Journal of Environmental Management 293 (2021) 112792

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Bio-products from algae-based biorefinery on wastewater: A review

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ARTICLE INFO

Keywords:
Microalgae
Wastewater
Circular bioeconomy
Bio-based products

ABSTRACT

Increasing resource demand, predicted fossil resources shortage in the near future, and environmental concerns due to the production of greenhouse gas carbon dioxide have motivated the search for alternative 'circular' pathways. Among many options, microalgae have been recently 'revised' as one of the most promising due to their high growth rate (with low land use and without competing with food crops), high tolerance to nutrients and salts stresses and their variability in biochemical composition, in so allowing the supply of a plethora of possible bio-based products such as animal feeds, chemicals and biofuels. The recent raising popularity of Circular Bio-Economy (CBE) further prompted investment in microalgae, especially in combination with wastewater treatment, under the twofold aim of allowing the production of a wide range of bio-based products while bioremediating wastewater. With the aim of discussing the potential bio-products that may be gained from microalgae grown on urban wastewater, this paper presents an overview on microalgae production with particular emphasis on the main microalgae species suitable for growth on wastewater and the obtainable bio-based products from them. By selecting and reviewing 76 articles published in Scopus between 1992 and 2020, a number of interesting aspects, including the selection of algal species suitable for growing on urban wastewater, wastewater pretreatment and algal-bacterial cooperation, were carefully reviewed and discussed in this work. In this review, particular emphasis is placed on understanding of the main mechanisms driving formation of microalgal products (such as biofuels, biogas, etc.) and how they are affected by different environmental factors in selected species. Lastly, the quantitative information gathered from the articles were used to estimate the potential benefits gained from microalgae grown on urban wastewater in Campania Region, a region sometimes criticized for poor wastewater management.

1. Introduction

On December 2020 human-made materials outweigh Earth's entire biomass (Elhacham et al., 2020). As the global effect of humanity accelerates, also the demand for food, energy and materials is expected to grow in the next decades: 60% more food, 50% more energy and 40% more water by 2050 (FAO, 2015). Therefore, together with lowering consumers' footprint, especially in developed countries, it is becoming imperative to explore alternatives to the alarmingly depleting of fossil resources. The solution is as old as life on earth: the photosynthesis. Using plant biomass for production of energy and added value products can help ensure sufficient supply of food for all, reduce dependence on non-renewable resources as well as help mitigate and adapt to climate change. This broad concept/model encompassing the production and use of biological resources, products, and processes to replace fossil resources and/or sustainably provide goods and services is referred as bio-based economy or bioeconomy (Bugge et al., 2016). In this context, microalgae have received a great deal of interest (Rumin et al., 2020) representing an emerging biological resource of great importance for its potential able to produce high-value products such as animal feeds, foods (supplements, nutraceuticals, vitamins, anti-oxidants, etc.), chemicals (cosmetics, biodegradable plastics, cosmeceuticals, etc.) and bio-fuels. Although the commercial production of microalgal products is still in its infancy, microalgal biotechnology has emerged due to the great diversity of the products that can be developed from the biomass (Dolganyuk et al., 2020; Hamed, 2016) thus raising the role of microalgae feedstock in the biobased economy (Vigani, 2020). Microalgae are microscopic plants which are naturally found in freshwater and marine environment and that can be grown using water resources through photosynthesis. They convert sunlight, CO_2 and nutrients into oil and biomass from which various biologically valuable products (proteins, lipids, polysaccharides, pigments and vitamins) can be obtained (Dolganyuk et al., 2020). Microalgae have been used as a human food source

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https://doi.org/10.1016/j.jenvman.2021.112792
Received 31 December 2020; Received in revised form 12 May 2021; Accepted 13 May 2021

Abbrevation index

ADE	Anaerobic digestion effluent
ADPP	Anaerobic digestate pulp-paper
ADMW	Anaerobic digestate municipal wastewater
BioH_2	Biohydrogen
BOMW	Bleached olive-oil wastewater
BP	Biogas production plants
CBE	Circular bio-economy
CH_4	Methane
CM	Cattle manure
CO_2	Carbon dioxide
CSM	Chicken manure supernatant
DW	Dry weight
EU	European Union
FE	Fermentation effluent
H_2	Hydrogen
HRAP	High rate algal ponds
HRT	Hydraulic retention times
HTC	Hydrotermal carbonization
HTL	Hydrothermal liquefaction
ISTAT	(Istituto nazionale di STATistica)
MAAS	Microalgae integrated with activated sludge
MS	Maize silage
MUFA	Monounsaturated fatty acids
MW	Municipal wastewater
N	Nitrogen
N+	Nutrient repletion
N	Nutrient depletion
P	Phosphorus
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PS	Primary sludge
PUFA	Polyunsaturated fatty acids
RED	Renewable Energy Directive
RS	River sediments
SFA	Saturated fatty acids
UWST	Urban secondary wastewater
WS	Wastewater sludge
WWTP	Wastewater treatment plant

or nutritional supplements for hundreds of years (García et al., 2017). Early experiments into the mass cultivation of microalgae may be traced to the 1940s and quickly publicized as the food source that could help feeding the ever-growing population (García et al., 2017). Later, with fluctuation in oil prices and energy dependence on foreign nations, microalgae were "revisited" for their potential to produce biofuels (Borowitzka, 2013; Mata et al., 2010). The meritorious sustainability attributes of microalgae-based production rely on the fast growth rate compared to the terrestrial crops, high protein content, and the ability to use non-arable resources for growth (IEA, 2017). For these reasons, in its relevant section (Part A of Annex IX), the Renewable Energy Directive (RED-II) lists microalgae as a feasible alternative to overcome the disadvantages of first and second generation biofuels (European Parliament and Council of the European Union, 2018). While first generation biofuels (i.e. food crop-based biofuels) received lukewarm reception due to their challenges at net energy and climate change benefit as well as competition of feedstock for food and fuel production, second generation biofuels (i.e. biofuels coming from woody biomass) remain not yet economically viable at commercial scale as they require sophisticated and expensive technologies (Mat Aron et al., 2020; Naik et al., 2010). These concerns have increased the interest in advanced generation biofuels from non-food feedstocks, such as microalgae, which potentially offer greatest opportunities in the longer term. However, the production of microalgae biomass for extraction of biofuels, is generally limited to areas with enough solar radiation, water, and nutrients, i.e. is an energy-intensive process; as a consequence, the price of microalgae-based biodiesel remains extremely high compared to its fossil equivalent (Alam et al., 2012; Bošnjaković and Sinaga, 2020). Moreover, Feng et al. (2016) estimated that, on average, 3494 kg freshwater is required to produce 1 kg microalgal biodiesel, therefore, the intensive use of freshwater to grow microalgae for bioenergy purpose on large scale may threaten freshwater availability in the future. By contrast, recycling wastewater to grow microalgae may drastically reduce water utilization to almost zero (Feng et al., 2016).

1.1. The circular bio–economy framework

The recent raising popularity of Circular Economy (hereinafter referred as CE), attempting to realign the linear take–make–use–dispose model of production and consumption with a circular model where residues and waste can be regenerated and re-fed back into the production system (European Commission, 2015; Ghisellini et al., 2016), further prompted investment in microalgae biofuels, some of which involved combined wastewater treatment. With two million tons of sewage and other effluents drain into the world's waterways every day, wastewater has been considered a serious problem for a long time (UNESCO, 2017). However, a paradigm shift, brought by the CE framework, has led to a new concept of wastewater, not as a problem but as a source of energy and other precious resources, including water itself. Globally, the most prevalent water quality problem is eutrophication, a result of high-nutrient loads (mainly phosphorus and nitrogen), which substantially impairs beneficial uses of water. The potential of using algae to bioremediate waste, including nutrients, metal, carbon dioxide and organic pollutants, has been recognized over many decades (Stiles et al., 2018). In 1950s, pioneer studies already showed that microalgae may be critical microorganism in wastewater treatment: directly through the uptake of organic and inorganic nutrients from waste and indirectly through the oxygenation of wastewater for aerobic microbes to further breakdown the waste (Paddock, 2019). In other words, they can reduce nutrient load in wastewater as they utilize nitrogen and phosphorous present in wastewater owing to their phycoremediation acumen. In a CE framework, this system has a twofold advantage: microalgae bioremediate nutrients in wastewater, thus avoiding eutrophication and promoting water recycle and, at the same time, using undiluted wastewater to grow microalgae allows to save freshwater and nutrients (Feng et al., 2016). In this sense, microalgae grown on wastewater are situated at the intersection of circular economy and bioeconomy and can be considered real factories (or biorefineries) capable of producing water to reuse as well as of recovering value added resources from wastewater (Goswami et al., 2020; Hussain et al., 2021). With the principles of bioeconomy and the principles of CE walking hand in hand, a new concept known as 'Circular bioeconomy' (hereinafter referred as CBE) has recently emerged signifying the convergence of circular economy and bioeconomy agendas (Hadley Kershaw et al., 2020; Santagata et al., 2021). (Integrated) biorefineries easing the use of wastes and facilitating the combined production of high value products, as microalgae grown on wastewater, are considered an important part of the CBE (Stegmann et al., 2020). The major objectives of research towards successful biorefineries are focused on identifying critical factors for large-scale development and deployment of microalgae that can achieve targeted levels of algal biomass productivity and composition and conversion efficiencies. Although microalgae-based biofuels and bio-product applications and their associated promises and challenges have been the subject of a number of recent literature reviews (Borowitzka and Moheimani, 2013; Dolganyuk et al., 2020; Hamed, 2016; Vigani, 2020; among others), to the best of Authors knowledge, there are no literature reviews discussing the potential bio-products may be gained from microalgae grown on urban wastewater.

1.2. The goal of the present study

With the attempt to cover this gap while taking into account the CBE concept, this study aims to perform a comprehensive literature assessment on the obtainable bio-based products, and to identify the research issues that need further investigation. For this scope, the study was organized according to the following points:

i) to identify those microalgae species that are suitable candidates for growing on urban wastewater,
ii) to improve the understanding of the main mechanisms driving formation of microalgal products (such as biofuels, biogas, etc.) and
iii) how are they affected by different environmental factors in selected species.

The study ends with a discussion on the current state of the art on the topic and an example of potential benefits gained by applying the wastewater grown microalgae concept to a real case. The rest of the article is organized as follows. Section 2 describes the methodology adopted for the analysis of publications, as well as the inclusion and exclusion criteria. Section 3 and Section 4 present the results and a discussion on the prospects and challenges to implement microalgae on urban wastewater have also been emphasized in this study. Based on the gained results, the last portion of the review is dedicated to an estimation of environmental advantages potentially gained by the implementation of this system in Campania Region. Finally, Section 5 presents the main conclusions and discusses the limitations of this study and the scope for future research.

2. Materials and methods

In order to fulfil the proposed objectives, an approach was developed consisting of several stages, as illustrated in Fig. 1. This work was elaborated using the bibliometric database Scopus (www.scopus.com), the largest abstract and citation database of peer-reviewed literature. To enable a systematic and thorough review of the existing literature, several steps were followed. The first was the choice of keywords. To avoid limiting the search and thus obtain a more comprehensive set of publications, the terms “Microalgae” OR "Algae" AND "Municipal" OR "Urban" AND "Wastewater" AND "Biofuel" OR "Biogas" OR "Biodiesel" OR "Biorefinery" OR "Bioplastics" OR "Fertilizers" OR "Cosmetics" OR "Food" OR “Feed”. This selection resulted in 472 publications, including research articles, review articles, conference articles, book chapters, and editorials. Later on, a refinement of the search was accomplished by firstly selecting a time interval between 1992 and 2020, as in 1991 the Directive 91/271/EEC concerning urban wastewater treatment was adopted (European Commission, 1991). This Directive aimed to protect the water environment from the adverse effects of discharges of urban wastewater and to establish the processes of collection, treatment, and discharge of wastewater.

Fig. 1 summarizes the inclusion criteria used for the selection of publications in this work. First of all, only articles and reviews were

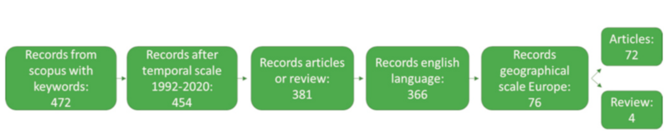

Fig. 1. Flow diagram for literature search.

selected, excluding conference papers, book chapters etc. Subsequently, only publications of European countries in English language were taken into consideration. The result of this selection yields 70 articles and 6 reviews.

The choice of focusing on the European geographical scale relies on the fact that, according to IEA (2017) Report on the State of Technology Review – Algae Bioenergy, more than half of the total commercial and research operations aimed at algae–based commodities are located in Europe (as for 2017).

The references cited in the selected publications were used as secondary sources; however, this resulted in only a few articles, which may indicate the wide-ranging of the initial research. The largest part of the selected 76 articles and reviews were published in the last 5 years, making up the final body of articles for which a more detailed content analysis was carried out.

A further screening of the articles was performed based on title, abstract and keywords: out of these 76 articles, 43 were inherent to topic while 33 were excluded because they were not related to the topic or did not present useful data for research. A content analysis of all these articles, based on careful reading, was then conducted to gather and analyse information on the production of products deriving from microalgae grown on urban wastewater.

The review focused on: aims of the studies, breakdown in product groups, factors influencing products yields. The type of urban wastewater to be used as substrate as well as the energy or energy–related products (biodiesel, bioethanol, biogas, biohydrogen, biomethane, biofertilizers and bioplastics) are comprehensively described and assessed. A comparative study on microalgal strains is also analyzed for a more efficient implementation of future processes.

3. Results

Out of the publications screened for this work, six review papers were detected. The topics of each review are shown in Table 1. Pittman et al. (2011) and Singh and Olsen (2011) assessed the potential of microalgae as a resource for biofuel production. Both studies concluded that the production of biofuels from microalgae is advantageous from an environmental point of view while from an economic point of view it is not viable yet because of the maintenance costs needed to ensure high and constant biomass production over time. Delrue et al. (2016) explored the use of microalgae for wastewater treatment by focusing on which microalgae are best suited for this purpose, and the uses of biomass for various industrial sectors, mainly biofuels. Milledge et al. (2019) assessed the use of macroalgae for the production of biogas through anaerobic digestion. Arias et al. (2020) evaluated the use of cyanobacteria for the production of polymers due to their ability to store PHA and proposed possible solutions to avoid contamination of green microalgae limiting the production of PHA. Finally, Guilayn et al. (2020) evaluated the technical feasibility of obtaining value added products from digestates from anaerobic digestion plants.

Several potential products can be identified: Fig. 2 breaks out bioproducts that can be obtained by microalgae grown on urban wastewater according to their approximate concentration in algal biomass, depending on the different species.

Fig. 2 shows the product categories found in the articles: forty-three articles concern biofuels (including 25 biodiesel, 16 biogas, 1 biohydrogen, 1 bioethanol) and seven concern other products. Regarding biodiesel, all data regarding biomass concentration (g/L - which indicates the maximum concentration of biomass reached in the experiment), biomass productivity (g/L/d - quantity of biomass produced in the unit of time of a day) and lipid content (% - the percentage of lipids present in the microalgal biomass) were identified and collected in Appendix A (not shown in the main text), while data on the lipid content (%) of the microalgae in the various types of wastewater are shown in Appendix B (not shown in the main text). As regards biogas, all the data on the yield of biomethane (mL CH_4/g - quantity of biomethane

Table 1
Outline of "Review" items regarding microalgae on wastewater.

Item	Authors	Title	Main topic	Source	Citations
1	Pittman et al. (2011)	The potential of sustainable algal biofuel production using wastewater resources	Biofuel production from microalgae grown on wastewater	Bioresource Technology	1447
2	Singh et al. (2011)	A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels	Conversion processes for biofuel production	Applied energy	380
3	Delrue et al. (2016)	The environmental biorefinery: using microalgae to remediate wastewater, a win-win paradigm	Use of microalgae to remediate wastewater and application of biomass obtained	Energies	84
4	Milledge et al. (2019)	A brief review of anaerobic digestion of algae for BioEnergy	Macroalgae anaerobic digestion	Energies	25
5	Arias et al. (2020)	Production of polymers by cyanobacteria grown in wastewater: current status, challenges and future perspectives	Production of polymers by cyanobacteria grown in wastewater	New biotechnology	13
6	Guilayn et al. (2020)	Valorization of digestates from urban or centralized biogas plants: a critical review	Valorization of digestates from biogas plants	Environmental sciences	1

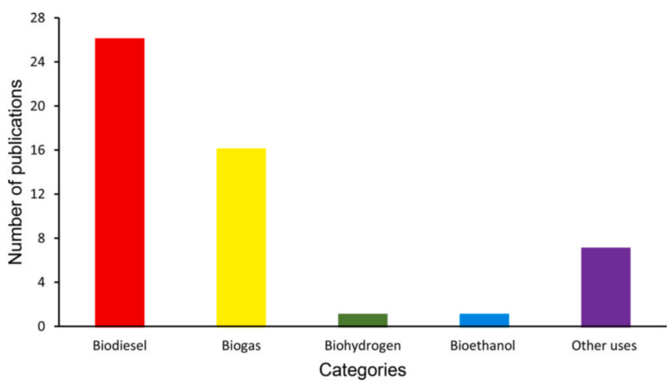

Fig. 2. Bio-product categories included in the review.

obtained from the anaerobic digestion of biomass) are included in Appendix C (not shown in main text).

3.1. Bioenergy

Microalgae extracts grown on wastewater can be converted into different forms of biofuels such as biogas, biodiesel, bioethanol, kerosene and biohydrogen through a series of conversion processes such as anaerobic digestion, transesterification, fermentation, gasification, pyrolysis, hydrothermal liquefaction (HTL), etc. (Speranza et al., 2015; Tsavatopoulou et al., 2019). As said earlier, algal biomass composition is made up of protein, lipids, carbohydrates, ash, and a range of minor constituents, such as nucleic acids, pigments, etc. Approximately 20–50% by weight of (dry) algae is made up of lipids, depending on type of microalgae (and any interactions between different microalgae) and growing conditions (Andersson et al., 2014). This makes microalgae suitable candidates to produce biofuels.

3.1.1. Liquid biofuels

3.1.1.1. Supply of biodiesel

3.1.1.1.1. Conversion routes. The bio-oils extracted from microalgal biomass have different characteristics compared to fossil diesel fuels (Andersson et al., 2014; Speranza et al., 2015). These cannot be used directly as fuels in diesel engines but first need to be extracted and then refined to obtain quality biofuels. There are several approaches to extract lipids from microalgal biomass, including solvent extraction, osmotic shock, ultrasonic extraction, etc. (Singh et al., 2012). Solvent extraction is a fast and efficient extraction method directly applied to dried biomass. This implies extracting bio-oil from microalgae with an organic solvent (such as hexane, ethanol or mixture of hexane-ethanol, benzene, cyclohexane, etc.) and obtain an extraction of fatty acids up to 98%. Osmotic shock consists of a sudden decrease in osmotic pressure causing cells breaking while, ultrasonic waves are used to create cavitation bubbles in a solvent shock waves and liquid jets breaking cell wall with a subsequent release of their contents into the solvent (Singh et al., 2012). With regards to refining, there are several processes to refine bio-oil, among these, the most advantageous is transesterification for its high conversion efficiency and low cost (Lin et al., 2011). This process allows to obtain a biodiesel very similar to the fossil equivalent (Singh and Olsen, 2011). The transesterification process can take place in one or two steps using acidic and/or basic catalysts. Depending on the process and on the type of microalgae employed, the yields of biodiesel varies significantly (Tsavatopoulou et al., 2019). Several studies have shown that under stressful conditions, such as nutrient deficiency, lipid content in microalgae increase (Ruiz et al., 2013; Zuliani et al., 2016). However, these culture conditions, while promoting the accumulation of lipids in the microalgae, also determine a lower yield of biomass (Ruiz et al., 2013). It is therefore of fundamental importance to identify the most suitable microalgal genera for growth on wastewater for the production of lipids to be used for biodiesel supply. Another process that allows to obtain biofuels is HTL (hydrothermal liquefaction) (Delrue et al., 2016; Hodaifa et al., 2013). This thermochemical process converts biomass into bio-oils (with yields ranging between 20% and 87%), gases, residual solids and an aqueous phase. The bio-oils obtained can be burned directly in a boiler or can be upgraded by hydrotreating into biofuels (Delrue et al., 2016). The advantage of this process is that it converts the entire biomass thus avoiding the selection of lipid-rich microalgae and processes such as biomass drying and lipid extraction (Delrue et al., 2016; L. Ferro et al., 2018).

3.1.1.1.2. Microalgae genera. The microalgal genera mainly found in this research are *Scenedesmus* and *Chlorella*, due to their resistance and adaptability to wastewater (Acién et al., 2016). Other genera are also *Desmodesmus*, and the marine microalgae *Tetraselmis* and *Nannochloporis*. There are different types of wastewater on which microalgae can be grown: i) primary wastewater, in which solids (pieces of plastic, wood, stones, paper, etc.) and, subsequently oils and fats, are removed; ii) secondary wastewater, where most of the organic substance is removed by biological purification with activated sludge from microorganisms such as bacteria that use the organic substance for their metabolic activity; iii) anaerobic digestate, which is an effluent deriving from the anaerobic digestion of sedimentation sludge; iv) centrate, which is the supernatant of the digestate obtained by centrifugation (Acién et al., 2016; Lima et al., 2020).

With regards to genera, *Scenedesmus* appears to be particularly suitable for biodiesel production as it has a high lipid content, an adequate fatty acid profile and a great ability to adapt to wastewater (Singh et al., 2012). As displayed in Appendix B, cultivation trials of *Scenedesmus* on wastewater have shown a lipid content ranging from 15.3 to 49.1%, with higher yields of lipid content in cultivations on secondary wastewater and digestates. This is due to the fact that secondary wastewater is less rich in nutrients than primary wastewater and favors the accumulation of lipids. Hodaifa et al. (2013) showed that, on secondary wastewater, the lipid content of *Scenedesmus* is higher than that on synthetic culture medium. When microalgae grow on secondary wastewater at different percentages (10–25%) added to 5% of BOMW (bleached olive-oil wastewater), lipid content rises to 33.2–49.1%. The accumulation of lipids in conditions of nutrient deficiency for this microalga was also demonstrated by Ruiz et al. (2013) who evaluated the growth of *Scenedesmus* on six secondary wastewater cultures with different hydraulic retention times (HRT). This study shows how high HRTs (nutrient deficiency stress) considerably increase the accumulation of lipids in microalgae; however, this condition also causes a slowdown in the productivity of biomass from 0.35 to 0.29 g/L/d. Nevertheless, the most suitable lipid content (27.7%) is given by cultivation with HRT 2.8 which provides a good balance between biomass productivity and lipid accumulation.

Several studies point out that cultivation on primary wastewater can also yield high lipid content. The growth of different species of *Scenedesmus* on primary wastewater was tested yielding a lipid content ranging between 16.61 and 22.48% and a biomass concentration from 1.24 to 1.36 g/L (Ferro et al., 2018). Similarly, the growth of *Scenedesmus dimorphus* in raw wastewater under critical stress conditions, i.e. three days of nutrient deprivation and salt excess stress (salinity 5%) was analyzed by Kudahettige et al. (2018). The stress of nutrient deprivation resulted in a decrease of biomass concentration from 0.33 to 0.27 g/L and an increase in lipid content from 17.4 to 29.6%, while salt excess stress caused a decrease in biomass from 0.33 to 0.26 g/L and an increase in lipid content from 17.4 to 28.9%. Nutrient deprivation and salt excess stress also increased the content of SFA (saturated fatty acids) compared to MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) (Kudahettige et al., 2018). In addition, Tao et al. (2017) assessed the lipid content of *Scenedesmus acuminatus* grown on different digestates (ADPP-anaerobic digestate pulp-paper, ADMW-anaerobic digestate municipal wastewater) with a lipid content from 19.9 to 35.9% and a biomass concentration ranging from 2.92 to 8.22 g/L, with higher biomass concentration on ADPP and higher lipid content on ADMW.

Cultivation trials of *Chlorella* on wastewater revealed a lipid content spanning from 12.2 to 35.7%, with higher yields in cultivations on secondary wastewater. A study carried out by Osundeko et al. (2013) showed a lipid content ranging from 27.7% for *Chlorella luteoviridis* to 35.7% for *Parachlorella iussii* and a biomass productivity range of 0.60–0.77 g/L/d, respectively, with cultivations on secondary wastewater. As for *Scenedesmus*, the higher lipid yields are due to the employment of secondary wastewater as cultivation medium. Furthermore, the authors claims that microalgae of the *Chlorella* genera are more suitable to be grown on wastewater given their high resistance to oxidative stress (Osundeko et al., 2013). In this last article, the fatty acid profile of *Chlorella luteoviridis* and *Parachlorella iussii* was also evaluated: they have a cetane number of 56.43 and 63.12, respectively. Considering that the standard cetane number for the production of quality biodiesel is at least 51, these algae appear to be suitable options to be taken into account. In a later study by the same author (Osundeko and Pittman, 2014), the growth of the above mentioned algae on secondary wastewater with the addition of 25% of concentrated liquor of activated sludge, containing substances that normally inhibit algal growth, was assessed in order to evaluate the resistance of these algae to oxidative stress. The study demonstrated that the addition of this liquor not only did not negatively affect the algal growth but even strengthened the algal growth. However, the addition of the liquor did not determine a higher accumulation of lipids. This suggests that only specific stresses have this type of effect.

Cultivation trials on primary wastewater also yielded high lipid contents. Ferro et al. (2018) evaluated the growth of different species of *Chlorella* on primary wastewater, obtaining a lipid content between 12.21 and 34.18% and biomass concentration from 0.80 to 1.15 g/L. The highest lipid yield was obtained from *Chlorella vulgaris*. The authors argues that this is due to the fact that the other two *Chlorella* species have stiffer cell walls which hinder lipid extraction. Tao et al. (2017) valued the lipid content of *Chlorella* on different digestates (ADPP, ADMW). The lipid content ranged from 21.7 up to 23% and biomass concentration from 2.02 to 2.91 g/L, with higher biomass concentration on ADPP and higher lipid content on ADMW.

Growth of *Desmodesmus* on wastewater have shown lipid contents ranging from 2.4 to 36.70%, with higher contents of lipid productivity in cultivations on primary wastewater. Samorì et al. (2013) compared the growth of this alga on primary and secondary wastewater in comparison to the one on Chu 13 culture medium. The study revealed that biomass productivity on primary wastewater (0.13 g/L/d) is comparable to the one obtained using synthetic culture medium (0.11 g/L/d). Although biomass productivity on secondary wastewater was lower (0.02 g/L/d), lipid content ranged from 4.9% for primary wastewater to 9.3% secondary wastewater. In this latter case, secondary wastewater gave the highest results in terms of lipid content as the stressful condition due to cultivation on less nutrient-rich water favored the accumulation of lipids. Ferro et al. (2018) tested the growth of *Desmodesmus* sp. on primary wastewater gaining a biomass concentration of 0.99 g/L and a lipid content of 36.70%.

Pereira et al. (2016) quantified the growth of the euryhaline microalga *Tetraselmis* sp. on primary wastewater with nutrient repletion (N+) and nutrient depletion (N-). The results displayed that the growth on N+ determined a higher biomass productivity (0.29 g/L/d) compared to growth on N- (0.25 g/L/d). However, lipid content on N- is higher than N+ (33 and 10%, respectively). The fatty acid profile of this microalga was also analyzed. More than 75% of the fatty acids were saturated and monounsaturated, which makes this microalga suitable for the production of biodiesel.

Nannochloporis gaditana growth was also investigated. Lima et al. (2019) tested its growth on primary wastewater and Guillard's modified culture medium.[1] The lipid content of cultivation on primary wastewater (21.61%) was slightly higher than the one obtained with growth on synthetic medium (21.57%). Silkina et al. (2019) evaluated the growth of *Nannochloporis oceanica* on F/2 culture medium and anaerobic digestate of municipal waste (AD municipal waste). The biomass and the lipid content resulted to be higher on F/2 medium (1.99 g/L and 24.5%, respectively) than on digestate (1.78 g/L and 17.9%, respectively).

The interaction of microalgae consortia grown on wastewater was also investigated. Koreiviene et al. (2014) examined the growth of *Chlorella* and *Scenedesmus* on sterilized primary wastewater at different concentrations (diluted and concentrated). The results of this study indicated that, while *Scenedesmus* grew more on concentrated wastewater, *Chlorella* on dilute wastewater. Therefore, an interaction of *Scenedesmus* and *Chlorella* may be a good solution as it would ensure optimal growth in relation to changes in biochemical composition of the wastewater over time. Furthermore, in accordance with what was found by Osundeko and Pittman (2014), the authors argue that, for *Chlorella,* the stress of nutrient deficiency does not cause the accumulation of lipids in the cells. However, this effect can be achieved through a shock with strong light or saline medium. Hultberg et al. (2016) assessed the growth of *Chlorella* and *Scenedesmus* on both primary and secondary wastewater. The study found that there are no significant differences in biomass concentration and lipid content between the two types of cultivation (0.53 and 0.57 g/L biomass and 11% lipids for secondary and primary wastewater, respectively). Zuliani et al. (2016) gauge the growth of *Chlorella vulgaris* and *Scenedesmus I* on three different digestates: dA (deriving from the treatment of agricultural waste), dB (deriving from the treatment of primary activated sludge) and dC (deriving from the treatment of urban wastewater). The results showed that dC digestate raised biomass productivity compared to dA and dB which, in turn, showed a significant increase in lipid productivity (stress condition). Therefore, the authors suggested a two-step growth option: first cultivation on dC to increase the biomass, then on dA and dB to increase the lipid content.

[1] Synthetic culture medium for the growth of microalgae.

Stockenreiter et al. (2016) suggested the symbiosis of multiple microalgae grown on municipal wastewater to maximize lipid productivity. The authors, in agreement with Koreiviene et al. (2014), considered that the positive effect of increased biodiversity on productivity was due to the fact that different species have different optimums and can complement each other in relation to changes in the culture medium over time. Iasimone et al. (2018) assayed the growth of microalgae (mainly *Cyanobacteria*, *Chlorella* and *Scenedesmus*) on raw wastewater with the addition of nutrients (L-low nutrients load, M-medium nutrient load, H-high nutrient load). The biomass concentration ranged from 0.15 to 0.21 g/L and the highest biomass concentration occurred with a culture medium poorer in nutrients (L). The lipid contents ranged from 16.6 to 23.4% with the highest values obtained with (L) and the lowest value with (H). Again, the availability of nutrients in the cultivation medium affected lipid yield and the highest accumulation of lipids occurred in cultivations in nutrient-poor medium.

3.1.1.2. Bioethanol. Microalgae biomass can also be used for bioethanol production. Bioethanol is produced through the fermentation of this. Microalgal biomass can be entirely used for the production of bioethanol or following the extraction of lipids for biodiesel production. Algal residues following the extraction of lipids are rich in starch/cellulose, and can be therefore used to produce bioethanol. An important factor in bioethanol production is the carbohydrate content of the biomass (Singh and Olsen, 2011). Microalgae can synthesize and accumulate large quantities of carbohydrates useful for the production of bioethanol (Maia et al., 2020). Some genera such as *Chlorella*, *Dunaliella*, *Scenedesmus*, *Chlamydomonas* and *Spirulina* are known to accumulate large amounts of starch, cellulose and glycogen (>50% DW) useful for bioethanol production (Chen et al., 2009; Ungureanu et al., 2020). Establishing the culture conditions of microalgae can influence the bioethanol yield. In fact, the manipulation of the culture conditions allows to establish the biochemical composition of the microalgae, favoring the accumulation of certain components compared to others (Cabirol et al., 2014). The main environmental factors that influence the biochemical composition are: light intensity, pH, salinity, temperature, and nutritional factors (de Farias Silva and Bertucco, 2016). Generally, to increase the concentration of carbohydrates, microalgae can be cultivated by applying strategies to reduce nutrient sources (Dragone et al., 2011; Kim et al., 2014). The cultivation of microalgae in conditions of lack of nutrients (nitrogen and phosphorus) increases the carbohydrate content in the biomass (Braga et al., 2018).

Carbohydrates are stored in the outer layer of the cell wall (pectin, agar, alginate), in the inner layer of the cell wall (cellulose, hemicellulose) and inside the cell (starch) (Lam and Lee, 2015).

A pre-treatment step is required for the extraction of carbohydrates from microalgal cells. (Velazquez-Lucio et al., 2018). The starch/cellulose can be extracted from cells using water or organic solvents and then used for fermentation to produce bioethanol (John et al., 2011). Among the various microorganisms used for fermentation there are mainly yeasts, thanks to their ability to transform sugars into alcohol. The yeast with the greatest industrial application for alcoholic fermentation is *Saccharomyces cerevisiae* (Walker and Stewart, 2016). There are three pathways for bioethanol production from such microorganisms: the traditional one involving hydrolysis and fermentation of biomass with bacteria or yeasts, the dark fermentation path and the use of engineered cyanobacteria or "photo-fermentation". The last path is impractical in nature, in fact it requires the use of genetic engineering (de Farias Silva and Bertucco, 2016).

The advantage of using microalgae for the production of bioethanol is that algal biomass, unlike the vegetable ones, does not have structures such as hemicellulose and lignin, thus easing the extraction process. Singh and Olsen (2011) reported a bioethanol yield of 4–10 g/L starting from microalgae residues after lipid extraction. The main bottlenecks for an industrial implementation are a lack of knowledge on genetically modified cyanobacteria as well as the need for more research on hydrolysis and fermentation technologies. This entails high costs for the production of bioethanol from microalgae (Ramos Tercero et al., 2014).

3.1.2. Gaseous biofuels

3.1.2.1. Biogas. Biogas is produced through the anaerobic digestion of microalgae biomass. The anaerobic digestion of biomass results in a biogas mixture made of 55–70% methane (CH_4) and the rest mainly CO_2 (Andersson et al., 2014; Menger-Krug et al., 2012). Nevertheless, an important factor to be considered is the biochemical composition of the microalgae (Ruiz et al., 2013). The composition in carbohydrates (5–23%), proteins (6–52%) and lipids (7–23%) varies with the species. When the macromolecular component is known, the theoretical yield of biomethane obtained through anaerobic digestion can be quantified (Singh and Olsen, 2011). Passos et al. (2015) reported the theoretical yield of biomethane for each macromolecular compound: protein yielding 0.85 L/g; carbohydrates 0.42 L/g and lipids 1.01 L/g. As rule of thumb, the yield of biomethane raises with the increase of lipid content in microalgal biomass and decreases when the protein content is high. A high percentage of protein could hinder digestion due to the toxic effect produced by the release of ammonia (Ruiz et al., 2013). Ruiz et al. (2013) assessed the biomethane yield from microalgae crops on wastewater at different HRTs confirming that the biomethane yield was higher in microalgae grown on wastewater with high HRT (stress condition) which causes a higher accumulation of lipids in algal cells at the expense of proteins. However, a higher lipid content in cells causes an extension of the time required for anaerobic digestion (Singh and Olsen, 2011). The production of biomethane through the anaerobic digestion process depends on the algal species used because when algal biomass is introduced into the anaerobic digester, algal cell walls can limit the accessibility of microorganisms to the intracellular content (Singh and Olsen, 2011). In this regard, there are several chemical and physical treatments that help improving the kinetics of methane production (Singh and Olsen, 2011). Co-digestion of biomass with other types of waste or sludge from wastewater treatment processes can improve biomethane production yield (Menger-Krug et al., 2012). Moreover, as in the case of bioethanol, microalgae biomass can be entirely used for the biomethane production or following the extraction of lipids for biodiesel production (Singh and Olsen, 2011). Biomethane can be used for a wide range of applications such as on-site combustion for heat and power co-generation, as a fuel for transportation or as a substitute for natural gas. If used as a transport fuel, the methane fraction must be higher than 95% and the gas must therefore be enhanced (Andersson et al., 2014). Several studies evaluated the production of microalgae in wastewater treatment plants and the use of biomass for the production of biomethane. Thorin et al. (2018) reckoned if co-digestion of microalgae-sludge in WWTPs could improve biomethane yield of sludge mono-digestion. The results displayed that co-digestion gave, on average, a higher yield of biomethane (317 mL/g) than that of the mono-digestion of sludge (304 mL/g). Olsson et al. (2018) did the same experiments and, contrary to what Thorin et al. (2018) found, the results showed that co-digestion reduced the yield of biomethane (168.2 mL/g) compared to that obtained from mono-digestion of sludge (199.8 mL/g). This divergence might be attributed to the composition of the microalgae population: in fact, different microalgae can give different yields of biomethane. The lower yield could also be attributed to a high heavy metal content which creates toxic conditions for biomethane production during anaerobic digestion. Tsapekos et al. (2018) evaluated the co-digestion of microalgae grown on municipal wastewater and piggery slurry. Results showed how co-digestion improved methane yield (216 mL/g) compared to single digestion of piggery slurry (176 mL/g). Moreover, the authors highlighted that the increase in biomethane yield can be attributed to the high content in the microalgal biomass of carbohydrates (easy to digest anaerobically) and lipids (which have a high theoretical potential of biomethane). Anbalagan et al. (2016) quantified biogas yields of microalgae integrated with activated sludge (MAAS) in a WWTPs with or without thermal pretreatment. Untreated MAAS gave a higher biogas yield (349 mL/g) than treated MAAS (308 mL/g). Arashiro et al. (2019) evaluated if biomethane yield deriving from the co-digestion of microalgae, grown on raw wastewater in HRAPs (high rate algal ponds), and primary sludge was higher than that of mono-digestion of primary sludge (PS). Different mixtures of microalgae-primary sludge were tested and the yield of biomethane varied between 237.6 and 258.3 mL/g. The co-digestion 75% PS-25% microalgae provided higher yields than that of mono-digestion of primary sludge (255 mL/g). Gutiérrez et al. (2015) assessed the biomethane yield from green microalgae grown in a HRAP treating urban wastewater and biomass recovered with natural floacculants. The yield of biomethane obtained with and without the use of floacculants ranged from 162 to 166 mL/g, with highest values detected with the use of floacculants. Moreover, both with and without floacculants, the CH_4 content in the biogas was 70%, therefore the biomethane content remained the same in both cases. Wieczorek et al. (2015) estimated the yield of biomethane from algae-bacterial maB-flocks taken from: biogas production plants (BP), wastewater treatment plants (MWTP), river sediments (RS). The biomethane yields were 186.55, 219.23 and 195.93 mL/g, respectively. Caporgno et al. (2015) evaluated the biomethane yields of *Chlorella kessleri* and *Chlorella vulgaris* grown on primary wastewater. The biomethane yields were 346 and 415 mL/g, respectively. Dębowski et al. (2017) reckoned the biomethane yield of *Chlorella* sp. grown on several ADEs (anaerobic digestion effluent). The yields of biomethane obtained ranged from 183 to 267 mL/g. The highest biomethane yield was obtained with microalgae grown on ADE from urban wastewater treatment. Wirth et al. (2020) focused on microalgae-bacteria consortia grown on municipal (MW-municipal wastewater), agricultural (CMS-chicken manure supernatant) and industrial (FE-fermentation effluent) liquid waste. The yields of biomethane gained ranged from 236 to 241 mL/g, with the highest biomethane yield obtained with CMS. Mendez et al. (2016) assessed the biomethane yield of *Chlorella vulgaris* and two cyanobacteria (*Aphanizomenon ovalisporum* and *Anabaena planktonica*) grown on urban wastewater. The yields of biomethane were 184.8 mL/g for *C. vulgaris* and 218.2–261.6 mL/g for cyanobacteria. Differences in biomethane yield are attributable to cell wall differences which make cyanobacteria more biodegradable than *C. vulgaris.*

3.1.2.2. Biohydrogen. Microalgae and cyanobacteria own the genetic, metabolic and enzymatic characteristics necessary for bioH_2 gas production. This is a renewable and non-polluting energy source as the combustion of H_2 only releases water (H_2O) as by-products. H_2 is considered a fuel of the future mainly due to its high conversion efficiency between 122 and 142 kJ/g, a value 2.75 times higher than combustible hydrocarbons (Patel et al., 2014; Rashid et al., 2013). However, this resource is not naturally available on earth (Goswami et al., 2020; Preethi et al., 2019). In recent decades it has been used for the generation of electricity through fuel cells or internal combustion engines.

The biological production of hydrogen (bioH_2) is more environmentally friendly and less expensive in terms of energy than conventional thermochemical and electrochemical processes such as gasification and water electrolysis (Batista et al., 2015). The conventional processes are much more impactful because they are energy-intensive and require high temperatures (970–1100 K). Moreover, these cause the release of large amounts of CO_2, therefore they are not recognized as eco-friendly processes on a commercial scale (Medisetty et al., 2020).

The production of bioH_2 from microalgae can take place through different processes: i) direct bio-photolysis, ii) indirect bio-photolysis, iii) photo-fermentation and iv) dark fermentation (Goswami et al., 2020). In direct bio-photolysis, microalgae split water into protons (H^+) and oxygen (O_2) in the presence of light. H^+ is converted into H_2 by hydrogenase, an enzyme that produces H_2 (Demirbas, 2009). The production of H_2 in this process is low because H_2 and O_2 are produced simultaneously and mix immediately, giving water as a by-product. Furthermore, H_2 production rates are hampered by the sensitivity of hydrogenase to oxygen (Show et al., 2011). This inhibitory effect can be resolved by adopting indirect bio-photolysis. Indirect bio-photolysis consists of two steps. In phase-1, cells do photosynthesis to accumulate organic compounds (mainly glucose) and oxygen evolves. This phase is also called the aerobic phase. In phase 2, cells degrade organic compounds stored under anaerobic conditions (Melis and Melnicki, 2006). Stage 2 is called the anaerobic stage. In the two-phase process, oxygen (in phase 1) and hydrogen (in phase 2) evolve separately. Regarding the fermentation processes, they are generally faster and lead to higher yields of H_2 (Hallenbeck, 2005).

Batista et al. (2015) was one of the first to test bioH_2 production from microalgae (*Chlorella vulgaris*, *Scenedesmus obliquus* and natural algal *Consortium*) grown on wastewater. The microalgae were subjected to nutritional stress to induce sugar production, later they were used as a substrate for dark fermentation from a strain of *Enterobacter aerogenes*. After nutritional stress the sugar content in the three microalgae was respectively 42.6, 21.9 and 28.6% while the H_2 production was 56.8, 40.8 and 46.8 mL H_2/g^{vs}. The study revealed how H_2 production is directly related to sugar content of microalgae. Singh and Olsen (2011) reported a theoretical maximum yield of H_2 production, starting from green algae, of about 198 kg H_2/ha/d.

However, only a few studies have been governed by the economic viability of large-scale H_2 production. The prices of H_2 gas are high compared to other fuels and are not pushed by the energy industries for production on a commercial scale. To attract investments and make it cheaper, one solution would be to invest in technologies (photo-bioreactors and metabolic engineering), carrying out technical-economic analyzes and optimizing process flows (Khetkorn et al., 2017; Show et al., 2018).

3.2. Other uses

In addition to the production of biofuels, microalgal biomass can also be used for other purposes (Table 2). These biomasses can be used entirely for these other purposes or, alternatively, after the extraction of lipids for the production of biodiesel. The growth of microalgae on wastewater allows to obtain good quantities of biomass that could be

Table 2
Other uses of microalgae biomass, processes and uses.

Other uses	Processes	Uses	Data source
Fertilizers	Absorption of wastewater N and P	Agricultural fertilizer	Moges et al. (2020) Wuang et al. (2016)
High value molecules	Pigment extraction	Nutraceutical and cosmetic products Natual colorant	Park et al. (2018) Prabakaran et al. (2020) Rahman et al. (2017)
Animal feed	Production of high energy content biomass	Farm feed Aquaculture feed	Becker (2007) Silkina et al. (2019)
Bioplastics	PHA absorption and storage	Production of bioplastics	Arias et al. (2020) Uggetti et al. (2018a), b
Biochar	Pyrolysis or HTC (hydrotermal carbonization) of microalgal biomass	Soil amendment Fertilizers	Arun et al. (2020) Delrue et al. (2016)

used for food purposes (Romero Villegas et al., 2017; Viegas et al., 2021) or for the production of high value molecules (Delrue et al., 2016). However, the use of biomass for these purposes requires further studies to verify its safety (Delrue et al., 2016). These biomasses are also capable of absorbing large quantities of N and P from the wastewater, this could suggest their use for the production of fertilizers (Moges et al., 2020). If the biomasses do not comply with safety regulations, they can be used for the production of biochar (Delrue et al., 2016). Additionally, some microalgae are capable of absorbing and storing polymers such as PHAs (polyhydroxyalkanoates), making them suitable for the production of bioplastics (Arias et al., 2020).

3.2.1. Fertilizers

The anaerobic digestion of microalgae biomass, in addition to the production of biogas, gives a nutrient-rich by-product such as digestate. This can be used as a fertilizer (Slepetiene et al., 2020). Another example that allows to recover N from microalgal biomass is gasification. With this process, nitrogen present in the microalgae forms ammonia which can be recovered in the aqueous phase and used as fertilizer (Singh and Olsen, 2011). Silkina et al. (2019) tested the growth of the *Nannochloporis oceanica* microalgae on different types of waste and obtained rather high yields of biomass (1.3–2.5 g/L), which can also be used as fertilizer. In fact, this biomass contained a high content of N and P that is biodegradable and could easily be used on the soil. Moges et al. (2020) evaluated the growth of *Chlorella sorokiniana* on blackwater (rich in N and P). This microalga was able to absorb between 77.8 up to 99.8% of N and 86.1 up to 99.5% of P. This implies that almost all of N and P was assimilated in the form of microalgae biomass which can be used as a biological fertilizer. Wuang et al. (2016) have demonstrated the potential as fertilizer of *Spirulina platensis* produced by the treatment of aquaculture wastewater. The growth of different plant species was tested using *Spirulina*, an industrial chemical fertilizer (Triple Pro 15-15-15) and their combination as fertilizer. Growth with *Spirulina* as fertilizer was almost comparable to that with industrial fertilizer. Spirulina-fertilizer combination enhanced growth performance. Finally, Arun et al. (2020) tested the use of biochar as an adsorbent of N and P from wastewater and its use as a fertilizer. In this study, 3.4 g of biochar was produced from 20 g of microalgae biomass (*Scenedesmus* sp.) grown on wastewater through hydrotermal carbonization process. The biochar obtained was used to absorb N and P from synthetic wastewater and was able to absorb 90% of P and 73% of N. This biochar was activated with P and N solubilizing bacteria to avoid leaching of N and P into the atmosphere and was tested as a fertilizer in comparison with DAP, a commercial ammonium phosphate. The growth of plants with biochar as fertilizer was higher than that obtained with DAP.

3.2.2. High value molecules

High value molecules such as pigments (phycocyanin, carotenoids, etc.) can be extracted from microalgal biomasses (Adarme-Vega et al., 2012; Eriksen, 2008). The phycocyanin pigment, extracted from *Spirulina*, can be used for its antioxidant and anti-inflammatory properties for the production of nutraceutical and cosmetic products (Park et al., 2018; Prabakaran et al., 2020). Also, this can be used as a natural blue colorant in certain food products (Rahman et al., 2017). However, the productivity of these molecules in wastewater is low because it requires specific culture conditions to be optimized. Furthermore, the strict regulations imposed by the pharmaceutical and cosmetic industries would hinder the use of biomass grown on wastewater for this purpose (Delrue et al., 2016).

3.2.3. Animal feed

Regarding the production of animal feed from microalgae biomass, Becker (2007) states that some microalgae have an amino-acid profile similar to that of traditional foods such as eggs, soy, etc. and could therefore be suitable for feed formulation. Zhou et al. (2012) identified a heterotrophic microalgal strain (UMN 231) collected from local waters suitable for the production of animal feed. Growing this strain on digested swine manure resulted in a biomass concentration of 0.83 g/L. The carbohydrate, lipid and protein content of this microalga was 14.7, 19.4 and 45.7%, respectively. Furthermore, the EPA (omega-3 polyunsaturated fatty acid) content of this microalgae was 3.75% of the total fatty acids. The high percentage of proteins and EPA make this alga suitable for the production of animal feed. Silkina et al. (2019) tested the growth of *Nannochloporis oceanica* on agricultural, aquaculture and municipal waste and calculated the calorific value of the gained biomass. The highest value (6.43 kcal/g) was obtained with growth on aquaculture waste while the lowest with growth on municipal waste (4.9 kcal/g). The authors suggested that this biomass could be suitable for feed supply to aquaculture and poultry. In fact, *Nannochloporis oceanica* contains omega-3 and could be a valid alternative to fish oil. Similarly, Romero Villegas et al. (2017) suggest the use of microalgae biomass produced on wastewater for the production of animal feed or for aquaculture. However, the authors also underline that more comprehensive analyses to evaluate the presence of toxic compounds or heavy metals in biomass are needed before commercializing them.

3.2.4. Bioplastics

Microalgae can also be used for the production of bioplastics. Cyanobacteria are able to assimilate and store PHA, a biodegradable polymer offering a valid alternative to fossil-based plastics. The most common polymer within this family is PHB (polyhydroxybutyrate) and its accumulation has been demonstrated in several cyanobacteria (Arias et al., 2020; Uggetti et al., 2018a). The main limitation for the production of polymers is the high cost of the nutrient source (Arias et al., 2020). Despite this, cultivation on wastewater can help overcoming this issue. However, cultivation on wastewater presents the problem of contamination of other microalgae that can affect the production of PHA. In this regard, some solutions to ensure the dominance of cyanobacteria in crops are: 1) low concentration of P in the culture medium (because cyanobacteria have greater affinity for P); 2) crops in closed systems less susceptible to contamination (Arias et al., 2020). A study conducted by Abdo and Ali (2019) reports how the growth of the cyanobacterium *Microcystis* sp. grown on domestic wastewater produced 0.2 g/L of dry biomass with an amount of PHB in it of 0.0067 g/L.

3.2.5. Biochar

Finally, when biomass grown on wastewater does not comply with chemical and biological safety regulations to be used for other purposes, it can be converted into biochar. Biochar can be used as a soil amendment. This allows to improve the negative effects of drought and salt stress on plants as it increases the water retention of the soil and improves its physical and biological properties (Ali et al., 2017). This product can be obtained through pyrolysis (Delrue et al., 2016). In addition to pyrolysis, another process for obtaining biochar is HTC (hydrotermal carbonization). The advantages of this process are that this is a CO_2 neutral process, has a low cost and does not require drying of the biomass (Arun et al., 2020).

4. Discussion

As already pointed out in the introduction, the need for an alternative substrate for microalgae growth has emerged because of their input requirements (mainly water and fertilizers) and cost. Indeed, microalgae production is usually an expensive process as it requires fertilizers (Singh et al., 2012) which contribute up to 20% of the total production costs (Delrue et al., 2016; Romero Villegas et al., 2017). Delrue et al. (2016) estimated the cost of biodiesel production from microalgae produced with industrial fertilizers (2.5 €/L) is five times higher than that the cost of diesel production from oil (0.6 €/L). Therefore, the authors state that it is necessary to use wastewater for the production of microalgal biomass to be economically feasible.

The mechanisms driving the formation of algae-based bio-products from wastewater are: the selection of the species suitable for growth on wastewater. According to the body of literature taken into account in this study, the most suitable genera for the growth on wastewater are *Scenedesmus, Chlorella, Cyanobacteria and Desmodesmus*, but also marine microalgae such as those of the genus *Nannochloporis* and *Tetraselmis*. Another important factor is the choice of the most suitable wastewaters and their content of nutrients such as N and P which are limiting factors for the growth of microalgae (Lima et al., 2020). The microalgae, in addition to growing on the waste of the water line, are also able to grow on the waste of the sludge line such as digestate and centrate, derived from anaerobic digestion. The cultivation of microalgae on these effluents can give high biomass yields as these effluents are rich in N and P (Peralta et al., 2019; Tao et al., 2017). Furthermore, in this way, the centrate (supernatant of the digestate), could be reintroduced into the water line, since it is impossible to return it to the environment given its high N and P load (Mantovani et al., 2020; Marazzi et al., 2019). Therefore, recycling nutrients from the centrate could also be an economical and ecological solution because it would allow to obtain microalgal biomass for a broad spectrum of by-products and a reduction in pollution due to the release of these wastewaters into the environment. A possible problem related to the cultivation on wastewater could be an excessive amount of ammonium, which in excessive quantities is toxic to microalgae. To overcome this problem, one approach could be to use zeolite to mitigate the toxicity of this substance. Zeolite has the ability to absorb ammonium present in wastewater and subsequently release it gradually for the growth of microalgae (Lu et al., 2019). Microalgal growth also depends on other factors like CO_2 availability (used as a source of inorganic carbon) which can cause an increase in biomass production from 30 to 50% (Lorenza Ferro et al., 2018; Uggetti et al., 2018b). Furthermore, CO_2 also participates in the stabilization of the pH which must be kept stable around a value of 8, as higher or lower values can negatively influence the metabolic activities (Arbib et al., 2013; Uggetti et al., 2018b). Generally, CO_2 is artificially supplied in microalgae cultivation. The supply of CO_2 has high operating costs that make the process expensive. An economic alternative, which could reduce the costs of growth, is the 'Industrial Symbiosis' (Andersson et al., 2014). By implementing this strategy, CO_2 from flue gases from nearby power plants or industrial sites could be recycled. Furthermore, this strategy would allow to reduce pollution due to greenhouse gases because it would reduce the CO_2 load emitted into the atmosphere (Andersson et al., 2014; Ho and Goethals, 2020). Finally, climatic conditions such as light intensity, temperature and photoperiod can also play an essential role in the growth of microalgae (González-Camejo et al., 2019; Iasimone et al., 2018). A key factor to consider in the production of algae-based bio-products is the biochemical composition of the biomass. If these biomasses are to be used for the production of biofuels, it is of fundamental importance to guarantee cultivation conditions that cause the accumulation of lipids, important macromolecules for the production of biofuels (de Farias Silva and Bertucco, 2016; Markou et al., 2012).

The factors influencing the accumulation of lipids in microalgae are stressful conditions such as nutrient deficiency (Doria et al., 2012; Ruiz et al., 2013) and salt excess stress (Kudahettige et al., 2018). As previously said, under nutrient deficiency, the protein content decreases, and the lipid content increases. This is due to the fact that in N deficiency the fixed carbon dioxide is converted into lipids due to the unavailability of N (Kudahettige et al., 2018). However, most of the time the conditions that favor lipid accumulation also result in lower biomass production, which is therefore why finding a good compromise between biomass production and lipid accumulation is advisable (Ruiz et al., 2013; Zuliani et al., 2016). In this regard, a strategy for obtaining high biomass yields under stress conditions is to adopt consortia of different microalgae that have different optimum conditions for their growth and allow to overcome the changes in the composition of wastewater over time (Hultberg et al., 2016; Koreiviene et al., 2014). In addition, species richness can also cause increased lipid accumulation in microalgae (Stockenreiter et al., 2016). Furthermore, as can be seen from Appendix B, cultivation on wastewater represents a valid solution if these biomasses are used for the production of biofuels, since it guarantees good lipid yields.

Besides being employed for biofuel supply, microalgal biomasses could also be used for other purposes, such as production of fertilizers, biochar, bioplastics, animal feed and the extraction of high-value molecules for use in the cosmetic and pharmaceutical industries. Currently, the products that can be safely obtained from microalgal biomass grown on wastewater are mainly fertilizers and biochar. To improve the efficiency of the use of raw materials, a solution could be to combine the production of fertilizers obtained from microalgae with fertilizers obtained from organic waste, this would allow a more sustainable use of resources. One such fertilizer could be vermicompost (Abdelhay et al., 2019). This type of fertilizer is produced from different types of waste such as livestock waste and other types of organic waste (Frederickson et al., 1997). The earthworm is suggested as an effective means of converting this waste into vermicompost. Vermicomposting is known to convert these wastes into more bioavailable forms and confer beneficial properties for the growth of plants (Hussain et al., 2016) and microalgae (Abdelhay et al., 2019). In fact, worm compost contains up to five times the nutrients available to the plant found in medium soil mixes (Abdelhay et al., 2019). An ecologically sustainable solution which follows the strategies of the circular economy could be to use microalgae grown on vermicompost to feed farmed fish (Abdelhay et al., 2019). Later, their farm waste could be used to produce the vermicompost again. The production of animal feed and the extraction of high value molecules for cosmetic and pharmaceutical industry is not yet a safe process, due to the fact that microalgae grown on wastewater absorb pollutants such as heavy metals (Leong and Chang, 2020).

For biomass downstream processing, the efficiency of traditional processes has long hindered the progression of microalgae towards products, so more advanced technologies are needed in order to obtain higher productivity and good quality products. In this regard, the use of technologies such as membrane-based technology and liquid biphasic flotation system could increase the recovery effectiveness of biomolecules from microalgal biomass and make the process more efficient from an economic and ecological point of view (Mat Aron et al., 2021).

However, even if there are still limits to be overcome, it is important to continue to investigate which technologies can be used in order to be able to allocate biomass to these market sectors as these could represent a new frontier of innovation.

Furthermore, one of the most important challenges in microalgal biomass production is finding an adequate technique to separate suspended algae from wastewater due to the high environmental and economic costs associated with this process (Kohlheb et al., 2020). Harvesting turns out to be the main bottleneck for large-scale microalgae cultivation, due to the small size of the microalgal cells (Ho and Goethals, 2020; Leite et al., 2020). There are mechanical (centrifugation, filtration, etc.) and chemical (flocculation, coagulation, etc.) methods for biomass harvesting (Lima et al., 2020; Mennaa et al., 2019). It is estimated that these processes contribute 20–30% to the production costs (Acién et al., 2016; Lima et al., 2020). In order to make large-scale production feasible, less expensive and impactful biomass harvesting technologies need to be developed. Net of the advantages, there remains an uncertainty regarding the fact that many studies are based on the extrapolation of data from pilot and laboratory scale levels and larger and more extensive demonstration scale data for the cultivation and improvement of algae remains a fundamental requirement.

4.1. *Practical applications and future research prospects*

A possible application for developing countries that need to make the most of resources could be to plan and apply circular economy strategies that allow them to be exploited and recovered following their use. Given the lack of energy and food resources in these countries, one possible way forward could be to use microalgae for wastewater treatment. This would make it possible to obtain low-cost energy resources in order to promote the development of rural areas and to obtain food resources for indigenous peoples and livestock. Furthermore, this type of approach would allow to carry out a wastewater purification at a low cost compared to that of conventional treatment plants available in Western countries. Finally, the application of this system could also increase the employment rate. However, in order to make this type of approach more efficient, there are still many obstacles to overcome, such as the need for greater technological development that makes the harvesting and processing of microalgae biomass on an industrial scale more effective at low costs.

4.2. Potential bio-based products. A real application

In recent years in Italy, at national level, progresses have been made for sustainable development through the implementation of strategies aimed to the circular economy (Ministero dell'Ambiente e della Tutela del Territorio e del Mare, 2017). However, despite clear progresses, an effective sustainable wastewater management strategy has not yet been developed (ARERA, 2019). Papa et al. (2017) highlighted how 60% of Italian WWTPs do not carry out any type of wastewater recovery. This occurs mainly in the Center and South of the country. A case to mention is certainly the Campania region (located in Southern Italy) which is periodically sanctioned due to poor waste management (European Commission, 2019). An example of deficiency in wastewater treatment in Campania Region is the sadly known Sarno river, which is the most polluted river in Europe (Lofrano et al., 2015). In order to implement a strategy for the recovery of the treated wastewater of the Campania Region, the theoretical growth of the *Scenedesmus* and *Chlorella* microalgae has been hypothesized on the total volume of urban treated wastewater with the aim of assessing which potential environmental benefits may be gained in a CE logic. Two different CE pathways were hypothesized: the production of energy and biochar from microalgae grown on urban wastewater. As emerged from the present work, the most suitable microalgal genera for the production of biofuels are *Scenedesmus* and *Chlorella*. This is due to the fact that these two microalgae are particularly appropriate for the treatment of wastewater due to their fast growth and high resistance to pollutants. Furthermore, these microalgae are not very sensitive to environmental fluctuations (Acién et al., 2016). Most of Campania's wastewater undergoes primary treatment by mechanical removal of coarse substances and secondary treatments by conventional biological treatment with activated sludge (Colella et al., 2021). According to the results of this study, the productivity of *Scenedesmus* biomass varies in a range of 0.15–0.38 g/L/d (Arbib et al., 2014; Ruiz et al., 2013) and a range of lipid content between 15.3 and 49.1% (Ferro et al., 2018; Hodaifa et al., 2013; among others). The productivity of *Chlorella* varies from 0.11 to 0.77 g/L/d (Arbib et al., 2014; Osundeko et al., 2013) and the lipid content between 12.21 and 35.7% (Ferro et al., 2018; Osundeko et al., 2013; and others). The theoretical volume of treated urban wastewater (411,114 × 10^6 L/a) in Campania was taken from Colella et al. (2021) which calculated the volume of wastewater treated by the largest plants in the Campania Region. The theoretical biomass and lipid productivity of *Chlorella* and *Scenedesmus* on one-year urban treated wastewater in Campania was therefore calculated. As it can be seen in Table 3, a biomass productivity between 61,667.1 and 156,223.3 ton/L_{tot}/a and a lipid productivity between 9435–76,705 ton/L_{tot} can be assumed for *Scenedesmus* and a biomass productivity between 45,222–316,557 ton/L_{tot} and a lipid productivity between 5521–113,011 ton/L_{tot} for *Chlorella*.

By applying this assumption, an average biodiesel production between 9435–76,705 ton from *Scenedesmus* and 5521–113,011 ton for *Chlorella* was calculated. According to data from the Italian Ministry of Economic Development, the consumption of diesel in Campania Region for engines, heating, thermoelectric and agricultural use in 2019 was equal to 1,706,492 ton (Ministero dello Sviluppo Economico, 2019a),

Table 3
Theoretical biomass and lipid productivity of *Scenedesmus* and *Chlorella* on the theoretical volume of urban wastewater treated in Campania in one year.

Microalgae	Urban treated wastewater (L/a)	Biomass productivity (ton/L_{tot})	Lipid content (%)	Lipid productivity (ton/L_{tot})
Scenedesmus	411,114 × 10^6	61,667–156,223	15.3–49.1	9435–76,705
Chlorella	411,114 × 10^6	45,222–316,557	12.21–35.7	5521–113,011

therefore, the production of microalgae on wastewater may allow to cover between 0.32 and 6.62% of the current diesel consumption in Campania Region. Moreover, to reduce the costs related to the extraction of lipids, it is also possible to produce biodiesel through an esterification/transesterification process of the entire biomass (Haas and Wagner, 2011) or, alternatively, use the extracted lipids as boiler fuel (Hu and Gholizadeh, 2020) to avoid the transesterification process. In addition to the production of biodiesel, the production of biomethane starting from the *Chlorella* biomass growth on wastewater was also estimated. According to the results, the yield of biomethane obtained from the anaerobic digestion of *Chlorella* ranges between 184.8 up to 415 mL CH_4/g (Caporgno et al., 2015; Mendez et al., 2016). The theoretical yield of biomethane obtainable from the anaerobic digestion of *Chlorella* produced on the wastewater of Campania in one year should give a quantity of biomethane between 8,357,118 and 131,371,487 m^3 CH_4. The data reported by the Ministry of Economic Development show how the natural gas consumption of Campania region in 2019 for industrial, thermoelectric and distribution network use amounts to 2,796, 000,000 m^3 (Ministero dello Sviluppo Economico, 2019b). The anaerobic digestion of *Chlorella* could therefore cover 0.29–4.69% of the total region natural gas consumption. Although it is unlikely that biofuel production from microalgae grown on wastewater would be a viable alternative to fully break the addiction to petroleum fuels, this option shows a double advantage: firstly, it can contribute to the reduction of environmental impacts of an harmful waste; secondly, it provides a small but non-negligible fraction of renewable energy to society.

Another possible option is provided by the production of biochar starting from *Scenedesmus*. Arun et al. (2020) reported a biochar yield of 3.4 g of biochar from 20 g of microalgal biomass *(Scenedesmus)*. According to the production estimates of *Scenedesmus* on the treated wastewater of Campania, a quantity of biochar between 10,483.41 and 26,557.96 ton could be produced to be used as soil amendment. According to ISTAT data, the consumption of amendment in Campania Region in 2019 amounted to 17,287 ton (ISTAT, 2019). Therefore, the production of biochar to be used for soil amendment could cover around 60% or, in the best case, the entire consumption of amendment in Campania Region with a surplus of 53%.

5. Conclusions

The aim of this study are clarified in Section 1.2 and we believe that the achieved Results fit these goals, as also pointed out in the Discussion.

The most suitable microalgae for growing on wastewater are found to be mainly *Chlorella* and *Scenedesmus* due to their high growth rate and high resistance to pollutants. However, other freshwater microalgae such as *Cyanobacteria* and *Desmodesmus* and marine microalgae such as *Nannochloporis* and *Tetraselmis* can also grow on wastewater.

As stated previously, the production of microalgae biomass on wastewater for the production of bio-products is influenced by several factors (species suitable for cultivation on wastewater, types of wastewater, climatic factors, etc.). The variety of products obtainable is affected by the biochemical composition of the microalgal biomass and by the possible presence of contaminants. The bio-based products obtainable are mainly biofuels such as biodiesel and biogas. This is due to the fact that this destination of biomass does not require particular regard for the presence of contaminants. Furthermore, these biomasses, when grown on wastewater, are rich in lipids.

In addition to the production of biofuels, microalgal biomass can also be used for the production of other products such as fertilizers, biochar, bioplastics, animal feeds and high value molecules for the cosmetic and pharmaceutical industry. For the production of these products there are limits to be overcome as the use of microalgal biomass grown on wastewater is not yet completely safe, however it would be worthwhile to implement the knowledge and technologies in order to be able to allocate the microalgal biomass also to these purposes to expand their range of use.

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.

Acknowledgments

The authors gratefully acknowledge the support from the Italian Ministry of Foreign Affairs and International Cooperation (Ministero degli Affari Esteri e della Cooperazione Internazionale - Direzione Generale per la Promozione del Sistema Paese; Grant number: PGR05278) and the Sino-Italian Cooperation Commission of the China Natural Science Foundation (Grant Number 801-71861137001). The Authors also gratefully acknowledge the support from the projects "Realising the Transition towards the Circular Economy: Models, Methods and Applications (ReTraCE)", funded by the H2020-MSCA-ITN-2018 programme (Grant Number: 814247) and "Promoting Circular Economy in the Food Supply Chain (ProCEedS)", funded by the H2020-MSCA-RISE-2018 programme (Grant Number: 823967).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.112792.

CRediT author statement

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