Waste Biomass Valor (2017) 8:129–139
DOI 10.1007/s12649-016-9604-3

REVIEW

Recent Achievements in the Production of Biogas from Microalgae

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Received: 2 February 2016 / Accepted: 31 May 2016 / Published online: 8 June 2016
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Abstract Microalgae are nowadays regarded as a potential biomass feedstock to help reducing our dependence on fossil fuels for transportation, electricity and heat generation. Besides, microalgae have been widely investigated as a source of chemicals, cosmetics and health products, as well as animal and human feed. Among the cutting-edge applications of microalgae biomass, anaerobic digestion has shown promising results in terms of (bio)methane production. The interest of this process lies on its potential integration within the microalgae biorefinery concept, providing on the one hand a source of bioenergy, and on the other hand nutrients (nitrogen, phosphorus and CO_2) and water for microalgae cultivation. This article reports the main findings in the field, highlighting the options to increase the (bio)methane production of microalgae (i.e. pretreatment and co-digestion) and bottlenecks of the technology. Finally, energy, economic and environmental aspects are considered.

Keywords Microalgal biomass · Anaerobic digestion · Biogas · Co-digestion · Pretreatment

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Abbreviations

BMP	Biochemical methane potential
CHP	Combined heat and power
CH_4	Methane
C/N	Carbon/nitrogen
CO_2	Carbon dioxide
COD	Chemical oxygen demand
E_i	Energy input
E_o	Energy output
HRT	Hydraulic retention time
LCA	Life cycle assessment
LCC	Life cycle costing
LCFA	Long chain fatty acids
OLR	Organic loading rate
SEM	Scanning electronic microscope
TEM	Transmission electronic microscope
VS	Volatile solids
VFA	Volatile fatty acids

Introduction

Anaerobic digestion has long been used to produce biogas from organic residues, such as sewage sludge, agricultural and industrial by-products. More recently, this technique has been applied to microalgae and to the microalgae residue after lipid extraction. In this process, complex organic molecules are firstly hydrolysed releasing long chain fatty acids (LCFA) and alcohols from lipids, sugars from carbohydrates and aminoacids from proteins. Simple organic molecules are then fermented producing volatile fatty acids (VFA) like propionic, butyric and valeric acids, among others, via acidogenesis and acetic acid via acetogenesis. Finally, (bio)methane is produced from acetate via acetoclastic methanogenesis and from hydrogen via hydrogenotrophic methanogenesis. The main products of the process are:

- a biodegraded stabilised effluent, known as digestate; and
- biogenic gas mainly composed of (bio)methane and carbon dioxide, with minor amounts of ammonia, hydrogen sulphide and water vapour, which constitute the so-called biogas.

The process takes place in anaerobic digesters, which are enclosed (generally mixed) reactors. It may be performed under three temperature ranges, namely psychrophilic (<25 °C), mesophilic (30–40 °C) and thermophilic (above 50 °C). Mesophilic digestion is the most widely used at industrial scale, as it is well-known and fairly stable. However, under thermophilic conditions there is a higher activity of extracellular enzymes responsible for the hydrolysis of organic compounds, which may enhance the reaction rate and/or biodegradability of the substrate.

With the very same objective, pretreatment techniques, including biological, chemical and physical methods, have been applied to biomass. The idea behind is to ease the hydrolysis of slowly biodegradable macromolecules, which otherwise may not be converted into bio(methane) within the typical reactor retention time (20–30 days). They have been applied to waste activated sludge to enhance bacteria cells lysis and release intracellular compounds [1], and to lignocellulosic biomass to disintegrate macromolecules in vegetable cell walls and release intracellular compounds [2]. They have also been tested on microalgae [3].

Another means of improving anaerobic digestion performance is by co-digesting complementary substrates altogether in the same reactor (Fig. 1). In this case, the aim is to equilibrate the substrate composition [i.e. carbon/nitrogen ratio (C/N)] in order to promote microbial growth, hence the reaction rate. In fact, the C/N ratio plays an important role in anaerobic digestion stability, and values between 15 and 30 have shown a positive effect on the methane yield [4]. Lower C/N ratios may lead to ammonia inhibition, while higher C/N ratios may cause nitrogen deficiency for biomass synthesis. Hence, the co-digestion of different substrates creates a synergistic effect by alleviating nutrients imbalance and attenuating potential inhibition effects of individual substrates. Thus, some highly energetic compounds such as fats which may not be digested as a sole substrate are most appropriate to improve the methane yield of less energetic ones. Indeed, lipids have the highest energy value (37.6 kJ/g), followed by proteins (16.7 kJ/g) and carbohydrates (15.7 kJ/g) [5].

The following sections will focus on the anaerobic digestion of microalgae, including pretreatment and co-digestion experiences attempted to improve the process performance. Energy, economic and environmental aspects, as well as challenges for future research will be highlighted.

Anaerobic Digestion of Microalgae

Both freshwater and marine microalgae species have drawn attention as anaerobic digestion substrate for biogas production. Intensive research has been developed during the last years, testing a range of microalgae strains, operational

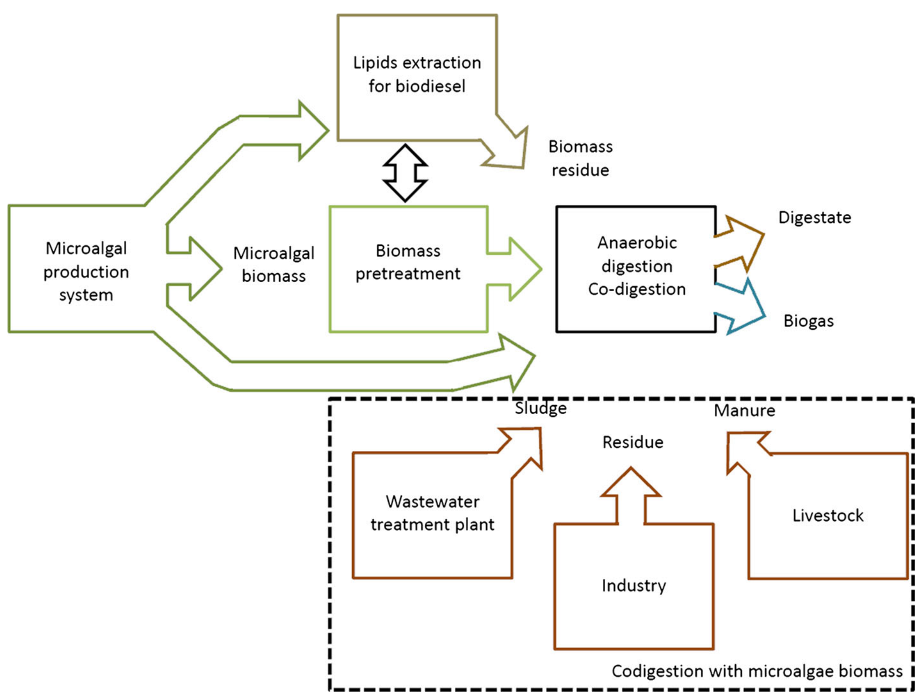

Fig. 1 Anaerobic digestion and co-digestion substrates

parameters and reactor configurations in order to enhance the (bio)methane production through anaerobic digestion [6, 7]. In fact, operational (i.e. bioreactor design, hydraulic retention time (HRT) and temperature) and cultivation conditions, which are responsible for variations in cellular proteins, carbohydrates and lipids contents, may lead to a wide variation in methane conversion [8].

Substrates

Due to the cell wall structure of different microalgae species, anaerobic digestion performance is highly strain specific [6], and so is the potential methane yield (Table 1). For instance, values up to 0.39 L CH_4/gVS were found for *Chlamydomonas reinhardtii*, while values about 0.1 L CH_4/gVS were obtained by digesting *Chlorella* and *Scenedesmus* biomass [6].

During the last years the feasibility of digesting the microalgae residue after lipid extraction for biodiesel production has been shown [9]. This option is gaining interest bearing in mind that the biomass residue represents approximately 65 % of the initial biomass, whose treatment or disposal would otherwise increase biodiesel production costs. Indeed, the microalgae residue still contains proteins and carbohydrates, which could undergo anaerobic digestion to produce biogas. For example, Yang et al. [10] obtained a methane yield of 0.39 L CH_4/gVS by digesting residual *Scenedesmus* biomass derived from oil extraction processes. This value is quite high in comparison with the values reported in Table 1, probably due to the pretreatment applied by the authors before digestion (8 g/L NaOH at 100 °C for 8 h). Additionally, the anaerobic digestion of residual *Scenedesmus* biomass after aminoacid extraction saved energy, fertilizer and carbon dioxide (CO_2) needs. In a recent study, a semi-continuous reactor operated at an OLR of 3.8 g VS/L day produced 0.29 L CH_4/g VS [11].

Table 1 Methane production from different microalgae species and microalgal biomass under mesophilic conditions (T < 40 °C). Adapted from [6] with permission © John Wiley and Sons Ltd (2012)

Microalgae species	Methane yield (L CH_4/g VS)
Chlamydomonas reinhardtii	0.39
Dunaliella sp.	0.32–0.44
Spirulina sp.	0.26–0.32
Scenedesmus oliquus	0.18
Chlorella vulgaris	0.15–0.35
Spirulina maxima	0.09–0.15
Scenedesmus residue after lipid extraction	0.1–0.14
Chlorella and *Scenedesmus* biomass	0.09–0.16
Microalgal biomass[a]	0.10–0.18

[a] It refers to microalgae-bacteria consortia grown in wastewater

The high methane yield was attributed to a physical pretreatment with a high-pressure homogenizer and enzymatic hydrolysis [11].

Products

The (bio)methane produced through anaerobic digestion, which accounts for about 60–70 % of the biogas, can be used as fuel gas to generate heat in a boiler or to co-generate electricity and heat in a combined heat and power (CHP) unit. Other interesting applications such as biofuel for transportation or natural gas grid injection require biogas upgrading techniques to increase the methane content (>90 % CH_4).

In order to close the flow of products, it would be particularly interesting to reuse the CO_2 released during biogas combustion to improve microalgae growth. In fact, inorganic carbon is a primary nutrient for microalgae and its limitation should be prevented to optimise microalgal growth. In this context, it has been shown that *Arthospira* sp. and *Chlorella vulgaris* were able to consume CO_2 directly from biogas in a range of concentrations between 2 and 56 % CO_2 (v/v) in the mixture [12, 13]. In general, the exploitation of biogas in a co-generation process can release a gas mixture characterised by low concentrations of toxic compounds (NO_x, SO_x, C_xH_y, CO, heavy metals and particles) that could be injected in the microalgae culture. However, this should be further explored because the literature on the subject is still scarce.

Besides biogas, the digestate is another anaerobic digestion product with interesting properties. In fact, this effluent is rich in phosphorus and organic nitrogen compounds. Many options for nutrient extraction from the digestate are nowadays being explored in order to produce high quality fertilizers (e.g. ammonia stripping for ammonium sulfate production or phosphorus recovery by struvite precipitation). These processes, which may be improved by the addition of organic or mineral flocculants, produce:

- a liquid fraction, rich in mineralised elements that can be re-used for microalgal culture; and
- a solid fraction, usually composted, dried and/or exploited as an organic amendment in crop fields.

In this respect, the liquid phase of dewatered digestate from sewage sludge and manure digestion was successfully used as nitrogen source for microalgae cultivation [14, 15]. Indeed, the growth rates of microalgae on digestate were similar to those obtained with urea [13]. Regarding the solid fraction of the digestate, Collet et al. [16] reported that an organic content composed of 120 kg of carbon, 4.5 kg of nitrogen, 0.6 kg of phosphorus and 0.5 kg of potassium would result in the production of 33 m^3/d of soil conditioner.

Anaerobic Digestion Within the Microalgae Biorefinery Concept

The recent interest in microalgae anaerobic digestion lies on the production of biogas and mineralisation of microalgae containing organic nitrogen and phosphorus. Indeed, microalgae anaerobic digestion offers a wide range of opportunities in terms of biomass treatment and product applications.

The integration of anaerobic digestion within the microalgae biorefinery concept provides, on the one hand, an important source of bioenergy and, on the other hand, nutrients (nitrogen, phosphorus and CO_2) and water for microalgae cultivation. Indeed, freshwater and fertilizer consumption significantly increase microalgae culture costs and, for this reason, they are among the main challenges for scaling-up microalgae biorefinery technologies.

The wide range of substrates and anaerobic digestion products allows the placement of this process at different stages of a biorefinery chain, promoting the generation of multiple products from microalgae biomass (i.e. (bio)-methane, fertilizers and nutrients for microalgae culture). In other words, the residues from a process could be used as input for another process, towards the zero waste approach.

For instance, anaerobic digestion can be conceived as:

- a sludge treatment and (bio)methane production process in a conventional wastewater treatment plants (in this case sludge is co-digested with microalgal biomass harvested from wastewater treatment units in order to produce (bio)methane and fertilizers);
- a treatment of the microalgae residue after the extraction of molecules for high-value products generation (the (bio)methane and fertilizers are here generated from the microalgal biomass waste);
- a source of nutrients for microalgae production (microalgal biomass could then be used for fuel or energy purposes).

Methods to Improve Anaerobic Digestion Performance

Anaerobic biodegradability is limited by microalgae cell walls, composed of slowly biodegradable macromolecules like cellulose and hemicellulose. Thus, either long HRT or pretreatment techniques are needed to enhance the anaerobic biodegradation rate and extent. Indeed, the methane yield of *Chlorella vulgaris* was improved from 0.11 to 0.18 L CH_4/g COD by increasing the HRT from 16 to 28 days [17], and from 0.10 to 0.18 L CH_4/g VS by increasing the HRT from 15 to 20 days in the case of microalgal biomass from wastewater treatment systems [18]. In practise, though, this would require a larger reactor with higher capital cost. In order to uncouple the retention time of solids and liquids, Zamalloa et al. [19] employed a hybrid flow-through reactor combining a sludge blanket and a carrier bed. This configuration was conceived to increase the retention time of microalgae, which require longer time than the liquid fraction to be degraded. Even if 0.28 L CH_4/gVS were obtained in this study, the authors concluded that microalgae biomass was not readily biodegradable under such conditions and pretreatments were recommended so as to enhance the methane conversion of biomass.

Pretreatment Techniques

Pretreatment methods have proved successful at enhancing the methane yield of complex biomass and/or cell structures, such as sewage sludge, lignocellulosic biomass and several strains of microalgae [1–3, 20]. Regarding microalgae, most species have a tough cell wall containing low biodegradable substances, which hinders the anaerobic digestion rate and extent. Recent studies have shown that microalgae pretreatment is effective at improving anaerobic digestion performance (Table 2). Some of them (thermal, microwave and enzymatic pretreatments) have already been tested in continuous reactors, while others (thermal hydrolysis, thermochemical and ultrasound pretreatments) have only been evaluated in biochemical methane potential (BMP) tests.

The thermal pretreatment at low temperature (<100 °C) has only been investigated in continuous reactors using microalgal biomass from wastewater treatment systems. In these systems, microalgae cells generally have a resistant and complex cell wall conferring a slow and/or low biodegradability. Nonetheless, the methane yield was increased by 30–70 % after thermal pretreatment at 60–100 °C [18, 21, 22]. Regarding the thermal pretreatment at higher temperature (>100 °C), the methane yield of *Nannochloropsis salina* increased by 108 % after thermal pretreatment at 100–120 °C [23], *Scenedesmus* sp. showed a 3-fold methane yield increase [24], while *Oocystis* sp., a microalgae species with a complex trilayer cell wall, grown in wastewater treatment ponds, showed a lower methane yield increase of 42 % after pretreatment at 130 °C [25]. Finally, the thermal hydrolysis at 170 °C and 8 bars for 30 min increased the *Scenedesmus* biomass methane yield by 83 % [26], and outcompeted the thermal pretreatment at lower temperature (55 °C) and ultrasonication in BMP tests [27].

The only mechanical technique that has already been studied in continuous reactors is microwave irradiation. It increased the methane yield of microalgal-bateria biomass grown in wastewater by 60 % (from 0.17 to 0.27 L CH_4/g VS) [28]. Electronic microscope techniques, such as SEM

Table 2 Microalgae pretreatment for improved anaerobic digestion

Microalgae species	Pretreatment conditions	Methane yield increase	References
Continuous reactors			
Scenedesmus sp. and *Chlorella* sp.	Thermal:	33 %	[21]
	100 °C, 8 h	(0.270 L CH_4/g VS)	
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Thermal:	70 %	[18]
	75 and 95 °C, 10 h	(0.180 L CH_4/g VS)	
Pediastrum sp., *Micractinium* sp. and *Scenedesmus* sp.	Thermal:	32 %	[22]
	60 °C, 2–6 h	(0.136 L CH_4/g VS)	
Nannochloropsis salina	Thermal:	108 %	[23]
	100–120 °C, 2 h	(0.130 L CH_4/g VS)	
Oocystis biomass	Thermal:	42 %	[25]
	130 °C, 15 min	(0.120 L CH_4/g VS)	
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Microwave:	60 %	[28]
	70 MJ/kg VS, 26 g TS/L	(0.272 L CH_4/g VS)	
Chlorella vulgaris	Enzymatic:	260 %	[31]
	Protease (0.585 UA), 65 g TS/L	(0.128 L CH_4/g COD)	
BMP tests			
Scenedesmus sp.	Thermal hydrolysis	246 %	[27]
	165 °C, 8 bar, 30 min	(0.320 L CH_4/g VS)	
Scenedesmus sp.	Thermal hydrolysis:	83 %	[26]
	170 °C, 8 bar, 30 min	(0.330 L CH_4/g VS)	
Chlorella vulgaris	Thermochemical:	65 %	[33]
	pH 2 (H_2SO_4), 120 °C, 40 min	(0.229 L CH_4/g COD)	
Chlorella vulgaris	Thermochemical:	73 %	[33]
	pH 10 (NaOH), 120 °C, 40 min	(0.241 L CH_4/g COD)	
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Ultrasound:	33 %	[29]
	106 MJ/kg VS, 19 g TS/L	(0.196 L CH_4/g VS)	

(scanning electronic microscope) and TEM (transmission electronic microscope) images showed how some microalgae cell walls remained intact; although intracellular organelles were strongly damaged after the pretreatment step, possibly easing the anaerobic biodegradability (Fig. 2) [28]. For ultrasound pretreatment, BMP tests of microalgal biomass grown in wastewater showed that the higher the applied specific energy the higher the final methane yield, with the highest value obtained for the trial pretreated at 106 MJ/kg VS (33 % increase) [29]. However, a comparative assessment of thermal and mechanical techniques using microalgal biomass from wastewater treatment systems showed how the thermal pretreatment (<100 °C) achieved the highest macromolecules solubilisation and methane yield increase [30].

The enzymatic pretreatment with protease increased *Chlorella vulgaris* methane yield by 260 % (from 0.05 to 0.13 L CH_4/g COD) in continuous reactors [31]. In BMP tests, the highest methane yield was attained when microalgae were pretreated using an enzyme mix composed by cellulase, glucohydrolase and xylanase (0.22 L CH_4/g VS) if compared to non-pretreated biomass (0.19 L CH_4/g VS) or biomass pretreated with cellulase alone (0.20 L CH_4/g VS). The best results attained with the cocktail were due to the synergistic effect among several macromolecules contained in the cell structure [32].

With regards to the thermochemical pretreatment, the methane yield increase was higher under alkali conditions (pH 10) with 73 % methane yield increase, compared to acid conditions (pH 2), with 65 % increase. Nevertheless, in this study the highest methane yield was reached after thermal pretreatment at 120 °C without chemical addition (93 % increase) [33].

Lab-scale experimental results suggest that microalgae pretreatment improves the anaerobic digestion performance and methane yield. Prospective research in pilot-scale reactors should elucidate the scalability of the techniques according to the energy balance of microalgae conversion to biogas.

Co-digestion

Microalgal biomass generally contains high amounts of nitrogen, hence very low C/N ratios around 6 [34].

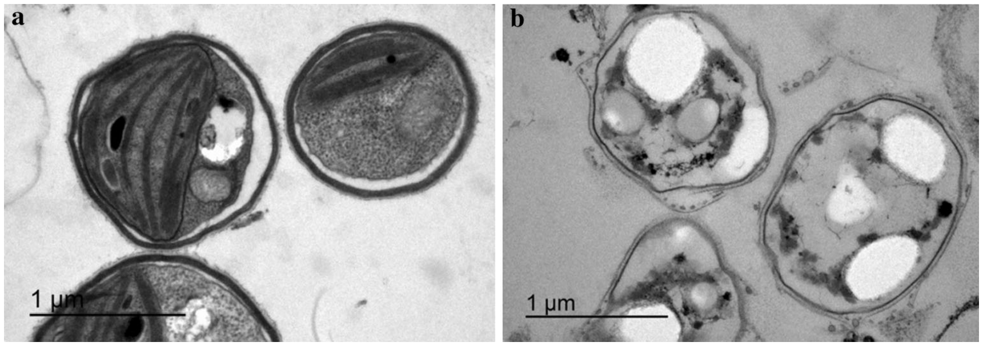

Fig. 2 TEM images of *Monorraphidium* sp. before (**a**) and after (**b**) microwave pretreatment. *Source*: Passos et al. [28]

Therefore, carbon-rich co-substrates may be added to enhance the methane conversion process (Table 3). For example, the addition of carbon-rich paper waste to a mixture of *Scenedesmus* spp. and *Chlorella* spp. doubled methane yield from 0.14 to 0.23 L CH_4/g VS [34]. Besides, the co-digestion of microalgae with other carbon-rich substrates can enhance the anaerobic digestion processes at high OLRs. For instance, experiments conducting continuous anaerobic co-digestion of *Scenedesmus* ssp. and *Opuntia Maxima* at 5.33 gVS/L day showed stable performance with high methane yield and no ammonia inhibition [35].

Concerning the microalgae residue after lipid extraction, the co-digestion with lipid-rich fat, oil, and grease waste increased the methane yield from 0.15 L CH_4/g VS (when only microalgae biomass was digested) to 0.54 L CH_4/g VS [36]. Likewise, the co-digestion of the *Chlorella* residue with waste glycerol from the transesterification process for biodiesel production showed a 4–7 % increase in CH_4 production [37]. The authors highlighted that some solvents used for oil extraction, such as chloroform, inhibited the methane production. Even if solvent effects can be reduced by rinsing to remove toxic solvents from biomass, it should be carefully selected when microalgae residues are reused for biogas generation.

Microalgae co-digestion may play a role within the microalgae biorefinery concept (Fig. 1). Moreover, when microalgae are produced as a by-product of wastewater treatment, sewage sludge is generated in the same process chain. In such a case, the co-digestion of primary sludge and microalgae may not only enhance anaerobic digestion (due to an increased C/N ratio), but it may also optimise waste management. A recent study showed that co-digestion of primary sludge (75 % COD) and *Chlorella vulgaris* (25 % COD) enhanced microalgae methane yield by 17 % in respect to theoretical values. Moreover, no ammonia inhibition was observed despite the high nitrogen content of microalgae, considering the higher C/N ratio of primary sludge in respect to *C. vulgaris* [38]. Additionally, co-digestion of *Chlorella* sp. with waste activated sludge improved the volatile solids reduction, hydrolysis efficiency as well as the biogas yield of microalgae by 10 % [39]. Similarly, the anaerobic co-digestion of a mixture of *Chlorella* sp. and *Scenedesmus* sp. (37 % VS) with sewage sludge (63 % VS) produced 23 % more methane than with sewage sludge alone [40].

Finally, the anaerobic co-digestion of microalgae with manure has recently been investigated. Although both substrates are characterized by low C/N ratios, some synergies have been pointed out with their co-digestion. For instance, the methane yield was increased by 8–74 % when microalgal biomass was digested with different quantities of swine manure as cosubstrate [41]. Similary, *Scenedesmus* biomass theoretical methane yield was increased by 50 % after co-digestion with pig manure, from 0.16 to 0.25 L CH_4/g VS. This fact may be attributed to the higher biodegradability of pig manure compared to microalgae [42].

Nutrients Starvation

Another approach to improve the methane yield is to try and modify the microalgae macromolecular composition by nutrient starvation during microalgae cultivation. For example, Mairet et al. [43] indicated that high carbohydrates content, especially simple sugars like glucose, could be advantageous for anaerobic digestion. In line with this, Markou et al. [44] increased the carbohydrates content through phosphorus limitation, observing how the methane yield ranged between 0.12 and 0.20 L CH_4/gVS according to the carbohydrate enrichment percentage. Similarly, an enhancement in the biogas production of *Chlamydomonas reinhardtii* due to the increase of its carbohydrates content after sulfur starvation was reported by Mussgnug et al. [45].

Energy, Economic and Environmental Assessment

Energy, economic and environmental aspects are important parameters for scaling-up the technology; thus this section will address these issues.

Table 3 Co-digestion of microalgae and other residues for improved anaerobic digestion

Microalgae species and co-substrates	Co-digestion conditions	Methane yield increase	References
Continuous reactors			
Algae sludge and waste paper	50 % VS of algae sludge and 50 % VS of waste paper	104 % (1.17 L CH_4/L d)[a]	[34]
Scenedesmus sp. and *Opuntia Maxima*	25 % VS of *Scenedesmus* sp. and 75 % VS of *O. maxima*	NP[b] (0.31 L CH_4/g VS)	[35]
BMP tests			
Lipid-extracted *Chlorella* sp. and glycerol	5.85 g *Chlorella* sp. and 0.21 g pure glycerol	20 % (0.27 L CH_4/g VS)	[37]
Chlorella sp. and WAS[c]	21 % of *Chlorella* sp. and 79 % of WAS	10 % (0.25 L biogas/g VS)	[39]
Chlorella sp. + *Scenedesmus* sp. and sewage sludge	37 % VS of microalgae and 63 % VS sludge	23 % (0.41 L CH_4/g VS)	[40]
Microalgal biomass and swine manure	14.6 % COD of microalgae and 85.4 % COD of swine manure	74 % (0.22 L CH_4/g COD)	[41]
Lipid-extracted *Chlorella* sp. and lipid-rich fat	50 % of *Chlorella* sp. and 50 % of lipid-rich fat	260 % (0.54 L CH_4/g VS)	[36]
Scenedesmus sp. and pig manure	50 % VS of *Scenedesmus* sp. and 50 % VS of pig manure	50 % (0.245 L CH_4/g VS)	[42]
Chlorella vulgaris and primary sludge	25 % COD of *Chlorella vulgaris* and 75 % COD of primary sludge	10 % (0.231 L CH_4/g COD)	[38]

In this study anaerobic digestion was carried out at 20 °C

[a] Results expressed as methane production rate

[b] Not presented

[c] Refers to waste activated sludge

Energy Assessment

In the previous section it has been shown that pretreatment methods may improve the anaerobic biodegradability of microalgae. To make them feasible, these techniques should not only improve the methane yield, but also the net energy production. In this sense, mechanical methods that employ electricity (i.e. microwave, ultrasound) seem less feasible than thermal pretreatments that use waste heat from CHP units fuelled by the produced biogas [3]. Furthermore, upon application of thermal pretreatments, heat could also be recovered while cooling down pretreated biomass from the pretreatment to the digestion temperature. Therefore, this review was focused on thermal pretreatments.

According to the literature, the anaerobic digestion of microalgal biomass (13.5 g VS/L) in lab-scale continuous reactors following a thermal pretreatment at 75–95 °C would lead to surplus energy generation; i.e. 20–30 % excess energy produced over the energy consumed by the process [18]. In fact, the thermal pretreatment of *Nannocloropsis salina* (200 g VS/L) at 120 °C only consumed 7 % of the energy produced; while electricity and heat generation increased by 100 % after applying the pretreatment step [23]. However, the thermal pretreatment of *Oocystis* sp. (14.5 g VS/L) at 130 °C showed a negative energy balance, due to the low methane yield obtained during the anaerobic digestion [25]. On the whole, it is troublesome to compare the energy assessment calculated using experimental data from studies using with different biomass concentration, reactor configuration and operations conditions.

For this reason, standard anaerobic digestion conditions were here defined to calculate the energy balance of different pretreatments based on literature results (pretreatment temperature and methane yield) from continuous lab-scale reactors (Table 2). It was supposed that biomass would be thickened to reach a concentration of 40 kg VS/m^3, the flow rate would be 10 m^3/day and the digester HRT 20 days. The energy balance (ΔE) was calculated as the amount of energy produced (energy output, E_o) subtracted by the amount of energy invested (energy input, E_i) in the process, as described in detail elsewhere [18]. The energy input included the electricity required for biomass pumping and reactor mixing, and the heat required to raise influent biomass temperature to the pretreatment temperature, subtracted by the heat recovered when cooling down pretreated biomass to mesophilic digestion conditions. Heat

Table 4 Energy assessment of the thermal pretreatment and mesophilic anaerobic digestion of microalgae in continuous reactors

Microalgae species	Control (without pretreatment)				Thermally pretreated					References
	Methane yield (m^3 CH_4/kg VS)	Ei (GJ/d)	Eo (GJ/d)	ΔE (GJ/d)	Pretreatment temperature (°C)	Methane yield (m^3 CH_4/kg VS)	Ei (GJ/d)	Eo (GJ/d)	ΔE (GJ/d)	
Chlorella sp. and *Scenedesmus* sp.	0.24	835	3093	2258	100	0.32	3019	4124	1105	[21]
Nannochloropsis salina sp.	0.13	835	1675	841	120	0.27	3855	3480	−375	[23]
Pediastrum sp., *Micractinium* sp. and *Scenedesmus* sp.*	0.10	835	1327	493	57	0.14	1221	1753	531	[22]
Oocystis sp.	0.12	835	1547	712	130	0.17	4273	2191	−2082	[25]
Stigeoclonium sp., *Monorraphidium* sp. and diatoms	0.18	835	2320	1485	75	0.30	1974	3866	1893	[18]
Stigeoclonium sp., *Monorraphidium* sp. and diatoms	0.18	835	2320	1485	95	0.31	2810	3995	1185	[18]

* In this study anaerobic digestion was carried out at 20 °C

losses through the reactor walls were also accounted for. The energy output considered the electricity and heat generated in a CHP unit fuelled by biogas, with a conversion efficiency of 35 % for electricity and 55 % for heat. Finally, the global energy balance was calculated by adding the heat and electricity balances. Positive values represent surplus energy generation, hence a self-sustainable process.

Results from the energy assessment are summarised in Table 4. As can be seen, the energy balance of control reactors without pretreatment would always be positive, meaning that digesters treating thickened microalgal biomass would be net energy producers. The results ranged from 500 to 2250 GJ/day. The thermal pretreatment would thus aim at further increasing the energy gain, by improving the anaerobic biodegradability of microalgal biomass. In this case, energy balances were only positive with pretreatment temperatures up to 100 °C (i.e. 57, 75, 95 and 100 °C) [18, 21, 22], and negative for higher temperatures (i.e. 120, 130 °C) [23, 25]. Positive values ranged from 500 to 1900 GJ/day. Differences between our calculated values and those published by the authors are due to the biomass concentration used for the calculations (i.e. 40 g VS/L in our case). If we compare the net energy generation with and without pretreatment, the results are more evident when microalgal biomass shows a low biodegradability, as compared to those of non-pretreated biomass with a high methane yield.

On the whole, the results suggest that the thermal pretreatment at low temperatures (<100 °C) is a promising technique for increasing the methane yield and net energy production, especially when microalgal biomass shows a poor anaerobic biodegradability, since microalgae anaerobic digestion depends highly on the predominant species, its cell structure and cell wall characteristics.

Economic Analysis

In terms of costs, different studies analysed biodiesel production from microalgae including the anaerobic digestion of residual biomass from lipid extraction. The cost of 1 L of biodiesel varied between 1.94 and 3.35€, being the capital cost for the cultivation step (60 and 30 % of the total cost for biodiesel production in photobioreactors and raceway ponds, respectively) the most influential parameter [46]. A Life Cycle Costing (LCC) comparing open ponds and closed photobioreactors for microalgae cultivation for biodiesel production showed that, even if both systems appeared to be financially unattractive, improving the process line (e.g. enhancing efficiency of CO_2 utilization and anaerobic digestion of residual biomass) could make the open pond systems profitable [47]. Moreover, the capital cost of the photobioreactor was estimated to be 100 times higher than the raceway pond capital cost [48]. Therefore, the production cost of microalgal biomass grown in photobioreactors was significantly higher compared to that of microalgae cultivated in raceway ponds (3.8–10 and 0.3–1.6 €/kg_{algae} for photobioreactor and raceway pond systems, respectively) [49].

Regarding biogas production from microalgae, the economic feasibility of growing and harvesting microalgae biomass to feed the digester and produce electricity also depends on the local power price [50]. Other drawbacks (such as the high water content, seasonal variations in biomass production and species composition, and the occurrence of inhibitory phenomena during anaerobic digestion), contribute to making it not yet economically feasible although it is more environmentally friendly than fossil fuels [51]. The economic feasibility of biogas production from microalgae may be improved by integrating microalgae production and wastewater treatment. In this case, the costs of microalgae production and harvesting might be covered by the wastewater treatment plant capital and operational costs [51, 52].

Environmental Assessment

From an environmental point of view, a Life Cycle Assessment (LCA) analysed the environmental performance of anaerobic digestion of microalgal biomass cultivated in high rate algal ponds [16]. Results showed that electricity consumption (especially for mixing and pumping in the cultivation step) and materials for the high rate algal ponds construction were the main source of impacts [16]. Moreover, cultivating algae in raceway ponds was responsible for the lower energy consumption and greenhouse gas emissions compared to closed photobioreactors [47].

Conclusions

From this overview of biogas production from microalgae, the following conclusions can be drawn:

- In spite of recent developments in the field of (bio)methane production from microalgae, the optimal scenario combining ease of cultivation, high biomass production and methane yield still has to be determined. Both fundamental and applied research is required at different steps in order to improve the potential of the process.
- Concerning microalgae culture, attention should be paid on strain selection and operating parameters optimisation in order to improve the production of the system while reducing capital and operating costs. Moreover, cultivation strategies aimed at increasing the methane yield of microalgae ought to be investigated.
- Regarding anaerobic digestion, pretreatments should be considered in order to improve the process performance and net energy production. On the other hand, the still limited knowledge on digestion of microalgal biomass residue after lipid extraction should be enhanced in order to promote nutrients recycling. Prospective research on digestate properties as substrate for microalgae growth and/or fertilizer is needed.
- The increasing interest in microalgae biogas production requires a detailed assessment of energy, costs and potential environmental impacts of the entire process chain, from biomass production to biogas exploitation. Pilot-scale experimental data would contribute to more realistic assessment of economic and environmental aspects.

Acknowledgments Authors want to thank the Spanish Ministry of Science and Innovation (MICINN) for financial support to this project (CTQ2014-57293-C3-3-R). Fabiana Passos appreciates her Post-Doctorate scholarship funded by the National Council for Scientific and Technological Development (CNPq) from the Brazilian Ministry of Science, Technology and Innovation. Marianna Garfí is grateful to Ministry of Economy and Competitiveness (Spain) (Plan Nacional de I+D+i 2008-2011, Subprograma Juan de la Cierva (JDC) 2012). Enrica Uggetti is grateful to the Ministry of Economy and Competitiveness for her scholarship (IJCI-2014-21594).

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