REVIEW

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Anaerobic digestion and agronomic applications of microalgae for its sustainable valorization

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Microalgae are considered potential candidates in biorefinery processes, and due to their biochemical properties, they can be used in the production of biofuels such as biogas, as well as for bioremediation of liquid effluents. The objective of this review is to study the current status of microalgae anaerobic digestion and agricultural uses (as bio-stimulants and biofertilizers), starting from microalgae cultivation. Indeed, the efficiency of these processes necessarily depends on the evaluation of different biotic and abiotic factors that affect the growth of microalgae. However, the adaptation and the optimization of process parameters on a large scale is also limited by energy and economic constraints. Moreover, the integration of biogas production processes with microalgae cultivation allows a nutrients and CO_2 virtuous loop, thus promoting the sustainability of the process. Finally, this paper provides a general overview of biogas and biofertilizers production combination, as well as the related challenges and recommended future research perspectives to complement the gap in the literature.

1. Introduction

Microalgae are a promising source for third generation biofuels. The production of microalgae consists of cultivation and harvesting stages. Biofuel production depends also on biochemical properties of microalgae which is highly related to cultivation and harvesting conditions. Light intensity, pH, temperature and nutrients availability as well as mixing

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conditions are among the main factors influencing the performance of the growth stage.[1,2]

Anaerobic digestion is among the most widely used biological processes converting organic matter to biogas. The efficiency of anaerobic digestion processes can be enhanced when applying a pretreatment to favor hardly degradable matter containing in microalgae.[3] However, optimization of pretreatment should be carried out to reduce energy and chemicals consumption. Moreover, pretreatment cannot remedy inhibition problems related to high proteins, heavy metals or polyphenols content in microalgae, that is why co-digestion can be seen as an interesting alternative leading to high methane production.[4] In addition, because of the low biomass productivity, monodigestion of microalgae cannot be economically attractive.[5]

In order to improve the performance of the microalgae production and utilization system, consideration must be given to optimizing processes in such a way that the nutrient and energy loop is closed. Extraction of high-value added matter from microalgae can be carried out first to generate a biostimulant for plants for *e.g.*, and then the solid residues can be subjected to anaerobic digestion. The resulted digestate can be used as biofertilizer or as a source of nutrients for microalgae biomass growth,[6] as well as the recycling of CO_2 contained in biogas produced from AD can lead to a more cost-effective cultivation stage.

However, more studies need to be oriented towards process life cycle assessment, in order to determine the best possible scenarios for the valorization of microalgae.

Therefore, the aim of this paper is to provide a general overview of the different aspects affecting the production and use of microalgal biomasses. In addition, this paper offers prospects for improving the valorization of microalgae residues by integrating anaerobic digestion and agronomic application of microalgae in an innovative eco-friendly biorefinery in cascade.

2. The potential of microalgae for bioenergy and biofertilization

Microalgae are photosynthetic microorganisms that live in freshwater or seawater. They can be distinguished in two types of microalgae: eukaryotes (green or diatom microalgae) and prokaryotes (blue-green algae). Depending on the growth environment and the adaptability of the species, microalgae can grow by consuming complex organic matter (heterotrophic mode), by consuming organic matter and absorbing light (mixotrophic mode) or by absorbing only light (photoautotrophic mode).

The growth conditions as well as the type of microalgae have an impact on the properties of microalgae. Typical species include *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Chlorella* sp., and *Botryococcus braunii*. Other interesting microalgae species for biotechnology development include *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Nannochloropsis*, and *Isochrysis* spp.[7] In general, microalgae are rich in fatty acids, proteins and sugars as presented in Table 1. In addition, some microalgal biomasses contain calcium (0.1–3%), magnesium (0.3–0.7%), phosphorous (0.7–1.5%), potassium (0.7–2.4%), sodium (0.8–2.7%) and sulfur (0.4–1.4%), as well as heavy metals such as copper (18–100 mg kg^{-1}), iron (1.4–11 g kg^{-1}) and zinc (28–64 mg kg^{-1}), as described by Tibbetts *et al.* (2015).[8] Among the major pigments and polyphenols in microalgae there are chlorophylls (0.5–1%), carotenoids (0.1–0.2%), and phycobiliproteins which are present specifically in cyanobacteria.[9]

Depending on the expected bio-product, the use and choice of microalgae species depends on their composition. The transformation efficiency of microalgae yield for energy, molecules and materials production are highly affected by operating conditions of the growth/harvesting and different process steps.[10]

3. Microalgae cultivation challenges

3.1. Effect of operating conditions on microalgae growth

According to Jankowska *et al.* (2017), the most important factors affecting microalgae growth are cultivation system, light intensity, temperature, pH level, CO_2 concentration and nutrients availability in addition to biological factors.[1]

3.1.1 Light. Microalgae production is based on light provision through photosynthesis process. In fact, light intensity, the utilization efficiency as well as the radio of irradiation and dark periods are the three main factors emphasizing the impact of light on microalgae growth.[1]

Optimization of light intensity is required to avoid photooxidation and photoinhibition. In fact, above the light saturation point, microalgal growth is slowed down and can even be stopped, while light deficiency can lead to photo limitation,

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Table 1 Biochemical composition of some microalgal biomasses

	Carbohydrates (% TS)	Proteins (% TS)	Lipids (% TS)	N (%TS)	P (% TS)	K (% TS)	Ref.
Botryococcus braunii	4–55	1.5	25–75	8.3	1.4	0.8	11–13
Chlamydomonas reinhardtii	59.7	9.2	15–18	—	—	—	14 and 15
Chlorella sp.	12–26	53	28–32	9.7	0.91	0.91	13 and 16
Dunaliella salina	32	57	6–25	7.0	0.15	0.43	16 and 17
Euglena gracilis	14–18	39–61	4–20	—	—	—	17
Isochrysis spp.	4–8	5–14	7–40	—	—	—	18 and 19
Nannochloropsis	9.3	48.3	31–68	7.0	0.7	1.5	16 and 17
Phaeodactylum tricornutum	47	37	18–57	8.3	1.2	2.4	20
Neochloris oleoabundans	37.8	30.1	29–65	6.3	—	—	8, 19 and 21

since, if present in excess, may cause the generation of harmful oxidative stress and reactive oxygen species (ROS). Liu *et al.* (2013) found that 150 μmol m^{-2} s^{-1} was the optimal light intensity above which microalgae growth rate was decreased.[22] Patel *et al.* (2019) found that the increase of light intensity from 35 to 150 μmol m^{-2} s^{-1} increased the biomass yield of *Chlorella protothecoides* by 124%.[2] However, in the presence of an external source of carbon, the growth was slightly improved (+13%) by light intensity increase.[2] In the same manner, the growth rate of both *Chlorella sorokiniana* and *Asterarcys quadricellulare* doubled after the application of a light intensity of 200 μmol m^{-2} s^{-1} compared to 50 μmol m^{-2} s^{-1}.[23] Light intensity acts not only on growth but also on the accumulation of carbohydrates and lipids. Ho *et al.* (2012) reported that carbohydrates content from *Scenedesmus obliquus* was improved by 153% when light intensity increased from 60 to 420 μmol m^{-2} s^{-1}.[24] In another study, lipids content using *Neochloris oleoabundans* was increased by 26% when light intensity varied from 50 to 200 μmol m^{-2} s^{-1}.[25]

In the case of high light intensity (greater than the light saturation point), the use of an intermittent light supply mode was recommended. In fact, the ratio of light to darkness periods is also an important parameter impacting the performance of a photo bioreactor (PBR). As explained in Patel *et al.* (2020), the microalgae absorb light energy under light period and fix CO_2 using adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) which have reducing powers favoring the generation of lipids within the mixotrophic cultivation system.[26] Compared to continuous illumination at optimal intensity, intermittency is less effective.[2] However, from an economic point of view, it reduces energy consumption.

Light absorption is also related to the distance to light source. Liu *et al.* (2013) reported that at biomass yield was maximal at a distance of 2 cm and decreased above this value.[22] Therefore, the position of the energy source must be determined in such a way that the received light intensity is equal to or less than the light saturation intensity. This finding was also reported in Richmond *et al.* (2003), the length of optical path can highly affect the light absorbance and thus the cultivation of photoautotrophic microorganisms.[27] The efficiency of light use is also related to the irradiance conditions, for example, the use of one or more light sources, placed on one side of the

bioreactor or on both sides which will increase the fraction of microorganisms exposed to light at any given time. However, as high cell density can negatively affect the light penetration into the culture,[27] harvesting needs to be done more frequently.

3.1.2 Temperature. Temperature is another factor affecting microalgal biomass production. It is reported to be highly related to growth rate when temperature did not exceed its optimal level. However, the effect of temperature varies depending on species. In Kurpan Nogueira *et al.* (2015) study, increasing temperature from 20 °C to 30 °C reduced the *I. galbana* biomass yield (−22%) while slightly enhancing lipids content (+6%).[28] This finding was not in agreement with Wu *et al.* (2013) who reported a decrease in lipid content (−12%) when increasing temperature from 25 to 35 °C during *Monoraphidium* sp. growth.[29]

Also, when increasing temperature from 15 to 23 °C, lipid content was reduced by 33%, while carbohydrate content was enhanced by 43%.[10]

As seen previously, the temperature tolerance of each species can be different depending on their adaptability and gene regulation sensitivity.[30] Photosynthesis is negatively affected by low temperature which reduce the carbon assimilation. However, at high temperatures, photosynthetic proteins can be degraded and cell size can be highly reduced because of the decrease in ribulose-1,5-bisphosphate activity.[31]

3.1.3 pH. pH can highly affect the physicochemical properties of microalgal cells. In addition, it is related to the dissolved CO_2 which is the most used inorganic carbon source, and the uptake of nutrients (mainly nitrogen and phosphorous). The optimal pH range for microalgae growth is around 6–8. *Chlorella vulgaris* was reported to have a broad pH tolerance range up to 10.[31] In contrast, Ying *et al.* (2014) reported the decrease in growth rate of *Dunaliella salina*, when pH drop from 7 to 6.[32] Thus, as for temperature, pH tolerance is specific to each species.

However, pH stress conditions are widely studied in literature to investigate the impact of pH variation on bioprocesses stability. Ho *et al.* (2014) reported that lipid content and quality in biomass enhanced when increasing pH levels, but as growth decreases, lipid productivity is reduced.[30] Carbohydrates content was also found to be affected by the culture pH. Extracellar carbohydrates content in *Skeletonema costatum* were

constant in the range of 6.5–8.0 and increased above this pH level contrarily to growth rate.[33]

3.1.4 CO$_2$ and nutrients. Carbon is a vital element for microalgae respiration and photosynthesis. It is the main part of the composition of all metabolites (lipids, carbohydrates and proteins).[34] Inorganic carbon source can be taken from atmosphere in form of CO$_2$. The concentration of CO$_2$ plays an important role in microalgae growth. Overall, the optimal concentration of CO$_2$ for microalgae growth is 0.038–10%.[35] In some cases, CO$_2$ addition is required to avoid photorespiration, where O$_2$ acts as Rubisco substrate which reduces CO$_2$ fixation.[34] As seen previously, CO$_2$ tolerance depend highly on microalgae tolerance. In addition, the use of CO$_2$ as inorganic carbon source is benefic for the ecosystem. Moreover, organic carbon can be added to stimulate lipids synthesis and biomass yield. In fact, Khanra *et al.* (2020) found that sucrose addition enhanced biomass yield and lipid content by 25% and 55% respectively.[36] In the same manner, glucose addition was found to increase lipid productivity (4-fold higher) while reducing chlorophyll *a* and carotenoid content.[37] Moreover, depletion of carbon affects the enzyme balance and thus, alters photosynthesis.[38]

Nitrogen is an important nutrient, since it is essential for proteins synthesis. The three main nitrogen sources used for microalgae growth are nitrate, urea and ammonium. Khanra *et al.* (2020) reported that the nitrate addition as source of N for *Chlorococcum* sp. growth, enhanced biomass yield and lipid content by 40% and 117% respectively.[36] Similarly, Zarrinmeher *et al.* (2020) reported that a concentration of 72 mg L^{-1} of nitrate improved proteins (+112%), lipids productivities (+71%) and growth yield (15-fold high) of *I. galbana* compared to starvation condition.[39] Ammonium was reported to be the easiest nitrogen source to assimilate. However, growth inhibition may occur if ammonium concentration exceeds 100 mg L^{-1}.[40] In mixotrophic growth conditions (with 2 g L^{-1} of glucose added), ammonium addition at 50 mg L^{-1} enhanced lipids and proteins content, while carbohydrates synthesis was inhibited. However, in autotrophic conditions, nitrogen reduced lipids content in microalgae.[41]

In the opposite side, insufficiency of nitrogen can highly improve lipid and carbohydrates content in microalgae. Under starvation conditions, microalgae degrade chlorophyll and proteins and convert its Skelton carbon to lipids and carbohydrates.[30] However, biomass yield was found to be reduced in nutrient-stressed cultures compared to nutrient-replete cultures. Especially, chlorophyll *a*, nitrogen, antioxidant activity and phenolic compounds content in biomass (*Chlorella*, *Tetraselmis* and *Phaeodactylum*) was negatively affected by the depletion of nitrogen.[42] This finding is in agreement with Ho *et al.* (2013) in which carbohydrates content doubled under N starvation of *Chlorella vulgaris*.[43]

As for nitrogen, phosphorous can highly affect biomass growth and chemical composition. It is essential for microalgae cells and nucleic acids synthesis. The most easily assimilated form of phosphorous is phosphates. Phosphorus addition at 0.04 g L^{-1} didn't result in a significant change in terms of *Podohedriella* sp. yield. However, chlorophyll has roughly tripled and proteins content was increased by 82%.[44] Phosphorous depletion was found to reduce biomass yield, P and chlorophyll *a* content of the microalgae. However, vitamins C and E content in biomass was increased.[42] Shashirekha *et al.* (2016) found that a C : N : P ratio of 0.2 : 0.14 : 0.8% was the optimal leading to maximized biomass *S. obliquus* yield in sugar mill effluent.[45] The behavior of microalgae in the case of nutrient deficiency or sufficiency differs according to cultivation strategy, nutrients demand and the ability of microalgal species to tolerate environmental stress. Other nutrients such as trace metals are also important for microalgae cultivation as they promote enzymatic activities. Among the trace metals, iron was found to be the most important for its contribution in chlorophyll synthesis, electron transport and nitrogen fixation.[46] The presence of iron with phosphorus was found to boost the production of microalgae under 0.05 μM Fe and 50 μM P.[47]

3.1.5 Systems configuration. Microalgae can growth using autotrophic, heterotrophic or mixotrophic modes. The autotrophic cultivation is the most common growth system. It consists in consuming inorganic carbon (CO$_2$) and light as source of energy. This cultivation strategy can promote pigments production, but the biomass yield can be limited compared to other growth modes.[2,37] In addition, light is the limiting factor in autotrophic cultivation. In contrast, heterotrophic mode is defined by the use of organic carbon instead of inorganic one as source of carbon and energy. Thus, light is not required for this type of systems. The growth rate and biomass productivity were shown to be high using heterotrophic cultivation. However, not all of microalgae species can be grown in these conditions. *Amphora*, *Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Chlorococcum*, *Crypthecodinium*, *Cyclotella*, *Dunaliella*, *Euglena*, *Nannochloropsis*, *Nitzschia*, *Ochromonas*, and *Tetraselmis* were found to be able to grow heterotrophically.[48] Moreover, contamination by other microorganisms can occur which may reduce microalgae productivity. Microalgae can use both organic carbon for respiration, inorganic carbon and light for photosynthesis, which is known as mixotrophic growth. *Spirulina platensis*, *Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Scenedesmus obliquus*, and *C. vulgaris* are among the most known microalgae species that can grow under mixotrophic conditions.[49] This system favors metabolites production while gathering advantages of both autotrophy and heterotrophy.[37,49]

Microalgae cultivation can be carried out in open ponds or closed photobioreactors. Photobioreactors are using light source for phototrophic microorganisms' growth. In this system, environmental factors are easy to control. Operating conditions may affect CO$_2$ (then pH), nutrients, heat transfer and light availability in the cultivation system.[1] Thus, mixing is among the most important parameters related to reactors functioning. Maximizing mixing condition favors biomass growth, as long as shear stress is taken into account, which is related to the culture medium velocity.[1,35] As previously mentioned, light path can also affect microalgae growth. For photobioreactors, construction materials can play an important role in light absorption.[48] Light attenuation should also be

controlled by modifying the residence time to avoid dark zones within the PBR and then a decrease in biomass productivity.[50]

Also, an optimized diameter should be considered to accommodate the uniform distribution of light within the reactor.[51] The modelling of PBR systems has already been reported in the literature[50,52] but has not been addressed in this review.

The raceway pond is the cheapest cultivation system with a cost investment of 0.13 to 0.37 M€ per ha.[53] It consists in an oval channel containing a paddle wheel to ensure the continuous water flow. It used sunlight for microalgae growth what makes the strength of this technique.

The most appropriate design of raceway ponds should enhance the productivity and thus, limiting the photo-inihibition and photolimitation. These latter are generally avoided by optimizing the water head and reducing pond depth, which favors the contact of microalgae with light. In addition, low water head can be operated by a propeller which consumes less energy compared to paddle wheels.[54]

The use of existing wastewater lagoons for microalgae cultivation was found to be the most profitable way to produce biomass at large scale.[55] In addition, nutrient supply, water and sunlight are provided. Utilization of photobioreactors is generally more productive, but some constraints related to reactor sizing should be considered. Norsker *et al.* (2020) reported that the maximal recommended length of tubular photobioreactor with diameters between 5–10 cm is around 80–120 m. The dimensions of the photobioreactors depend mainly on dissolved oxygen tolerance and flow velocity. Larger diameters were found to result in lower biomass concentration and density and lower diameters are uneconomical in terms of mixing energy because of shear stress.[48] In contrast, open raceway ponds are the most frequently used systems at industrial scale due to their cost effectiveness. It can extend from 1000 to 5000 m^2,[53] for which the maximal depth is 0.45 m.[56] However, contamination issues, harvesting difficulty and environmental conditions changes may be the challenges encountered by this type of systems.

When using open ponds, environmental changes including, temperature and humidity, can affect the productivity of biomass. In addition, loss of water through evaporation can occur.[56] The use of sunlight can highly reduce the costs of microalgae production. However, this abundant energy source is not fixed and cannot be controlled. The variability of its intensity can lead to an unreliability of the process and therefore to a non-optimized production of microalgae, especially in cases where the objective is to produce products with high added value.[57] For heterotrophic cultivation, the use of organic carbon source such as glucose, can lead to extra costs.

3.2. Biological contamination issues

Biological contaminants affecting the growth of microalgae are classified into 4 categories: zooplankton, microorganisms (bacteria, fungi and protozoa), other microalgae species and viruses.[58] Contaminants can also be distinguished according to their contamination mechanisms. Microalgae grazers are found in all-natural aquatic environments. They present the greatest threat on microalgae growth. By ingesting microalgae, they cause reduction of biomass production. Park *et al.* (2016) reported that *Chlorella kessleri* can be highly infected by rotifers such as *Brachionus calyciflorus*. The solution given in the study suggests that addition of chlorine can help preventing pond crash.[59] Also, the capacity of *Brachionus calyciflorus* to consume more than 500 cells of *Chlamydomonas reinhardtii* per hour and per rotifer was reported.[58]

Grazers prevention can be carried out by sterilizing the medium before introduction in photobioreactors.[60] However, the risk of contamination is high in open ponds compared to photobioreactors. In case of open ponds, the use of greenhouses has shown a positive effect on the reduction of the risk of wind infection by cysts and grazers eggs that are threatening microalgae.[60] Other chemical treatments such as pesticides were studied, but their cost and impact on environment shows that their application in a larger scale may be inefficient. Moreover, the increase of CO_2 concentration was found to be effective in controlling and eliminating grazers.[61]

In literature, the use of algal–bacterial co-culture based on crashed rotifer culture, had a positive impact on protecting *Microchloropsis salina* against *Brachionus plicatilis*. After months of repeating the assays, the algal–bacterial co-culture was no longer effective in protecting microalgae grown in open ponds. This was due to the absence of certain genus in the non-protective community. However, studies need to be carried out to ensure the reliability of this practice.[62]

4. Biogas production from microalgae

Anaerobic digestion (AD) is the biological process converting organic methane to biogas which is composed mainly of methane and CO_2 (Fig. 1). As microalgae is rich in organic matter, its anaerobic digestion had attracted researchers' interest since 1982. Considering that, 1 g of carbohydrates, proteins and lipids produce 415, 496 and 1014 mL of methane. Thus, theoretically, *Chlorella* sp. can produce around 600 mL of
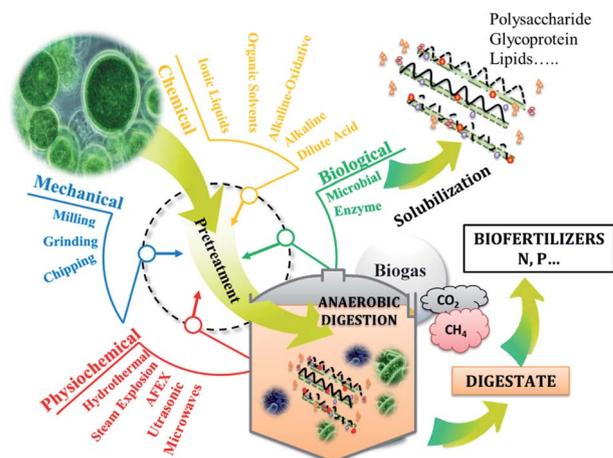

Fig. 1   Pretreatments and biogas production from microalgae.

methane per gTS. Mussgnug *et al.* (2010) found that the methane potentials of *Chlamydomonas reinhardtii*, *Chlorella*, *Dunaliella salina* and *Euglena gracilis* were 587, 335, 505 and 485 mL g^{-1} VS respectively.[63] Other algal biomasses such as *Isochrysis* sp. had lower methane potential (135 mL g^{-1} VS) compared to the others,[64] as long as it contained low carbohydrates (4–8%TS) as shown in Table 1.

Anaerobic digestion process is the succession of 4 stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In which the complex organic matter is broken down by the microorganisms into monomers, then volatile fatty acids, then into acetate, hydrogen and CO_2, ultimately leading to the production of methane.[65] The recalcitrance of microalgal cell walls can be the reason behind the low methane production.[66] Thus, pretreatments may be needed to increase solubilization of organic compounds contained in microalgae biomass and therefore, enhance the hydrolysis yield. The latter can be considered as the limiting step of AD process in the case of hardly biodegradable matter.

Many factors can affect the AD process, such as temperature (mesophilic or thermophilic), pH (6.5–7.5), C to N ratio (20–25) and operating conditions (organic loading rate, hydraulic retention time and mixing conditions). In the case of microalgae, Zamalloa *et al.* (2012) reported that thermophilic AD (54 °C) resulted in lower methane production from *Scenedesmus obliquus* and Phaeodactylum tricornutum compared to mesophilic temperature (33 °C).[67] C to N ratio in *Chlorella vulgaris* was found to be around 17,[68] while *Stigonematales* sp had lower C to N ratio (around 4.7).[69] This suggests that digestion of microalgae with other organic substrates with higher C to N ratio can be carried out to enhance biogas production, which is known as codigestion. By its simplicity and low energy and chemicals requirements, AD can be an efficient bioprocess for microalgae transformation into bioenergy. When AD of microalgae is applied as downstream process, steps such as dewatering and drying of microalgae are not required. In the case of an existing digester within the wastewater treatment plant, the co-digestion of microalgae with sewage sludge can be feasible and profitable.[70]

4.1. Pretreatment of microalgae

Pretreatment is a well-known practice in microalgae valorization such as anaerobic digestion (Fig. 1). Hence, the pretreatment of microalgae prior to its valorization is an essential step in order to increase macromolecules accessibility and biodegradability by anaerobic microorganisms. Application of pretreatment steps allows modifying the supramolecular structure of microalgae matrix, thereby changing the natural binding characteristics of microalgae materials and increasing the carbohydrates, proteins, lipids… accessibility for enzymatic or chemical biological action. For the last few years, several physicochemical pretreatments have been developed and applied to microalgae biomass for this purpose, including ultrasonic, mechanical, microwaves, chemical, biological, thermal and combined pretreatments. However, although most of them are known to be effective in hydrolysing the matrix and enhance sugars recovery, they are energy consuming and not always cost effective.[71] Microalgae biomass cell is composed of the skelton containing cellulose, mannan and xylans.[3] In addition, cell walls are composed of polysaccharide and glycoprotein matrix protecting microalgal cells.[72] However, the composition of cell walls varies from species to species. *Dunaliella* sp. has no cell wall while *Chlorella* and *Scenedesmus* sp. can further contain algeanan which is a lignin-like polymer.[3,73] The robustness of this wall obviously depends on the growing conditions, such as the attack of the grazers or variable environmental conditions.[74] For this reason, the key driver for the successful conversion of microalgae into energy and high values added compounds is the selection of efficient pretreatments that permit to maximize the sugars, lipids and proteins recovery and to minimize their degradation with the consequent formation of toxic derivatives. Thus, the AD of microalgae may require pretreatments of which some recent results are summarized in Table 2.

Thermal pretreatment of *Chlorella vulgaris* at 120 °C for 40 min improved organic matter solubilization leading to an enhanced methane production by 93%.[75] In the same manner, subjecting *Nannochloropsis salina* to 120 °C for 2 h resulted in 150% more biogas produced.[76] However, according to Mussgnug *et al.* (2010), thermal pretreatment at 105 °C for 24 h can lead to reduced biogas production by 18%, which may be due to volatilization of organic matter.[63] Applying microwaves to a coculture of *Chlorella* and *Scenedesmus* sp. can result in high solubilization of VS with a 13% higher methane production.[77] However, Schwede *et al.* (2013) reported that microwaves can lead to higher methane production (+40%) from *Nannochloropsis salina*. Microwaves are generally used to substitute conventional heating that consume more energy and take more time. However, the effectiveness of conventional heating can be higher than that of microwaves as reported in Schwede *et al.* (2013). In this case, it is necessary to consider whether the energy dissipated by the conventional heat pretreatment is compensated by the increase in methane production. Ultrasonic pretreatment is generally used to disrupt flocs and when applied to microalgae, it was found to improve solubilization of VS, but negative or low effect on methane production was reported.[78,79] Acid and alkaline pretreatments were also studied and a methane production increase by 15 and 16% were respectively found.[64,70] However, by combining thermal and chemical pretreatments, methane production can be enhanced as found in a previous work.[75] Moreover, biological pretreatment can achieve high methane production increase. It is the most eco-friendly pretreatment since it is generally carried out at mild conditions. Ciudad *et al.* (2014) found that fungi pretreatment enhanced methane production of *botryococcus braunii* by 67%,[80] while an increase of 87% of methane production was reported after the biological pretreatment of *chlorella vulgaris*.[81] Methane production from *Scenedesmus* sp. was also increased by 68% after enzymatic pretreatment.[82] The efficiency of pretreatment depends highly on the methane production increase, costs related to energy and chemical consumption as well as the impact of pretreatment on digestate management.

Table 2 Effect of microalgae pretreatments on its anaerobic digestion

Biomass	Pretreatment	Conditions	Results	Ref.
Consortia (*Scenedesmus* and *Chlorella*)	Microwaves	300 W, 3 min (50 °C) AD: batch test at 35 °C for 45 d 900 W, 3 min (98 °C) AD: batch test at 35 °C for 45 d	+280% of VS solubilization +13% of methane produced +799% of VS solubilization +78% of methane produced	77
Microalgae-based wastewater treatment system	Enzymatic	1% Enzyme mix (cellulase, glucohydrolase and xylanase) at 37 °C for 6 h AD: batch test at 35 °C for 45 d	+243% of soluble VS +15% of methane produced	3
Isochrysis galbana	Chemical	TS (45 g L^{-1}) 0.02% H$_2$SO$_4$ at 40 °C for 16 h AD: batch test at 30 °C for 15 d	+15% of biogas produced	64
Nannochloropsis Salina	Ultrasounds	TS (35%) 200 W, for 45 s AD: batch test at 38 °C for 40 d	+64% of soluble VS −29% of methane produced	79
	Microwaves	TS (35%) 600 W, 2450 MHz (until boiling) AD: batch test at 38 °C for 40 d	+130% of soluble VS +40% of methane produced	
	Thermal	TS (35%) 100 °C for 8 h AD: batch test at 38 °C for 40 d	+116% of soluble VS +58% of methane produced	
Phaeodactylum tricornutum	Ultrasounds	TS (67 g L^{-1}) 21 MJ kg^{-1} TS 36 MJ kg^{-1} TS 52 MJ kg^{-1} TS AD: batch test at 33 °C for 29 d	+11% of methane +10% of methane +11% of methane	78
Chlorella vulgaris	Thermochemical	TS (16 g L^{-1}) H$_2$SO$_4$ (4 M, pH 2) at 120 °C for 40 min AD: batch test at 35 °C for 30 d	+600% of released carbohydrates +11% of released proteins +65% of methane produced	75
	Thermochemical	TS (16 g L^{-1}) NaOH (4 M, pH 10) at 120 °C for 40 min AD: batch test at 35 °C for 30 d	+400% of released carbohydrates +94% of released proteins +73% of methane produced	
	Thermal	TS (16 g L^{-1}) 120 °C for 40 min AD: batch test at 35 °C for 30 d	+340% of released carbohydrates +17% of released proteins +93% of methane produced	
Nannochloropsis salina	Thermal	TS (16–30%) 120 °C for 2 h AD: semi-continuous at 38 °C, 1.96 kg per VS per m^3 per d for 120 d	+150% of biogas produced	76
Botryococcus braunii	Biological	TS (0.5 g L^{-1}) White-rot fungus (*Anthracophyllum* 1000 U L^{-1} for 24 h AD: batch at 30 °C for 55 d	+67% of methane produced	80
Chlamydomonas reinhardtii	Thermal (drying)	105 °C for 24 h AD: natch at 38 °C for 30 d	−18% of biogas produced	63
Scenedesmus sp.	Enzymatic	TS (16 g L^{-1}) Alcalase (0.2 mL g^{-1}) at 75 °C for 30 min. AD: batch test at 35 °C for 25 d	+68% of methane produced	82
Chlorella vulgaris	Biological	COD (20 g L^{-1}) 0.7 g L^{-1} of cellulase-secreting bacteria was added at 40 °C for 24 h. AD: batch test at 30 °C for 30 d	+87% of methane produced	99
Scenedesmus almeriensis and *Chlorella vulgaris*	Thermal pretreatment	WAS:microalgae = 25 : 1 60 °C for 24 h AD: batch at 37 °C for 50 d	sCOD increase (74-fold) −50% of methane produced	98
Macroalgae	Alkaline	TS (3%) 37% of HCl at 121 °C, 10 bars for 30 min. AD: batch at 37 °C for 50 d	+16% of methane produced	70

4.2. Codigestion of microalgae

As mentioned previously, codigestion aims to adjusting C to N ratio, humidity and diluting inhibitory compounds such as polyphenols.[83] Thus, codigestion of microalgae can improve methane production depending on methane potential of the co-substrate. Codigestion of microalgae with sludge was much studied. Microalgae can be used in WWTPs to treat wastewater effluents (N and P removal), their co-digestion with sludge in already existing digester in WWTPs appears to be an effective and cheaper way to valorize them. Many studies have investigated the effect of different microalgae to sludge ratio on methane production synergism. However, on a real scale, this ratio cannot exceed 50%. It is generally between 5/95 and 20/80 (based on VS).[4] In order to achieve the optimal C to N ratio of microalgae and septic sludge mixture, algal proportion should be within the range of 25–75% and a 3-fold improved methane production was observed compared to microalgae mono-digestion.[84] Similarly, Wang *et al.* (2013) reported that *Chlorella* sp. ratios of 4% and 11% resulted in enhanced methane production compared to microalgae alone (73–79%).[85] The synergetic effect of sludge and *Chlorella* sp. codigestion is not always observed, which was the case in.[86] Codigestion of sludge with 4% of *Chlorella* sp. resulted in higher dewaterability of digestate compared to monodigestion of microalgae. In addition, it was richer in ammonium and phosphorus, which suggests that its agronomic quality was improved due to sludge addition.[85] In parallel, addition of microalgae to sludge AD can highly affect digestate composition. Solé-Bundo *et al.* (2019) found that caffeine and triphenyl phosphate were removed from codigestion effluent by 92% and 64%.[87] Other organic wastes such as agricultural residues, animal wastes and food waste were studied as co-substrates of microalgae digestion. Agricultural residues are known for having a relatively high C to N ratio.[88] Thus, their codigestion with microalgae can improve the methane production. Potato peels wastes codigested with *Chlorella vulgaris* at a ratio of 25/75 resulted in 32% higher methane production,[89] where the C to N ratio increased when increasing potato peels proportion. Wheat straw was also used as cosubstrate for microalgae biomass containing mainly *Chlorella* sp. at a ratio of 50/50 (VS basis), AD was found to be slightly synergetic with 7% of additional methane production.[90] Moreover, codigestion of *Microcystis* spp. with corn straw was studied to enhance C to N ratio. It was shown that at a C to N ratio of 20/1, methane production was maximal with an increase of 62% compared to microalgae monodigestion.[91] Passos *et al.* (2018) reported that codigestion of *Chlorella* sp. with coffee husks provided 17% more methane production compared to theoretical production determined based on methane potential of both substrates.[92] However, in the literature, many studies are focused on certain species such as *Chlorella* sp. It is assumed that other microalgal biomasses, especially those rich in proteins such as *Dunaliella salina* and *Euglena gracilis*, should be studied in codigestion with carbon-rich organic substrates to adjust their C/N ratio, and avoid inhibition by ammonia.[93] Recently, codigestion of *Dunaliella salina* and olive mill solid waste was studied at a ratio of 5/95

and resulted in 29% high methane production compared to olive mill solid waste alone.[94] When mixing *Scenedesmus* sp. with pig manure at a ratio of 1/1, methane production was enhanced by 50% compared to microalgae alone, however, no synergetic effect was observed.[95] Food waste is also a carbon rich material, that is widely used as cosubstrate of sludge. It was already studied in codigestion with a mixture of *Chlorella vulgaris* and *Scenedesmus obliquus*. Results showed that at a ratio of 12/88 (microalgae to food waste ratio), methane production was enhanced by 20% compared to monodigestion of food waste.[96]

Anaerobic digestion can be integrated to microalgae valorization processes, such as lipid extraction and biodiesel production. In fact, microalgae residues from biodiesel production can be digested alone or codigested with other organic wastes. Relatively low synergy of methane production was obtained after the codigestion of lipid spend microalgae and glycerol at a ratio of 67/3. This was explained by the low C to N ratio of the mixture and also the potential inhibitory impact of the used solvent for trans-esterification.[97] Lipid extraction can be seen as a pretreatment step, as it can enhance biomass accessibility improving, thus, its methane potential, which was reported in.[95] Moreover, codigestion of lipid extracted microalgae with pig manure had the same effect as raw microalgae codigestion with pig manure at similar mixture ratio,[95] which raises the question of the effectiveness of combining co-digestion and molecules extraction of microalgae.

4.3. Combining codigestion and pretreatment

Theoretically, the hard digestibility of microalgae and some organic wastes can reduce the benefit of their codigestion. Thus, pretreatments can be carried out to one substrate or to the mixture. In fact, Mahdy *et al.* (2015) reported that thermal pretreatment at 120 °C for 40 min of *Chlorella vulgaris* before its mixture with primary sludge, improved methane production by 10%.[82] Similarly, pretreated microalgae mixed with sludge and fats, oil and grease resulted in 15% higher methane production compared to untreated microalgae.[86] However, the authors do not recommend the pretreatment as it has generally negative energy balance which must be compensated by a greater improvement in methane. Thermal hydrolysis (120 °C for 60 °C) of the mixture of microalgae and coffee husks increased methane production by 15%. However, it is assumed that the impact of pretreatment on individual substrates was more important (18% and 31% for microalgae and coffee husks respectively).[92] In some cases, the effect of pre-treatment is reversed. In fact, when low temperature pretreatment (60 °C for 24 h) was applied to microalgae and sludge mixture at a ratio of 1/25, methane production was reduced by 51% compared to untreated mixture.[98] However, previous researches assumed that there is no need to pretreat microalgae prior its codigestion. Moreover, additional costs related to chemicals and energy consumption can be generated.

5. Microalgae for agriculture

5.1. Impact on soil properties

Fig. 2 illustrates the different agronomic applications of microalgae. Soil degradation can be triggered by the extensive

agricultural practices in the fields which may affect 30% of the cultivable soils.[100] The application of microalgae enhances soil organic matter through production of exopolysaccharides which favors the growth of soil's fauna and flora. In addition, the inoculation of microalgae promotes the solubilization and mineralization of nutrients due to its photosynthetic activity.[101] Yilmaz and Sönmez (2017) found that the use of *Chlorella vulgaris* as biofertilizer added to vermicompost increased the soil aggregates stability and organic carbon by 19%.[102] Table 3 presents the effect of some microalgae use as fertilizer on soil nutrients. *Chlorella vulgaris* and *Scenedesmus quadricauda* were previously used as biofertilizers. It was found that the enzymatic activity of soil was enhanced by the addition of living microalgae as well as their extracts.[103] However, the use of live microalgae allows the *in situ* growth of these microorganisms at soil level, without the need for a separate cultivation process.[104] In addition, this technique reduces the costs related to the extraction of biostimulants or the drying of the algal biomass. Moreover, combining cyanobacteria with microalgae resulted in increased desert soil stability.[105] In the same manner, the application of microalgal consortia was found to enrich soil with the essential nutrients (N, P and K).[106] However, compared to cyanobacterial consortia, *Chlorella* application was less effective in enriching soil with nutrients.[106] Moreover, Dineshkumar *et al.* (2018) found that nitrogen, phosphorous and potassium and soil enzymes activities were positively affected by the use of *Chlorella* and *S. platensis* as biofertilizers, but the impact of *S. platensis* was the highest.[107] In addition, the use of microalgae and cyanobacteria was found to reduce the required N fertilizer dose.[107] Other essential nutrient such as iron, magnesium and zinc were found to be positively affected by microalgae and cyanobacteria addition.[108] Moreover, algal biomass can generate siderophores which promote ferric iron chelation and thus make it more accessible to plants and soil' microbial communities.[101]

Microalgae can promote the formation of biological soil crusts which help maintaining soil's biota and thus fertility. Yet, the greatest interest in using living microalgae in soils arises in their application to degraded soils such as desert soil. In fact, microalgae can be associated with minerals to form water-stable organomineral formulation which enhance arid soil fertility.[109] In addition, microalgae can survive the severe conditions due to their cellular structure especially the presence of cysts. For instance, *Protosiphon botryoides*, was reported to survive and was still active after 43 years in a dried soil content.[110] In the same context, Perera *et al.* (2018) highlighted the benefit of using the engineered consortia of cyanobacteria/microalgae for rehabilitating desert ecosystems.[105] However, the agronomic potential of microalgae may be limited by their growth which may be affected by soil moisture, pH (which need to be slightly acidic to basic) and temperature.[100] More studies should be carried out to investigate the mechanisms of soil fertilization by microalgae, especially in arid environments.

5.2. Impact on plant growth and properties

As seen previously, microalgae application can affect soil properties and thus increase availability of nutrients to the plants. In addition, it provides assimilable nitrogen to plant through atmospheric nitrogen fixation.[111] As the first step of plant growth is germination, healthy seedlings can be obtained due to microalgae application.[103] Some literature results showing the impact of microalgae and cyanobacteria on plant growth, are given in Table 3. Uysal *et al.* (2015) investigated the impact of *Chlorella* sp. in wheat and maize germination. It was found that an enhancement of 17% and 40% of germination rate was noticed for wheat and maize respectively.[112] Moreover, germination rate of bell pepper was increased by 36% after the application of both *Dunaliella salina* and *Phaeodactylum tricornutum*.[113]

Chlorella vulgaris and *Spirulina platensis* were found to enhance dry weight of maize plant by 30% and 35% which was increased to achieve 38% and 49% when adding a chemical fertilizer.[114] Wuang *et al.* (2016) found that *Spirulina platensis* enhanced dry weight of red spinach plant and its chlorophyll content and when mixed with chemical fertilizer, the application efficiency was enhanced.[115] Similarly, microalgae was reported to have higher impact on plant weight and pigments compared to inorganic fertilizer.[116] However, Jimenez *et al.* (2020) found that microalgae enhanced tomato plant weight and chlorophyll *a* by 32% and 13% compared to unfertilized soil, while industrial fertilizer had similar effect on tomato plant weight but higher impact on chlorophyll *a* (+69% with respect to control).[117] Also, it was reported that microalgae use had significant impact on nitrogen leaching (7% of loss) compared to industrial fertilizer (50% of loss).[117]

In fact, during their growth, microalgae uptake macro and micro nutrients and store them in their cells,[111] which explains their potential compared to chemical fertilizer. This effect depends strongly on species and their growth conditions,[103] dosages[118] and crop plant.[112] An algal liquid fertilizer was obtained using 500 mg of dried *Chorococcum* sp. biomass in 200 mL of distilled water. This algal liquid was found to have the best effect on 4 plants growth with a concentration of 50%.[118]

Moreover, the use of fresh or dried biomass was reported to have different impacts on lettuce growth in which case, fresh microalgae was recommended.[119] The enhancement of plant growth after microalgae/cyanobacteria inoculation is owed to their metabolites, which are capable of initiating plant metabolic reactions, such as respiration, photosynthesis, nucleic acid synthesis, chlorophyll production and ion absorption.[120] This finding was in agreement with Dineshkumar *et al.* (2019), in which cyanobacteria and microalgae mixtures with cow dung enhanced pigments and nitrogen, potassium and phosphorus contents in the maize plants.[114]

However, another study has shown that elemental composition of tomato plants were not significantly affected by microalgae addition as fertilizer, while nitrogen content was slightly increased due to industrial fertilizer incorporation.[117] The results of using microalgae in soil fertilisation differ according to the cultivation and agronomic tests conditions. However, the interest in using these species as biofertilisers remains high. More research studies are needed to understand much better the different interactions between these species
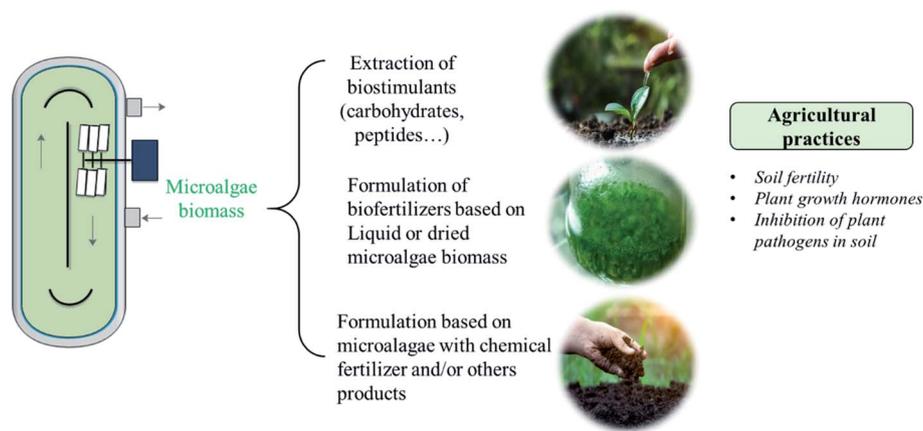
Fig. 2 Microalgae uses in agriculture.

and the soil/plant and the impact of biofertilisation on the nutritional quality of the fruit of these plants.

Despite the positive impact of applying microalgae biomass and extracts as fertilizer, the optimization of its concentration should be considered in order to maximize its advantage for plant growth. A 60% concentration of microalgae led to higher length, dry weight and chlorophyll content in tomato plant.[121]

Without any regard to the effectiveness of microalgae application, the use of microalgae as biofertilizer was found to be less beneficial compared to NPK fertilizer, which is mainly caused by microalgae cultivation and harvesting processes.[124] In the absence of optimization of these processes, the use of algae in agronomy may be less economically and environmentally attractive. In addition, the abiotic factors related to the growth environment can highly affect both the productivity and the biochemical composition of the microalgae.

To improve the profitability of the conversion of microalgae into valuable products for agronomy, the selection of microalgae strain to be used should be done in the basis of its composition and growth conditions. In addition, depending on extraction methods, several biofertilizers and biostimulants formulations can be realized.

For the commercialization of microalgae products, it is recommended to adopt a quality management system and the standardization of microalgae production processes to control the efficiency of the produced microalgae-based fertilizers.[125] Good field practices of these biofertilizers must be established, also the choice of plants and the frequency of treatment play a role in the effectiveness of these products.[124]

6. Closed-loop valorization of microalgae

6.1. Microalgae digestate as fertilizer

Generally, the composition of digestate depends on substrate composition, inoculum sources, AD conditions and configurations. Table 4 presents some typical properties of digestate. Digestate can be used as an organo-mineral fertilizer substituting the mineral fertilizers, due to its macronutrients elements (nitrogen, phosphorous and potassium).[126] In

addition, digestate is often rich in metal trace elements that are essential for plant germination and growth. However, an excessive heavy metal concentration or salinity can limit the use of digestate as fertilizer or amender. According to EU regulations, the limit concentrations of biosolids to be applied to soil should not exceed 2, 1000, 800, 10, 200, 1000 and 3000 mg kg^{-1} TS for Cd, Cu, Pb, Hg, Ni, Cr and Zn respectively. Moreover, digestate is much cheaper than mineral fertilizer, with a cost saving of 390 € per ha.[127]

Microalgae digestate can be used as biofertilizer as it is rich in organic matter, nutrients and heavy metals as presented in Table 4. Microalgae digestates was found to meet the European material requirements for an organic amendment. Available forms of nutrients in liquid and solid fractions of digestate from lipid-extracted *C. muelleri* were reported in a previous study.[131] In fact, 55% of nitrogen was found in solid digestate while 68% of phosphorus was released in liquid fraction. Phosphorus was found to be more available in algal digestates compared to dairy manure.[132] Even if the nitrogen was immobilized in the solid fraction, ammonium content in the dissolved organic matter fraction accounted for 40%, which highlights the fertilizing potential of microalgae digestate.[131]

Moreover, most *Oocystis* sp. cells were not degraded in digestate. This suggests that: (i) hydrothermal pre-treatment (130 °C for 15 min) improves the degradation of the organic matter contained in the microalgae and thus its methanogenic potential and also, (ii) to preserve organic matter for soil amendment with microalgae digestate, pre-treatments will not be favorable.[133] In addition, the presence of living microalgae can promote soil biofertilization. This finding was also reported in another study, where *Stigeoclonium* sp., diatoms *Nitzschia* sp. and *Amphora* sp. were present in digestate. The microalgal cells degradation during anaerobic digestion depend highly on algal stains.[134]

In addition, Solé-Bundó *et al.* (2017) study showed that microalgae digestate increased the growth index of cress by 10% which was lower than the growth index after codigestion residue application (75% VS of sludge and 25% VS of microalgae). Despite its lower nutrient content compared to monodigestate, the codigestion residue was found to present less phytotoxicity

Table 3 Effect of microalgae/cyanobacteria application for soil fertilization in literature

Species	Crop/soil	Conditions	Results	Ref.
Microalgal consortia comprising native unicellular strains of sewage (species of *Chlorella*, *Scenedesmus*, *Chlorococcum*, *Chroococcus*)	Wheat	$N : P : K = 120 : 60 : 60$ kg ha^{-1})	+38% of available N in soil at harvest stage +400% of available P	106
		75% of N and full dose of PK	+20% of available K	
Native filamentous strains isolated from sewage wastewater (species of *Phormidium*, *Anabaena*, *Westiellopsis*, *Fischerella*, *Spirogyra*)		50 g of biomass in 6 kg of soil	+52% of available N in soil at harvest stage +480% of available P +25% of available K	
Chlorella vulgaris	Onion	3 g of dried biomass per kg of soil	N in soil (+14%) P in soil (+3%) K in soil (+29%) Fe in soil (+21%) Zn in soil (+83%) Mg in soil (+149%)	108
Spirulina platensis		Mixed with 10 g of cow dung manure	N in soil (+17%) P in soil (+3%) K in soil (+12%) Fe in soil (+36%) Zn in soil (+114%) Mg in soil (+91%)	
Calothrix elenkinii	Rice/sandy clay loam (semi-arid climate)	Sterilized soil	Dry weight (+26%) Available N in soil (−14%)	122
		Unsterilized soil	Dry weight (+36%) Available N in soil (+13%)	
Acutodesmus dimorphus	Tomato	Greenhouse conditions at approximately 28 °C, in 85% relative humidity	+180% of branches number	123
		50 g of dry microalgae before 22 d of transplant	+150% of flower buds numbers Total fresh plant weight (10-fold)	
Chlorella vulgaris	Maize/sandy loam soil	3 g of dried biomass per kg of soil	Fresh weight (+38%) Dry weight (+30%) Chlorophyll *a* (+240%) Chlorophyll *b* (+225%)	114
		3 g of dried biomass per kg of soil Mixed with cow dung manure	Fresh weight (+57%) Dry weight (+38%) Chlorophyll *a* (+270%) Chlorophyll *b* (+275%)	
Spirulina platensis		3 g of dried biomass per kg of soil	Fresh weight (+41%) Dry weight (+35%) Chlorophyll *a* (+240%) Chlorophyll *b* (+250%)	
		3 g of dried biomass per kg of soil Mixed with cow dung manure	Fresh weight (+87%) Dry weight (+49%) Chlorophyll *a* (+270%) Chlorophyll *b* (+350%)	
Chlorella vulgaris	Maize	0.5 L of biomass diluted in 400 L of water	Plant height at 30th day (+60%) Germination rate (+40%)	112
	Wheat		Plant height at 30th day (+50%) Germination rate (+17%)	
Spirulina platensis *Chlorella vulgaris*	Rice/clay loam soil	Soil drench application of microalgae (+75% of recommended nitrogen)	Weight (+29%) Weight (+10%)	107
Nannochloropsis oculata	Tomato	Greenhouse conditions. Nutrients: 3600 mg per N per plant 4600 mg per K per plant	Dry weight (+42% compared to inorganic fertilizer)	116

Table 3   (Contd.)

Species	Crop/soil	Conditions	Results	Ref.
			Carotenoids (+50% compared to inorganic fertilizer)	
		Inorganic fertilizer 678 mg per P per plant	Dry weight (+23% compared to organic fertilizer)	
		Organic fertilizer: 1746 mg P per plant	Carotenoids (no significant difference compared to organic fertilizer)	
		Microalgae: 7900 mgP per plant		
S. platensis	Red spinach	5 g of biomass per pot	Chlorophyll (+34%)	115
			Dry weigh (+156%)	
		5 g of biomass per pot	Chlorophyll (+54%)	
		+Triple Pro 15-15-15, (0.3 g per pot per week)	Dry weigh (+430%)	
Phaeodactylum tricornutum	Bell pepper	NaCl (0 mM)	Germination (+36%)	113
		NaCl (25 mM)	No significant effect on germination	
Dunaliella salina		NaCl (0 mM)	Germination (+36%)	
		NaCl (25 mM)	No significant effect on germination	
Chlorella sp.	Wheat/Desert soil	Microalgae grown in wastewater	Plant height (+100%)	103
Scenedesmus sp.			Plant height (+100%)	
Tetraselmis sp.		Microalgae grown in seawater	Plant height (+77%)	
Nannochloropsis sp.			Plant height (+100%)	
Monoraphidium sp.	Tomato	170 kg per N per ha	Plant weigh (+32%)	117
			No effect on elemental composition of plants	
Chlorella vulgaris	Lettuce	Fresh 0.5 g kg^{-1} of soil	−4% of total pigments	119
		1 g kg^{-1} of soil	+30% of total pigments	
		2 g kg^{-1} of soil	−4% of total pigments	
		Dried 0.5 g kg^{-1} of soil	−9% of total pigments	
		1 g kg^{-1} of soil	−17% of total pigments	
		2 g kg^{-1} of soil	−14% of total pigments	

compared to untreated microalgae digestate, while the digestate of thermally pretreated microalgae had no impact on cress growth,[130] which suggest that the impact of codigestion with sludge on digestate agronomic properties was higher compared to the impact of pretreatment.

6.2.   Digestate as culture medium

As seen previously, mixotrophic mode was reported to be more advantageous concerning microalgae growth and productivity. As this mode requires nutrients and organic matter supply, wastewater, industrial and agricultural effluents as well as digestates can be used as cheap culture media for microalgae growth.[124] The optimized use of effluents as culture media can improve the cost-effectiveness of microalgae cultivation making it more competitive with industrial fertilizers.[124] At the same time, the treatment of these effluents can be carried out.

In fact, living or non-living algae can be used in heavy metals removal from wastewater. Biosorption using microalgae is more environmentally friendly if compared to surface modified biomass. It is also more cost effective with the possibility of converting the heavy metals to low toxic forms.[135] Using *Chlorella vulgaris* as biosorbent for Cr(VI) removal was found to be

effective. At a dose of 1 g L^{-1} of algae and 50 mg of Cr per L for 4 hours and pH 2, the biosorption efficiency achieved 80%.[136] Under the same conditions, *Spirulina* sp. was found to remove effectively chromium after 30 min.[137] Thus, the efficiency of heavy metal biosorption and operating conditions can be different from species to others. Also, pretreatment of microalgae may be needed to enhance its selective removal.[138]

Carbon, phosphorus and nitrogen from wastewater can also be removed by microalgae. *Chlorella vulgaris* and *Scenedesmus obliquus* are among the most studied biomasses for nutrients removal. Galan *et al.* (2020) reported that phosphorus and ammonium were removed from agricultural runoff with 100% and 93% efficiency respectively.[139] However, the use of microalgae for pesticides such as terbutryn, diuron, diazinon and imidacloprid removal was not effective.[139] Moreover, the use of microalgae for biosorption of toxic compounds can highly affect its ultimate conversion to biofuels, use for food or agriculture.[138] For this reason, the high nutrients and turbidity of the digestate can hinder its use as medium for microalgae culture. As light is the limiting factor for microalgae growth, suspended solids in digestate can absorb light and then increase its attenuation.[140] High turbidity can be overcome by pre-treating digestate with

Table 4 Digestate properties adapted from literature

Parameters	Typical digestate[128,129]	Digestate from microalgae[130]
pH	7.3–9.0	7.6
Matter profile		
TS (%)	1.5–45.7	2.9
Organic matter (%TS)	38–77	53
Organic carbon (%TS)	27–45	22
Nutrients profile		
NH_4^+ (g per N per kg per TS)	3.3–453.3	27.6
C/N	2–24.8	3
TKN (g per N per kg per TS)	31–140	80.6
Ca (g per CaO per kg per TS)	0.2–66	8.9
K (g per K_2O per kg per TS)	19–95	5.2
Mg (g per MgO per kg per TS)	1–47	4.2
Na (g per Na_2O per kg per TS)	0.7–25	9.4
P (g per P_2O_5 per kg per TS)	2–42	3.9
Cd (mg kg^{-1} TS)	—	2.7
Cu (g kg^{-1} TS)	0.01–0.27	0.59
Pb (mg kg^{-1} TS)	—	49
Hg (mg kg^{-1} TS)	—	1.7
Ni (mg kg^{-1} TS)	—	127
Cr (mg kg^{-1} TS)	—	75
Zn (g kg^{-1} TS)	0.07–2.2	0.59

centrifugation and filtration for examples which generates additional costs.[141] However, the most used method to reduce turbidity is dilution. Rajagopal *et al.* (2021) reported that above a digestate concentration of 30%, the high N content inhibited microalgae growth.[142] Dilution is also used to reduce the ammonia supply when its concentration in digestate exceed 100 mg L^{-1}.[141] In the same manner, C to N and N to P ratios in digestates should be maintained into the optimal ranges of 4–8 and 14–16 respectively for an effective microalgae growth.[141] Also, if CO_2 from anaerobic digestion is used for microalgae growth, attention should be paid to high concentrations of NO_x and SO_x.[143] Many studies have been carried out to assess the effect of digestate on microalgae growth as reported in Table 5. Digestate from different sources: manure, crop residues or municipal wastes showed different impacts depending on microalgae species, the dilution factor and growth conditions. Generally, a dilution factor above 1 : 10 is used.[144] Jimenez *et al.* (2020) reported that an increase in dilution factor enhanced the specific growth of *Monoraphidium* sp. as well as ammonium and phosphorus removal efficiencies.[117] However, in the case of using digestate from cattle slurry and cheese whey mixture, the final specific growth of three different species of microalgae was found to be reduced when increasing dilution factor. In fact, growth was faster when higher dilution factors were used, but after the 7th day, microalgae growth was slowed down which may be due to low nutrients concentrations in digestate and their fast consumption by microalgae.[145] However, the effect of digestate dilution on biomass growth can depend on microalgae strains.[146] In the case of *Synechocystis* sp., biomass productivity was improved by digestate dilution, contrarily to *N.*

salina.[146] Digestate source may also affect microalgae growth, which is obviously related to the composition of the substrates.[6]

Compared to synthetic media, digestate was reported to have a higher impact on microalgae growth and productivity.[147] However, Kobayashi *et al.* (2013) found that biomass productivity after digestate addition was 40% lower compared to Bold's Basal medium. This latter is rich in mineral nutrients and vitamins which may be more profitable compared to a diluted digestate.[148] Nitrogen removal from digestate was also found to increase when increasing digestate dilution.[148,149] The impact of using digestate as nutrient source for microalgae growth was found to be profitable for its agriculture application.[117] However, further studies should be carried out to determine to what extent this practice can be beneficial for the soil and the plant.

Rajagopal *et al.* (2021) studied the energy and nutrient benefit of coupling anaerobic digestion of chicken manure and microalgae production using liquid digestate. It was suggested that the nutrients provided by the digestates were sufficiently beneficial to have microalgae exploitable in the extraction of high value-added molecules.[142]

7. Challenges and recommendations

The main challenges of microalgae anaerobic digestion are: (i) low yield of biomass; (ii) inhibition occurrence due to high N and salinity content in microalgae is marine; and (iii) need for pretreatment in case of microalgae with thick cell wall; (iv) cultivation and harvesting costs.

Anaerobic digestion process requires the use of an available organic matter to feed the digester in case of continuous and

Table 5 Digestate use as nutrient source for microalgae growth

Digestate	Microalgae	Digestate dilution and pretreatment	Growth conditions	Results	Ref.
Mixture of crop residues and animal manure	*Monoraphidium* sp.	Separated with screw press, centrifuged and filtered with a 25 μm filter	33.75 μmol m^{-2} s^{-1} 21–23 °C	Specific growth = 0.03 d^{-1} NH$_4^+$ removal = 34%	117
		1 : 20	Continuous	P–PO$_4^{-3}$ removal = 0%	
		1 : 30		Specific growth = 0.05 d^{-1} NH$_4^+$ removal = 66% P–PO$_4^{-3}$ removal = 77%	
		1 : 50		Specific growth = 0.13 d^{-1} NH$_4^+$ removal = 100% P–PO$_4^{-3}$ removal = 92%	
Mixture of cattle slurry and cheese whey	*C. vulgaris*	1 : 10 1 : 25	200 μmol m^{-2} s^{-1} Air/CO$_2$ = 97/3 (v/v)	Specific growth = 0.64 d^{-1} Specific growth = 0.49 d^{-1}	145
	S. obliquus	1 : 10 1 : 25	25 °C	Specific growth = 0.49 d^{-1} Specific growth = 0.23 d^{-1}	
	N. oleoabundans	1 : 10 1 : 25	Continuous	Specific growth = 0.27 d^{-1} Specific growth = 0.26 d^{-1}	
Cattle manure	*C. sorokiniana* (UTEX 1230)	Centrifuged	160 μmol m^{-2} s^{-1} at 25 °C	Biomass productivity (-40% compared to Bold's Basal medium)	148
		1 : 10	Continuous	P–PO$_4^{-3}$ removal (+318% compared to Bold's Basal medium)	
Agroindustrial wastes	*Phaeodactylum tricornutum* *Pavlova lutheri*	Ultrafiltrate digestate	120 μmol m^{-2} s^{-1} at 22 °C Continuous	Specific growth = 0.24 d^{-1} (compared to 0.1 d^{-1} for f/2) Specific growth = 0.08 d^{-1} (compared to 0.05 d^{-1} for f/2)	147
Municipal wastewater and organic waste Sewage sludge	*Chlorella vulgaris*	1 : 30 1 : 10	70 μmol m^{-2} s^{-1} at 25 °C Light: dark (16 : 8)	Biomass dry mass = 2.1 g L^{-1} Biomass dry mass = 0.5 g L^{-1}	6
Animal manure and other organic wastes	*Neochloris oleoabundans*	1 : 20	50 μmol m^{-2} s^{-1}	Nitrogen content in supernatant = 4633 mg per N per L	149
		1 : 28	25 °C	Nitrogen content in supernatant = 4386 mg per N per L	
		1 : 50	Light: dark (10 : 10)	Nitrogen content in supernatant = 4229 mg per N per L	
Tetraselmis sp.	*Tetraselmis* sp.	80 mg L^{-1}	210 μmol m^{-2} s^{-1} 23–25 °C Continuous	0.5×10^6 cells per mL (compared to F/2 with 1.8×10^6 cells per mL)	150
Food waste	*Chlorella vulgaris*	1 : 10 Autoclaved cooled, and centrifuged	8000 lux 24–26 °C Continuous	Removal of NH$_4^+$ (40%) P–PO$_4^{-3}$ removal (28%)	151
Agro-industrial wastes	*Chlorella vulgaris* *A. obliquus*	1 : 10 Centrifuged and filtered	200 μmol m^{-2} s^{-1}, 25 °C Continuous	0.161 d^{-1} 0.145 d^{-1}	144
Municipal wastewater	*Synechocystis* sp.	3%	200 μmol m^{-2} s^{-1}	Biomass productivity = 151 mg per L per d	146
		6%	25 °C	Biomass productivity = 110 mg per L per d	
	N. salina	3%	Continuous	Biomass productivity = 83 mg per L per d	
		6%		Biomass productivity = 92 mg per L per d	

semi-continuous reactors. As microalgal biomass yield is generally around 0.5 g L^{-1},[152] monodigestion of microalgae does not appear to be a profitable way to valorize microalgal biomass because of the low methane productivity. Higher biomass recovery efficiency require the use of more expensive harvesting techniques such as centrifugation,[153] which is only preferred in the case of microalgae for high value-added products.[152]

Moreover, salinity can highly affect methanogenesis which may reduce methane production by 50% at a Na^+ concentration around 10 g L^{-1}.[154] As the optimal sodium content is around 0.23–0.35 g L^{-1}, monodigestion of marine microalgae can lead to operational problems and even to the failure of the anaerobic digestion process.[155] Co-digestion can, however, remedy this problem by using a co-substrate with a lower salt content such as sewage sludge or agricultural residues. Codigestion is also beneficial in case of protein-rich microalgae in order to avoid ammonia inhibition related to protein degradation through anaerobic digestion stages.[156] In fact, above 1.5 g of ammonium per liter, a reduction of methane production can occur.[157] In general, codigestion enables the dilution of potential toxic elements that may hinder anerobic digestion, in addition, it can regulate the C to N ratio leading to more optimal biogas production. Some other problems related to microalgal biomass bioaccessibility can be remedied when applying pretreatments as previously mentioned. The use of an effective pretreatment may require energy and chemicals consumption which generate additional operational costs. Energy and nutrients consumption during cultivation stage is among the most important concerns limiting the application of AD at large scale.

Previously, wastewater and digestate use as culture media showed positive impacts on microalgae growth when a dilution is carried out to reduce the toxicity of the elements that may be present in these liquids. In addition, the use of a renewable energy source such as solar or wind power to cover the needs of the upstream processes can greatly reduce the energy inputs.[158] So far, microalgae AD is not really economically viable.[5] Codigestion of microalgae residues after lipids or proteins extraction can be more interesting, even if the methanogenic potential of these molecules will be deducted from that of the raw microalgal biomass.[159] Anaerobic digestion and the use of microalgal residues as fertilizers after extraction of molecules of interest are rather considered as management strategies in the framework of the circular economy.

Fig. 3 presents a suggested system for a more sustainable and cost-effective valorization of microalgae. As mentioned previously, the co-digestion of agricultural or municipal solid wastes with microalgae residues after extraction of lipids, proteins or any other value-added molecules. The generated digestate can be then, used as fertilizer (solid fraction) or as nutrient source for microalgae growth (liquid fraction). The main advantage of this system is the recovery of energy, nutrients and CO_2 from anaerobic digester which are required for microalgae growth. In addition, to the treatment of microalgae residues which suggest that this process can reach the "Zero waste" objective if well optimized. However, a complete study needs to be carried out regarding technical, economic and energy calculations before establishing technological solutions in large-scale biogas production facilities.[17] This study should also take into account potential co-substrates that can boost
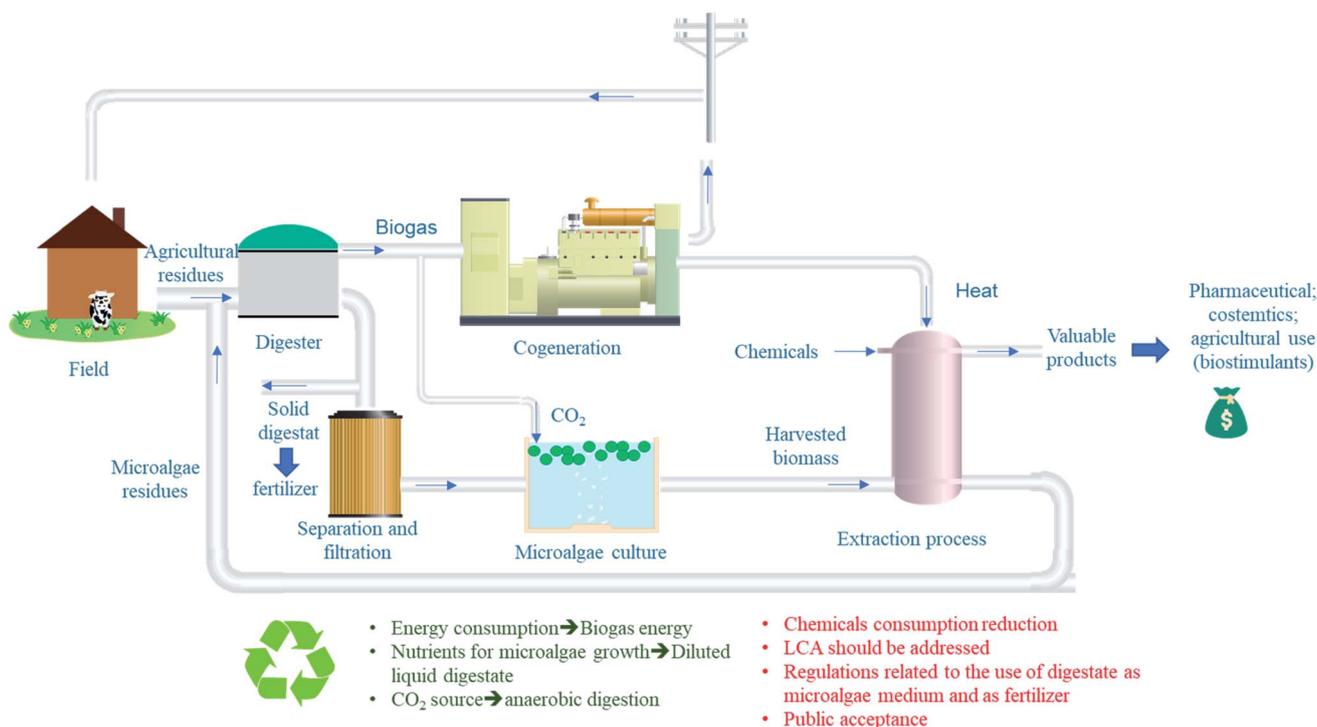

Fig. 3　Co-digestion and agronomic applications to valorize microalgae-derived residues.

methane production, their availability in a given geographical area and the costs of transporting these substrates and microalgae. A possible solution to reduce these costs would be the production of microalgae at the WWTP or within the farm in the case of using agricultural residues as cosubstrates. Moreover, sanitary aspect of using digestate for microalgae nutrients should be studied as well as its impact on microalgae properties and its eventual commercial value. As far as regulations are concerned, the use of microalgae for agronomic purposes and their residues should be reviewed and introduced. It is also necessary to study at what level the public will accept this practice, especially as some of the by-products will be returned to the soil.

8. Conclusions

The production of microalgae and their integration into a bio-refinery system is among the emerging and much studied strategies. However, their industrial application remains limited, as does their energy and economic efficiency. It is therefore recommended (i) to optimize the conditions of the different stages of the process to favor metabolites accumulation, (ii) to recycle nutrients from anaerobic digestate and CO_2 from biogas to reduce microalgae cultivation costs, (iii) to use microalgae residues for biogas production and soil fertilization for its organic and mineral components while meeting the environmental and economic requirements of this practice.

Abbreviations

AD	Anaerobic digestion
ATP	Adenosine triphosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
PBR	Photobioreactor
TS	Total solids
VS	Volatile solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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