., 2020)
Industrial wastewater	9 – 480	5 – 45	1.4 – 10.7	(Zheng et al., 2018)
Industrial wastewater	1.1 – 10.9	0.6 – 5.8	3.0 – 4.3	(Slade et al., 2004)
Agricultural wastewater	109 – 239	15.3 – 29.5	7.1 – 8.1	(Wang et al., 2010a)
Municipal wastewater (raw sewage)	40.7	5.7	5.9	(Wang, Min, et al., 2010)
Municipal wastewater	15 – 90	5 – 20	3.3	(Cai et al., 2013)
Municipal wastewater (secondary effluent)	34.5	2.5	13.8	(Ruiz-Marin et al., 2010)
Municipal wastewater (secondary effluent)	19.1 – 49.4	0.32 – 2.9	17.0 – 53.2	(Sukačová et al., 2015)
Municipal wastewater (secondary effluent)	23.1 – 35.4	3.8 – 4.9	6.1 – 7.2	(Su et al., 2011)

nutrient for the synthesis of nucleic acids and proteins (Fattore et al., 2021). The preferred form of nitrogen is thought to be ammonium, because its assimilation does not involve a redox reaction, requiring less energy. Therefore, microalgae tend to deplete ammonium earlier than nitrates. This phenomenon means that urban wastewater with high ammonium concentrations can be efficiently used for microalgae cultivation (Cai et al., 2013).

On the other hand, phosphorus is another important nutrient for algal growth as it influences the composition of the biomass, being directly related to the content of carbohydrates and lipids present (Khan et al., 2018). This nutrient is part of the DNA, RNA molecules (Fattore et al., 2021). Phosphorus is an important compound for the growth of photosynthetic microorganisms and constitutes about 1 % of the dry weight of microalgae on average (Borchardt and Azad, 1968).

However, in the natural environment there are limitations of these nutrients, which causes stress on the microalgae and their adaptation to it. One of the most studied effects is the lack of nitrogen, which leads to lipid accumulation in algal species such as *Nannochloropsis sp*. (Fattore et al., 2021). On the other hand, it also produces negative effects such as loss of photosynthesis capacity and therefore on biomass productivity. Both phosphorus and nitrogen can be considered as limiting factors in the growth of microalgae, and an excess of either in the wastewater or the culture medium could significantly influence the structure of microalgae (Barsanti and Gualtieri, 2005).

In a photobioreactor in which microalgae are grown using secondary effluent from domestic wastewater, very low nitrogen and phosphorus concentrations (between 10 and 15 mg/L and 0.5 and 1 mg/L, respectively) are generally found. It is important to take into account the choice of microalgae to be used in this type of system, as they must adapt adequately to the environment, as well as eliminate nutrients efficiently by accumulating lipids in the cells of the culture (Xin et al., 2010). Previous studies have shown that one of the microalgae species that can survive in low nutrient environments and therefore can adapt and grow well in secondary wastewater effluents is *Scenedesmus sp*. (Jiménez-Pérez et al., 2004).

In this way, microalgae can sequester carbon from carbon dioxide sources supplied by aeration when the C/N ratio in the wastewater or culture medium is inadequate based on the molecular formula of the algae ($C_{106}H_{263}O_{110}N_{16}P$) (Li et al., 2019; Stumm and Morgan, 1981). In a previous study, the influence of C/N ratio on biomass and lipid production and nutrient removal was tested, where results showed that organic matter promoted mixotrophic algal growth and nutrient removal (Gao et al., 2019). In addition, the addition of carbon dioxide contributes to pH regulation, thus improving nutrient removal by the microalgae (Park et al., 2011).

3.2. pH

This parameter also directly influences photosynthesis because microalgae are able to produce larger amounts of biomass in alkaline media, where carbon dioxide sequestration is more favoured. Generally, the optimal pH range for microalgae growth is between 6 and 8.7, although it is important to note that there are species for which the optimal pH and salinity are specific (Khoo et al., 2020).

It has previously been studied how high pH values can be positive for microalgae cultures at the time of harvesting, as they induce the phenomenon of self-flocculation (Iasimone et al., 2018). In this way, the sedimentation of the microalgae is favoured without using chemical flocculants. However, it should be noted that very high pH values also lead to the death of microalgae due to the accumulation of nitrites in the medium.

3.3. Temperature

Temperature control in the culture is important because extreme values could lead to inactivation of the microalgae. This is because high temperatures could reduce the rate of photosynthesis, while low temperatures could lead to a decrease in the kinetics of the cellular enzymatic process, thus reducing growth in the system (Huang et al., 2019; Khoo et al., 2020).

For most species, the optimum temperature range is between 20 and 30 °C, except for those that are able to survive extreme temperatures (Covarrubias et al., 2016; Suthar and Verma, 2018).

3.4. Light

Light is an especially important factor in microalgae cultivation as the process of photosynthesis affects the biochemical composition and the yield of biomass production. However, it is important to keep in mind that periods of darkness and a certain light intensity are needed for each species to avoid photoinhibition and to achieve optimal growth of the microalgae (Khoo et al., 2020).

Photolimitation occurs when the growth of microalgae is conditioned by insufficient light. In this case, increasing the light intensity (without reaching the saturation value) would improve the growth of the microalgae. On the other hand, photoinhibition occurs when the light intensity is higher than the saturation value (X. Chen et al., 2011; Wahidin et al., 2013). When this effect occurs, the light is too stressful and leads to reduced growth or even death of the microalgae. This effect can be counteracted by shortening the cyclic light/dark periods.

Other authors studied the optimum photoflux for various microalgae species, obtaining an optimum photoflux of approximately 100 $\mu Em^{-2}s^{-1}$ y 35 $\mu Em^{-2}s^{-1}$ for the microalgae species *Euglena gracilis* and *Amphidium* sp., respectively. Thus, they demonstrated that the optimal growth rate varies widely for different microalgae species (Kitaya et al., 2008).

Maximum photosynthetic efficiencies can be achieved when the light/dark period is close to the turnover time of the photosynthetic unit. Optimum light/dark regimes vary between 12:12 h and 16:08 h cycles for most cultures (Wahidin et al., 2013). Biomass productivity, growth rate and nutrient removal efficiency are related to the light intensity and photoperiod conditions to which the microalgae are exposed (Lee et al., 2015; Li et al., 2019). These requirements vary depending on the species, culture density and culture conditions.

Jabri et al. (2021) investigated the influence of light/dark cycles at constant temperature on the biomass concentration of a culture of *Nannochloropsis* QU130. They showed that light cycles produced a decrease in the biomass of the culture of 50 % (volumetric biomass productivity of 0.67 kg $m^{-3}day^{-1}$) compared to the crop under constant light conditions and continuous maximum volumetric biomass productivity of 1.1 kg $m^{-3}day^{-1}$). In another study, the biomass productivity of *C. reinhardtii* algae was evaluated by varying the light/dark cycles (flux density 220 $\mu molm^{-2}s^{-1}$), showing that microalgae growth is negatively affected by long periods of darkness (Takache et al., 2015). Lima et al. (2020) found that the growth rate of the algae *Nannochloropsis gaditana* increased from high to low flashing light periods, with the maximum growth rate under continuous light. However, his results show that at low and medium flashing light periods the total lipid content of the culture increased.

The importance of light in microalgae cultures can be seen in the results obtained by Wahidin et al. (2013) in the study of the effect of light intensity and photoperiod on the growth of the microalgae *Nannochloropsis* sp., grown in sterile seawater enriched with marine microalgae medium. The study used different light intensities at 23 °C and an initial pH of 8.2 (Wahidin et al., 2013). Table 3 shows how light intensity influences the specific growth rate, the optimal light intensity for this microalgae species was found to be 100 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$ in 16:8-hour light/dark photoperiods. It is important to note that in the laboratory it is relatively easy to control light penetration. In a large-scale microalgae wastewater treatment system, it will be more difficult to achieve good light penetration due to the turbidity of the medium and the self-shading of the microalgae biomass produced (Fallahi et al., 2021).

Table 3
Results of maximum cell density and specific growth rate of Nannochloropsis sp. obtained by Wahidin et al. (Wahidin et al., 2013).

Light intensity ($\mu mol \cdot m^2 \cdot s^{-1}$)	Photoperiod (cycle L:D, h)	Maximum cell density ($\times 10^7$ cells/ml)	Specific growth rate μ ($days^{-1}$)
50	24:0	3.0	0.299
	18:6	2.1	0.276
	12:12	1.3	0.236
100	24:0	3.8	0.308
	18:6	6.5	0.339
	12:12	3.0	0.268
200	24:0	3.6	0.264
	18:6	4.5	0.288
	12:12	4.8	0.299

3.5. *Agitation or mixing*

In the cultivation of microalgae, it is very important that the distribution of nutrients, biomass and temperature, among others, is homogeneous, as this avoids the appearance of dead or shaded areas, facilitating the distribution of light and preventing cell sedimentation. In the same way, the photosynthesis of the microalgae is favoured as they are in continuous movement between areas of light and shade, thus improving biomass production (Iluz and Abu-Ghosh, 2016; P. L. Show et al., 2017). However, it should be noted that excessive agitation could lead to cell damage.

4. Circular economy

Microalgae can be easily integrated into a process where carbon dioxide emissions are zero and wastewater is remediated, using the biomass produced as a source of energy or future biofuels, contributing to sustainable development (Serrà et al., 2020).

4.1. *Use of algal biomass in the agro-food industry*

4.1.1. *Human health, cosmetics and food*

Microalgae are being commercialised in the nutraceutical market, where they are included as ingredients in beverages and functional foods (Wells et al., 2016). Due to the numerous nutritional benefits of microalgae (being a great source of polyunsaturated fatty acids, proteins, vitamins, amino acids, minerals, etc.), they are considered to be one of the most auspicious sustainable food sources (Rahman, 2020). In human and animal nutrition, the most used microalgae species are green algae such as *Chlorella vulgaris*, *Dunaliella salina, Isochrysis galban or Haematococcus pluvialis*. On the other hand, cyanobacteria have also demonstrated a wide potential, with *spirulina (Arthrospira*) being one of the most suitable species (Kholssi et al., 2021).

Microalgae are used in different Asian countries, especially in China, Japan and Korea, as a primary food source. It is also used in other continents for its high nutritional value, but only a limited number of microalgae species can be used in human food due to strict food safety regulations and market demand (Alam et al., 2020). In addition to these obstacles, microalgae biomass from wastewater treatment is considered a waste product in many countries and cannot be used as a human dietary supplement.

According to Regulation (EU) 2015/2283 (Comisión Europea, 2015), algae are included as novel foods that require prior authorisation granted by the competent authorities in order to be placed on the market. On the other hand, the CTN 319 committee, which focuses its activity on monitoring the standards and technical reports that are being developed within the European Committee for Standardisation, is currently working on the processing of algae and specifications for food, feed and non-food use (cosmetics, pharmaceuticals, biodiesel, etc.).

Microalgae have great potential in the field of pharmaceuticals. High-value bioactive molecules are obtained naturally from microalgae by means of a biological process that cannot be reproduced chemically. Microalgae produce antibiotics of various chemical types, as well as neurotoxic and hepatotoxic compounds (Alam et al., 2020). One of the main pigments in microalgae is chlorophyll, which has healing and anti-inflammatory properties and is therefore often used in food and pharmaceuticals (Ferruzzi and Blakeslee, 2007). Another natural pigment found in microalgae biomass is carotenoids, which are responsible for a wide variety of dyes. These dyes are used in foods such as ice cream, sweets, soft drinks and dairy products, and even in some cosmetics such as eyeliners and lipsticks (Spolaore et al., 2006).

In the cosmetics industry microalgae are widely used due to their adaptability. Some species are used as dyes, to even out the skin or to protect and increase collagen formation in the skin (Yarkent et al., 2020). The main cosmetic products based on microalgae are refreshing care products, anti-ageing or regenerative creams, sun protection creams or hair care products (Kholssi et al., 2021).

4.1.2. *Biofertilizers*

In the search for ecological alternatives in agriculture, biofertilizers have been developed to reduce the use of chemical fertilisers. Biofertilizers formulated from microalgae stand out among the many biofertilizers available, as they increase crop yields while improving soil fertility (Guo et al., 2020). Several studies have confirmed the increased productivity of crops where biofertilizers based on microalgae or cyanobacteria are applied, as well as improving the nutritional value of the product obtained (Dineshkumar et al., 2018). By introducing this type of biofertilizer into the soil, carbon enrichment occurs, which improves the quality of the soil and the quality of the nutrients present (Guo et al., 2020).

In addition to all the advantages mentioned above, it is important to highlight that microalgae crops represent an alternative to physical and chemical processes for the elimination of environmentally harmful compounds. In this case, the use of microalgae-based biofertilizers is based on their renewability and capacity to partially replace and reduce part of the chemical fertilisers added to the soil, such as pesticides (Ali et al., 2021; Kholssi et al., 2021).

4.2. *Energy recovery of microalgae biomass*

The integration of microalgae biorefineries to produce high-value products could boost the economy in favour of cost-effective biofuel production (Kholssi et al., 2021). The main biofuels obtained from microalgae are bioethanol, biodiesel, biohydrogen and biomethane (Arun et al., 2020). Some of them will be discussed below.

4.2.1. *Gaseous fuels*

In addition to liquid biofuels, gaseous fuels such as biogas or biohydrogen can be produced from the biomass of microalgae used for urban wastewater treatment (Goswami, Mehariya, Obulisamy, et al., 2021).

For biohydrogen production, sugars produced in algae cells after being subjected to nutrient stress conditions can be used for dark fermentation by bacteria (Batista et al., 2015). It can also be produced naturally by green microalgae through direct and indirect photolysis. In the process of direct photolysis, by splitting water into protons and electrons by sunlight, biohydrogen is produced through a photochemical reaction (K. Y. Show et al., 2018). However, direct photolysis has lower productivities compared to other biohydrogen production methods because this compound can only be obtained transiently. This disadvantage is caused by the simultaneous production of oxygen by the microorganisms, which suppresses the production of photosynthetic hydrogen (Kumar Sharma et al., 2021; K. Y. Show et al., 2018). Biohydrogen is a clean and environmentally friendly biofuel produced by using cyanobacteria or microalgae, although it has certain limitations for its production on an industrial scale (the low biomass fixation rate, its production cost, the presence of oxygen, and the complex biorefinery) (Bhattacharya and Goswami, 2020).

Batista et al. (2015) investigated the production of biohydrogen from microalgae grown in wastewater. They used three species of microalgae (*Chlorella vulgaris, Scenedesmus obliquus* and a consortium of natural algae) which were subjected to nutritional stress and then fermented for biohydrogen production. The production obtained for each of the species studied was 56.8, 40.8 and 46.8 mL H_2/g of volatile solids.

For biogas production, anaerobic microorganisms are used to digest the microalgae biomass (Bhattacharya and Goswami, 2020). Biomethane is a component of biogas that is produced by removing carbon dioxide and hydrogen sulphide. Biomethane is used as bioelectricity in industry, transport vehicles and can also be used as a domestic fuel (Goswami et al., 2021c). Caporgno et al. (2015) obtained biomethane yields of 346 and 415 mL/g, for the microalgae species *Chlorella kessleri* and *Chlorella vulgaris*, respectively, grown in primary wastewater.

4.2.2. *Bioethanol*

This microalgae-based biofuel is considered third generation. It has already been demonstrated that their production can be produced sustainably on a large scale (N. Hossain et al., 2019). Bioethanol can be produced from the carbohydrates contained in algae biomass obtained after urban wastewater treatment (Goswami et al., 2022; Srivastava et al., 2021).

One of the advantages of microalgae biomass in bioethanol production is that it does not contain lignin in its cell wall and therefore no advanced pre-treatment is required in the biorefinery process (Bhagea et al., 2019). Nowadays, several methods have been developed to produce biofuels, such as thermochemical, chemical or biochemical conversion or direct combustion (Culaba et al., 2020). Once the sugar present in the microalgae is extracted, it is converted into simple fermentable sugars, which can be transformed into bioethanol through microbial fermentation using *Saccharomyces cerevisiae* (Bhattacharya and Goswami, 2020), Singh and Olsen (2011) obtained bioethanol yields of 4 to 10 g/l from microalgae residues after lipid extraction.

In terms of quality, microalgae-based bioethanol has advantages over conventional bioethanol. Therefore, in the future, microalgae-based bioethanol could be available on a commercial scale (Choo et al., 2020).

4.2.3. *Biodiesel production from microalgae oil*

Microalgae are mainly composed of carbohydrates, lipids and proteins. In some cases, the lipid content is high and can be extracted and transformed into biodiesel by chemical or enzymatic transesterification (W. H. Chen et al., 2015). This extraction represents about 30 % of the cost of biodiesel production, so it is of great interest to optimise the process (V. Kumar et al., 2019). As shown in Table 4, the average lipid content of microalgae is between 20 and 50 % of their dry weight (Sati et al., 2019).

Table 4
Lipid content of some microalgae species (Chisti, 2007; Ramluckan et al., 2014; Scott et al., 2010).

Microalgae	Lipid content (% dry weight)
Botryococcus braunii	25 – 75
Chlorella sp.	28 – 32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16 – 37
Dunaliella primolecta	23
Ellipsodium sp.	27
Hormidium sp.	38
Isochrysis sp.	25 – 33
Monallanthus salina	greater than 20
Nannochloris sp.	20 – 35
Nannochloropsis sp.	31 – 68
Neochloris oleoabundans	35 – 54
Nitzschia sp.	45 – 47
Phaeodactylum tricornutum	20 – 30
Scenedesmus sp.	20 – 21
Schizochytrium sp.	50 – 77
Tetraselmis sueica	15 – 23

The most commonly used solvents for conventional lipid extraction methods are ethanol, *n*-hexane, dimethyl ether, 1-butanol and chloroform/methanol mixtures, *n*-hexane/isopropanol, among others (Mat Aron et al., 2021). These methods continue to be used today due to their high recovery yields of lipids from microalgae, as well as their ease of use (Khoo et al., 2020). The ideal solvent must meet certain requirements: low boiling point, insoluble in water, high level of specificity for the intracellular lipids to be extracted, volatile, non-toxic, inexpensive, etc. (Garoma and Janda, 2016; Sati et al., 2019). Generally, ethanol is used as a conventional organic solvent. It allows the extraction of 20 to 90 % of the lipids contained in the microalgae (Chisti, 2007). Ramluckan et al. (2014) performed lipid extractions using the Soxhlet extraction method obtaining lipid yields of 11.76 % for the 1:1 chloroform:ethanol binary mixture and 10.78 % for the best single solvent, chloroform.

Due to the environmental problems presented by organic compounds, new biodegradable, renewable and less toxic solvents, known as green solvents, have emerged. One of the most used solvents of this type is supercritical carbon dioxide. Table 5 shows some lipid recovery data using this solvent. Its effectiveness is higher compared to Bligh & Dyer's method (Al-Otoom et al., 2014). Lipid yields by weight of 34 % for *Schizochytrium limacinum*, 33 % and 45 % for *Nannochloropsis* sp. or 51 % for *Crypthecodinium cohnii* using SC-CO_2 under optimum conditions have been obtained (Patel et al., 2020).

Other emerging green solvents are ionic liquids. These solvents are are suitable for the extraction of microalgae oil, (Choi et al., 2014; Khoo et al., 2020) achieving yields between 90 and 100 % in both dry and wet microalgae (Orr and Rehmann, 2016). In the experiments carried out by Y. H. Kim et al. (2012), it was found that the total lipid content extracted by weight from *C. vulgaris* by the conventional method of Bligh & Dyer was 10.6 % compared to 12.5 % obtained using ionic liquids. Besides, in Du et al. (2013)'s research, the extraction yield of the exchangeable solvent (*N*-ethylbutylamine) was 31 % higher than the Bligh & Dyer extraction method.

Biodiesel is a diesel fuel derived from vegetable or animal oils, generally composed of long-chain fatty acid methyl esters. The detailed chemical composition, in particular the chain length of the fatty acids, depends on the source of the oil. Biodiesel is generally produced from oil by chemical transesterification, where the glycerol to which the long-chain fatty acids in the source oil are esterified is replaced by another alcohol (Scott et al., 2010).

Table 5
Lipid recovery data from microalgae using supercritical CO_2 (Mat Aron et al., 2021).

Species	Temperature (°C)	Pressure (MPa)	Lipid recovery (%)	Reference
Brevundimonas diminuta	50	35	3.7	(Gomez-Gomez et al., 2020)
C. vulgaris	50	45 – 75	97.0	(Obeid et al., 2018)
N. oculata	50	75	83.0	(Obeid et al., 2018)
Chlorella vulgaris	60	60	75.2	(S. Hossain et al., 2020)
P. valderianum	40	35	30.5	(Chatterjee and Bhattacharjee, 2014)
Nanocloropsis oculata	60	40	100.0	(Crampon et al., 2013)
S. oblicuo	50 – 80	30 – 80	77.9	(Lorenzen et al., 2017)
S. obtusiusculus	50 – 80	30 – 80	44.0	(Lorenzen et al., 2017)

Microalgae are considered as a high energy and low cost biofuel feedstock, being a sustainable renewable source alternative to fossil fuels (Khoo et al., 2020; Ríos et al., 2012). The use of this biodiesel would lead to a decrease in gas emissions, as it provides the same performance as biodiesel from petroleum and does not contain sulphur (Chew et al., 2017). It is important that the microalgae used for production have a high oil productivity rate, which means that the mass of oil produced per unit volume per day is high. This in turn depends on the growth rate of the microalgae and the oil content of the biomass (Chisti, 2007). Caporgno et al. (2015) obtained biodiesel yields for *C. vulgaris* biomass of 11.3 g/100 g volatile solids.

Furthermore, the cultivation of microalgae has certain advantages over other energy cultures, which makes the conversion of microalgae into biodiesel profitable for industries (Koberg et al., 2011; Schenk et al., 2008):

- Certain species of microalgae do not require fresh water, which is an increasingly scarce commodity, and can grow in salt water or even polluted water, such as sewage.
- They contain more energy per unit weight than other biofuel crops and grow faster.
- They do not displace food crops because they can grow on land that is not suitable for food production.
- From microalgae crops, not only biofuels are produced, but other by-products of economic interest can also be obtained.
- They can be produced in batches throughout the year and can provide a continuous supply of oil.

However, the large-scale production of biodiesel from microalgae presents some obstacles, such as the large amount of water and nutrients required. Therefore, the ideal approach would be to combine microalgae cultivation with wastewater bioremediation, thus reducing the cost of production and the environmental impact (Gao et al., 2022; Khoo et al., 2020).

In addition to being biodegradable and non-toxic, biodiesel produces fewer emissions of hydrocarbons, carbon monoxide, carbon dioxide and particulates into the environment because it is made up of fatty acid methyl esters (FAME). However, emissions of nitrogen oxides are increased compared to diesel (Koberg et al., 2011; Valente et al., 2010). The presence of high levels of saturated fatty acids in the biomass used in the production of biodiesel indicates that the product could be stored for long periods of time as it would have good resistance to oxidation (Cancela et al., 2019; Koberg et al., 2011).

An economic study for a microalgae biodiesel production plant in China was carried out by Sun et al. (2019). Under the plant design conditions they have established, 6.7 t/day of microalgae biodiesel would be produced, generating USD 2.4 million profit per year. The unit cost of biodiesel production in 2019 was USD 2.3/kg, estimating the future cost for 2023 to be USD 1.9/kg, with costs decreasing as cumulative biodiesel production increases. This type of biofuel is one of the most promising. This is due to the considerable potential of the feedstocks such as the growth characteristics of microalgae and their high oil content.

5. Research needs and future directions

Microalgae are grown in photobioreactors as well as in open ponds, without competing for soil with other crops. Their photosynthetic efficiency (6 %) is the highest among biomass types and carbon dioxide absorption reaches 1.7 t CO_2 per t of microalgae produced (Stengel and Connan, 2015). In addition to reducing the level of CO_2 in the atmosphere during cultivation, biodiesel produced from microalgae helps reduce the risk of global warming by reducing CO_2 emissions into the atmosphere. Biodiesel was found to reduce CO_2 emissions by 78.5 % compared to petroleum diesel fuel (Voloshin et al., 2016).

One of the challenges of efficient large-scale microalgae cultivation is the amount of water and nutrients required by the microalgae. Therefore, it has been suggested to use wastewater for microalgae cultivation, bioremediating this water and alleviating excessive water consumption. However, the characteristics of such wastewater need to be taken into account, as it may require pre-treatment to remove microbes that compete with the microalgae for nutrients (Udayan et al., 2022). Another problem is that not all emerging pollutants or heavy metals in wastewater can be completely removed by microalgae (Chai et al., 2021). It should also be considered that a very high turbidity of the wastewater to be treated can lead to a high level of turbidity (Amenorfenyo et al., 2019).

Another obstacle in the commercialisation of microalgae-based products are techno-economic considerations, indicating the need for large initial investments for the cultivation and harvesting of microalgae. This cost could be reduced by solving challenges present at each stage of the product. For example, the main challenges in terms of the physical methods employed in the process are the production time and the high amount of energy required, so more effective microalgae biomass harvesting techniques are needed (Udayan et al., 2022). Microalgae biomass presents many possibilities in fields such as medicine, the food industry and biofuels. Even so, there is still a need for rules to regulate its commercialisation, making it possible to incorporate new products into the market as these products have many benefits for the environment and human health.

To ensure the economic and environmental viability of microalgae-based product development processes, further studies are needed to develop more robust microalgae cells. In the same way, it is necessary to develop synergistic treatment methods integrating microalgae to increase the performance of wastewater bioremediation(Chai et al., 2021). Further studies on the integration of wastewater treatment systems using microalgae into existing treatment systems are desirable to provide more opportunities for their implementation in conventional wastewater plants.

6. Conclusions

Microalgae-based wastewater treatment systems have attracted considerable attention due to its environmental benefits. These systems facilitate the recovery of nutrients from wastewater as well as being CO_2 sinks, contributing to the reduction of the greenhouse effect. This allows to introduce the circular economy principles in the wastewater treatment sector. In addition, sustainable bio-based products can be efficiently obtained from microalgae biomass. These products that can be obtained depend on the biochemical composition of the microalgae biomass and on the transformation methods. Some of the products produced from microalgae are liquid or gaseous biofuels, biofertilizers, human health products, cosmetic products or food supplements.

CRediT authorship contribution statement

Verónica Díaz: Conceptualization, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing. **Juan Carlos Leyva-Díaz:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Mari Carmen Almécija:** Methodology, Data curation, Visualization, Writing – original draft, Writing – review & editing. **José Manuel Poyatos:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **María del Mar Muñío:** Conceptualization, Project administration, Funding acquisition, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Jaime Martín-Pascual:** Conceptualization, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This work corresponds to a review paper

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