BIOGAS PRODUCTION FROM ALGAL BIOMASS: A REVIEW

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Abstract
The objective of this work is to provide a comprehensive study on algal biomass as feedstock for biogas production. Algae-derived biofuels are seen as one of the most promising solutions to mitigate climate change and as alternative to fast depleting of fossil fuels and oil reserves. Microalgae and macroalgae underwent an intense academic and industrial research, thanks to their capability to overcome the drawbacks related to the first and second generations of biomass resources. Major advantages of algae are: no competition with food crops for arable land, high growth rates, low fractions of lignin which reduces the need for energy-intensive pretreatment and compatibility with biorefinery approach implementation. However, some disadvantages such as the presence of high water content, seasonal chemical composition and the occurrence of inhibitory phenomena during anaerobic digestion, make algal biofuels not yet economically feasible although they are more environment friendly than fossil fuels.

Keywords: Macroalgae; Microalgae; Anaerobic Digestion; Methane; Biogas; Pretreatment.

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1. Introduction

The first generation biofuels are made from edible feedstock like corn, soybean, sugarcane, and rapeseed. The use of these resources for energy production was blamed for a rise of food prices. Second generation of biofuels from waste and dedicated lignocellulosic feedstocks have advantages over those of first generation. The major benefits are higher stock yields and lower land requirements in terms of quality and quantity. The main problem associated with lignocellulose conversion to biofuels is its strong resistance to degradation. Thus, second generation biofuels still lack of economic viability at large scale. Third generation biofuels feedstock is represented by micro- and macro- algae, which present further advantages over the previous two. This marine biomass shows the prospect of high yields requiring no use of arable land [1-3]. It has been proven that macroalgae can reach 2-20 times the production potential of conventional terrestrial energy crops [4], while microalgae commonly double their biomass within 24 h [1]. In addition, a negligible or low amount of lignin makes them less resistant to degradation than lignocellulosic feedstocks, and avoids the need for energy-intensive pretreatments before fermentation [5].
Furthermore, estimates indicate that the energy potential of marine biomass is more than 100 EJ per year, higher than the land-based biomass accounting only for 22 EJ [6]. In terms of carbon capture during photosynthesis, macroalgal primary productivity rates are approximately 1600 g Cm^{-2} y^{-1}, comparing favourably to a global net primary productivity of crop land of 470 g Cm^{-2} y^{-1} [7]. Approximately half of the dry weight of the microalgal biomass is carbon [1], which is typically derived from carbon dioxide absorption. Therefore, producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide from the atmosphere. It has been proposed that microalgal biomass production can potentially make use of some

of the carbon dioxide that is released by power plants when burning fossil fuels [1, 8]. Macroalgae can be converted to biofuels by various processes including thermal processes and fermentation. The most direct route to obtaining biofuel from macroalgae is via anaerobic digestion (AD) to biogas [7]. On the other hand, microalgal biomass has been mainly investigated as substrate for biodiesel production. Thus, the literature available on the subject results to be poor. However, it is emerging a re-interest for AD of microalgae due to the algal biomass compatibility with integrated production of other fuels and co-products within biorefineries [9, 10]. In addition, according to [10], regardless of species and operating conditions, the proportion of methane in the produced biogas is around 70%. This reveals that a good quality of conversion of the microalgal organic matter into methane is achievable.
The production of biogas through AD offers significant advantages over other forms of bioenergy production. It has been evaluated as one of the most energy-efficient and environmentally beneficial technology for bioenergy production [11]. Biogas generation can drastically reduce greenhouse gases compared to fossil fuels by utilization of locally available resources. The digestate represents an improved soil conditioner which can substitute mineral fertilizer [12].
Compared to other fossil fuels, methane produces fewer atmospheric pollutants and generates less carbon dioxide per unit energy. As methane is comparatively a clean fuel, the trend is toward its increased use for appliances, vehicles, industrial applications, and power generation [6]. Reijnders and Huijbregts reported that methane presents the higher heating value when compared to the most common transport fuels, such as biodiesel, bioethanol and biomethanol [13]. However, hydrogen which holds the highest heating value (LHV equals 120 MJ kg^{-1}) is not well developed commercially for production and use, and is more difficult to produce from biomass [6].
Biogas production from algal biomass needs to overcome certain feedstock-related obstacles.

As algae have much higher water content when compared to terrestrial energy crops, they are more suitable for wet AD processes [14]. On the other hand, the main disadvantages associated with such elevate moisture content are the use of limited organic loading rates (OLR) of the digesters as well as short term storage of biomass [4, 15, 16]. Another crucial parameter is their wide variation in nutrients content, which is related to several environmental factors. Most of them vary according to season, and the changes in ecological conditions can stimulate or inhibit the biosynthesis of such nutrients [17]. For this reason, many studies concluded that the seasonal variation of their composition restricts the use of marine biomass as feedstock for biofuels [15, 17-20]. Also, the unbalanced nutrients in algal biomass (e.g. low Carbon/Nitrogen ratio) were regarded as an important barrier in the AD process [21].
During AD, some process-related obstacles can also develop. Inhibitory phenomena can result from the accumulation of volatile fatty acids (VFAs) [22, 23], ammonia (NH_4^+ and NH_3) [24], and production of sulphide (H_2S) [25]. Besides, as the hydrolysis is considered the main limiting step of AD, a pretreatment is needed in order to improve the methane yields [26]. In general, the pretreatment step is required to be both effective and economically feasible in terms of overall process [4, 15, 16, 27-29]. In fact, the high pretreatments cost has been identified as one of the key barriers for commercialization of lignocellulosic biofuels [30].

This review aims to provide an overview of the major obstacles related to the exploitation of both microalgae and macroalgae biomass as feedstock for methane production through AD, gathering the main solutions reported in the literature. Biochemical composition of algal biomass, operational process-related parameters and occurrence of inhibitory phenomena are dealt with in this review.

2. Macro and microalgae production

Algal biomass can be cultured or acquired from natural, eutrophicated and degraded water bodies [31]. In 2010, the world production of seaweeds was estimated at 19 million tonnes, where *Laminaria japonica* was the most cultivated at 6.8 million tonnes [32]. The current uses of seaweeds are predominantly in the food, feed, chemicals, cosmetics and pharmaceutical sectors in Asian countries such as China, the Philippines, North and South Korea, Japan and Indonesia [33]. When the only outcome product is energy, the cultivation of algal biomass is unlikely to be economically viable [4, 34], and thus many studies have been carried out in order to make it feasible. The main solution seems to exploit the bioremediation capacity of this kind of biomass [35-37]. Nowadays the eutrophication, with excessive amount of N, P, CO_2 and insufficient amount of dissolved O_2, is becoming a serious problem in coastal seawater environment [37-39]. Seaweeds can be used as nutrients remover. Therefore, there is a great potential to remove large amount of C, N, and P nutrients with extensive seaweed cultivation [37, 40]. Seaweeds produced from these cultivations can then be used for high-value products [41] or as feedstock for bioenergy conversion processes. Furthermore, there is potential for macroalgal cultivation in offshore renewable energy facilities, such as wind farms [42]. Sharing the infrastructure with an offshore enterprise can be beneficial from planning, design and operation perspectives [43]. Nevertheless, conflicts and operations incompatibilities may arise, and be addressed by ensuring prior suitability of the offshore site for seaweed cultivation [44].

In many countries, an excessive natural growth of macroalgae has been observed as result of the progressive eutrophication of coastal water [45, 46]. The drift and consequent degradation of this resource is considered a pollution problem, which can be addressed through the exploitation of this kind of biomass as feedstock for AD [47, 48]. Another option is represented by the collection of storm cast weed from beaches, which is more developed in countries such as the UK and Ireland [44]. Hughes et al. [44] consider this as the most readily available feedstock for the generation of biofuel on a small, localised scale. However, it is underlined that the biomass of beach-cast would unlikely be sufficient for larger scale exploitation of this resource for bioenergy purposes [44]. Besides, it must be considered that this source of biomass does not guarantee a constant and homogeneous feedstock supply as it depends on variable climatic conditions [31].

In the case of microalgae, the two most common systems used for cultivation are raceway ponds and photobioreactors. The former are made of a shallow closed loop recirculation channel, in which mixing and circulation are produced by a paddlewheel, while the latter are culture systems where the light has to pass through the transparent reactor's walls to reach the cultivated cells [1, 49]. The economic feasibility and biomass productivity are considered the major differences between the two systems. The raceway ponds are less expensive to build and operate, while in terms of biomass productivity the photobioreactors allow higher biomass recovery [1]. Raceway ponds permit to achieve biomass productivity ranging between 10 and 25 g m^{-2} d^{-1}, on the contrary photobioreactors can produce from 25 to 50 g m^{-2} d^{-1} of biomass [50]. Even though several kinds of photobioreactors have been developed [49, 51], attempts of constructing such a system that would be also cost-effective have so far been unsuccessful [31]. Thus some studies have pointed out that the economic feasibility may be improved by integrating microalgal production and wastewater treatment [34, 52-55]. By using this approach, the costs of algal production and harvesting are covered by the wastewater treatment plant capital and operational costs [56]. High rate algal ponds are shallow (0.2-1 m), open raceway ponds and are used to treat municipal, industrial and agricultural wastewaters [56]. Microalgae assimilate nutrients and through photosynthesis, produce dissolved oxygen that is used by bacteria to oxidize wastes [50]. The subsequent

harvest of the algal biomass permits to recover the nutrients from the wastewater [56], which can be used as substrates for biofuel conversion processes. Wang et al. [57] demonstrated this concept by testing the cultivation of green algae *Chlorella* sp.in municipal wastewaters from different process treatment stages. The results showed that growing algae in centrate, which is the wastewater generated in sludge centrifuge, has a potential in terms of both nutrients removal and biomass cultivation for biofuel production. However, the implementation of these two constitutes an issue, since most wastewater facilities are embedded in urban infrastructure. In the case of coastal cities, it has been proposed to locate offshore membrane enclosures for growing algae in marine environments where wastewater is already discharged [52]. This is the case of the OMEGA system which consists of floating photobioreactors made of flexible plastic, designed to grow freshwater algae using wastewater effluent as the growth medium. A 2 year feasibility study in northern California, USA, indicates that algal productivity in prototype floating photobioreactors using secondary wastewater effluent ranged from 4 to nearly 30 g biomass m^{-2} day^{-1}. However, the economic feasibility has still to be determined.

At the same time, some critical parameters can affect the algal production in wastewaters. These factors have been extensively reviewed by Park et al.[53]. Light intensity, temperature, pH, CO_2 availability, dissolved oxygen, nutrients supply and zooplankton grazers and pathogens are considered to influence the algal growth rate and chemical composition, compromising further use of microalgae. Another factor which limits the development of economically feasible production system is the microalgae harvesting. Due to small size, low specific density and negative charge on the cell surface (particularly during exponential growth), microalgae result very difficult to remove [50, 56]. According to the TS content of the final algal slurry, microalgae collection can be divided into primary and secondary harvesting. The first permits to obtain algal slurry with TS between 0.5 and 6%, by using sedimentation or flotation. For thicker slurry (TS between 10 and 20%), secondary harvesting techniques such as centrifuge or belt filter press are the most recommended [50]. Wiley et al. [50] pointed out as the end use of the algal biomass influences the choice of harvesting. For instance, primary harvesting is more suitable for biogas production as AD process can tolerate high levels of moisture content. All the mentioned techniques are related to suspended algae, the use of attached cultures may offer several advantages [58]. When algal biomass is grown as a surface attached biofilm, the biomass is naturally concentrated and more easily harvested. This can lead to less expensive removal of the biomass from wastewater, and cheaper downstream processing in the production of biofuels and bioproducts [59]. Christenson et al. [59] developed a rotating algal biofilm reactor which allowed a biomass production ranged from 5.5 g m^{-2} day^{-1} at bench scale to as high as 31 g m^{-2} day^{-1} at pilot scale. In the same work, also an efficient spool harvesting technique was developed in order to obtain a concentrated product with TS content between 12 and 16%.

3. Algal Chemical Composition

Knowing the algal chemical composition permits to calculate the methane potential and ammonium yields that can be obtained by AD [10, 60]. The AD process involves diverse community of bacteria that act as an integrated metabolic unit to produce methane (~60%) and carbon dioxide (~30%) through a series of sequential and concurrent reactions. The end-products of one group's metabolism are used as substrate by the next group. The biological process involves four main phases, namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Algal biomass is rich in nutrients such as carbon, nitrogen and phosphorus, which are essential nutrients for anaerobic microorganisms. Nevertheless, the literature has identified several key factors in the biochemical composition of algal biomass affecting biogas production, such as moisture content, lipids, carbohydrates, proteins, ash content and

lignin fraction.
It is well known that algal biomass exhibits very high level of moisture content. It typically ranges between 78 and 90% [14, 17]. Thus, the compatibility of this kind of biomass with AD process as well as the impossibility of allowing high OLR. A drying step has been suggested, but it would negatively impact on the overall process cost [14].
Sialve et al [10] reported the theoretical yields of methane from lipids, proteins and carbohydrates. Lipids show the highest value of 1.014 L CH_4 g^{-1} VS (Volatile Solids), when compared with proteins (0.851 L CH_4 g^{-1} VS) and carbohydrates (0.415 L CH_4 g^{-1} VS). It has been identified that microalgae present high lipids content, within the range of 3-20% d.w. (dry weight) [10, 61-63] with peaks of 90% d.w. (under certain growth conditions) [36, 64]. For this reason, microalgae can be regarded as a valid feedstock for AD purposes [65]. However, as lipids fermentation presents slower hydrolysis rates, microalgae have been mostly adopted for oil production [1, 66, 67].
On the contrary, lipids level in macroalgae has been found to be very low, i.e. between 0.4 and 3.5% d.w. [17, 68], but exhibit higher values of carbohydrates. Carbohydrate content ranges between 3 and 40% d.w., depending on genera and season [62]. The carbohydrates synthesis is related to the periods of maximum growth, increased photosynthetic activity and a reduction in proteins content [17]. For instance, the carbohydrates content of *Laminaria digitata* peaked in June (69.1% d.w.) as result of the increased rate of photosynthesis, whereas the lowest level of carbohydrates was reached in early spring since most carbohydrates have been used up during winter [18]. Macroalgae contain different types of carbohydrates depending on genera [69]. Brown seaweeds lack of easily fermentable sugars [14]. For this reason, it would not be feasible pursuing a standard AD. Consequently, a pretreatment is required in order to break the polysaccharides into monomers prior to hydrolysis. On the other hand, green and red seaweeds have high levels of easily accessible sugars. Those are represented by floridean starch and xylan in red macroalgae [69] and starch in green macroalgae [14]. This suggests a boost of the AD process.
Some microalgae species are also rich of carbohydrates, up to 64% d.w. [61, 70]. These tend to increase when algal cells are subjected to high light intensities [36]. Also the temperature seems to have the same effect, although this may vary among species [71]. Carbohydrates of microalgae can be found in the form of starch, cellulose, sugars, and other polysaccharides [66], which makes them suitable for anaerobic fermentation [72].

Another factor depending on environmental conditions is the ash content (non-degradable matter), that oscillates between 10 and 40% d.w. ([17-20, 70, 73]). The highest ash content value was found in winter and early spring in conjunction with a reduction of carbohydrate synthesis. The opposite behaviour was observed during summer ([17-20, 74]). Therefore, it was concluded that the ash fraction is inversely correlated to the carbohydrates level [17]. Renaud et al. studied the growth and nutritional content of tropical Australian microalgae, finding a linear relationship between percentage of ash and temperature [71]. In this regard, the most suitable conversion processes for algae would be digestion technologies, as these are the most tolerant to ash presence [19].
As well as ash, also lignin is a non-degradable compound. Macroalgae present very low fractions of lignin and higher fractions of hemicellulose and cellulose ([14, 19]). For example, *Ulva* sp., exhibited lignin and hemicellulose amount of 1.3% and 9% d.w. respectively, while the cellulosic fraction was estimated at 15.7% d.w. [15]. Other species such as *Gracilaria cervicornis* and *Sargassum vulgaris* showed a fiber amount of 5.6 and 7.7% d.w., respectively [17]. *Laminaria japonica* exhibited negligible amounts of lignin, while hemicellulose oscillated between 31 and 55%, and the cellulose fractions between 16 and 30% [75, 76].

Microalgae contain almost no lignin [72, 77]; lignin amount was found to be less than 2% [78]. Instead, cellulose and hemicellulose contents were found 7.1% and 16.3%, respectively. Indeed, low fraction of lignin facilitates enzymatic access while providing high hydrolysis rates in both ethanol and biogas production [15].

4. C/N Ratio

A C/N ratio ranging from 20 to 30 is considered optimal for AD. If this ratio is very low, nitrogen will be released and accumulated in the form of ammonium ion (NH_4^+). Excessively high concentrations of NH_4^+ will increase the pH levels in the digester leading to a toxic effect on methanogens population [79].

The C/N ratio in algal biomass is around 10/1 [15, 21, 27, 48, 73, 80-83], which is too low for the digestion. In order to avoid excessive ammonia accumulation, addition of carbon rich materials is required in order to improve the digestion performance. Adding 50% (based on volatile solid) of waste paper to algal sludge increased the methane production rate up to 1170 mL L^{-1} d^{-1}, which corresponds to an improvement of more than 100%. Results suggested an optimum C/N ratio for co-digestion of algal sludge and waste paper ranging between 20 and 25/1 [21]. Zhong et al. [84] observed that the addition of corn straw to the digestion of Taihu blue algae at a similar ratio of 20/1 increased methane yield by 62% at 325 mL $g^{-1}{}_{VS}{}^{-1}$. Similarly, blends of *Saccharina latissima* and straw produced the maximum methane yield when the C/N ratio was around 30 [27]. Results from the co-digestion of post transesterified microalgae (*Chlorella* sp.) residues and glycerol showed an increase of the CH_4 production by >50% (compared to residues digestion only) when then C/N ratio was about 12 [85].

The value of the C/N ratio can be manipulated by applying selected growth conditions. In cultivated *Ulva lactuca*, Bruhn et al [4] found a positive correlation with the incoming irradiance, reaching a C/N ratio of 24. Furthermore, Habig et al [86] obtained a C/N ratio of about 30, when growing *Ulva lactuca* under nitrogen starvation. Although such technique leads to higher ratios, a possible drawback is represented by a lower algae production rate [87].

5. Organic Loading Rate (OLR)

The OLR is defined as the amount of VS or chemical oxygen demand (COD) components fed per day per unit digester volume [79]. Chandra et al. [79] suggest that higher organic loading rates can reduce the digester's size and consequently, its capital cost. However, sufficient time should be allowed for the micro-organisms to break down the organic material and convert it into gas. Generally, the methane yield is constant and maximal when the process is operated at low OLR and high hydraulic retention time (HRT). When HRT is instead reduced, an increase in OLR could result in imbalances in the bacterial population, leading to VS accumulation and digester failure [85]. It can be concluded that suitable OLR and HRT must be chosen depending on the nature and composition of the algal substrate. Hence, characteristics of each species make the difference for a given loading rate or HRT [10], as reported in Table 1.

Ras et al [87] noticed a 4-fold increase in the methane productivity of *Chlorella vulgaris*, when increasing the HRT from 16 to 28 days. In this case, the authors chose a low value of loading rate in order to keep the free ammonia and VFA concentrations below inhibitory levels. Therefore, it was observed that a considerable leeway existed in increasing the residence time and/or the OLR without affecting the degradation process. The authors suggested that increasing the OLR by 2.5 times at a HRT of 28 days should lead to a methane productivity of 450 mL L^{-1} reactor d^{-1}. However, it was pointed out that increasing the feeding rate has also an influence on the ammonium concentration in the reactor.

The methane potentials of cyanobacteria and *Chlorella* sp. have been investigated in eight different lab scale reactors at 25°C by Jegede [88]. The author studied the relation between OLR and methane production at a fixed three-days HRT. It was observed that methane production rates increased when feeding the reactors with an OLR up to 7 g VS, above this threshold the methane production dropped. An interesting work [89] addresses the concept of a closed loop system for conversion of solar energy into energy-rich biogas and electricity. In order to evaluate the totality of this concept, a simulation of a closed cycle setup, involving an algal growth unit, anaerobic digestion and microbial fuel cell was installed. The AD unit operated at mesophilic temperature in plug flow, with low volumetric loading rates of algae (10 mg L^{-1} d^{-1}), influent concentrations of 2 g COD L^{-1}, and with a virtually indefinite residence time. Under those conditions, the results suggested that up to 0.5 Nm produced per kg algal VS. biogas (with up to 65% CH_4) could be.

The work of Ehimen et al [85] concluded that the best combination of substrate loading rates and HRT is at 5 kg VS/m^3 digester and 15 days, respectively. This study investigated the codigestion of post-transesterified microalgae residues and glycerol at mesophilic condition. *Ulva* sp. mixed with manure in a completely stirred digester at 35°C, showed a low production of methane, due to a low loading rate. This low loading rate was due to the physical impossibility of adding more algae in the suspension fed to the digester [15]. As mentioned in *Section 2*, algae present very high moisture content. This represents a significant obstacle to increase the OLR of macroalgae-fed digesters. For example, fresh *Ulva lactuca* has the TS and VS content of 12.8% and 7.3%, respectively, which do not allow a loading rate in a continuously fed system to be more than approximately 4–5 g VS L^{-1} d^{-1} at 15–18 days HRT. Drying is an effective technique, able to increase the TS/VS content and results in a 5-9 times higher specific methane production when compared to wet biomass. Furthermore, a higher TS/VS ratio would allow a higher OLR in a continuous system without lowering the HRT [4].

Dried *Ulva lactuca* biomass was co-digested with cattle manure in a lab-scale continuously stirred tank reactor. The highest methane production was observed when the algae concentration in the mixture was 40%. However, an increase of *Ulva lactuca* content in the reactor to 50% gave no further yield improvement [16]. Similarly, it was found that elevated feeding concentrations of *Ulva* sp. caused instability during the methanogenesis, due to VFAs overload. The biggest methane yield was achieved with the lowest OLR, at thermophilic condition. Unlike *Ulva* sp., the thermophilic reactor containing *Laminaria* sp. showed a gradual rise of methane yield as the feeding concentration was increased. The maximum yield was found at the highest OLR. Differently, in the mesophilic reactor the methane yield was rather stable (average 140 L g^{-1} VS) despite it was fed with similar feedstock concentrations. The reason for such behaviour was attributed to the temperature [90]. In another study, *Laminaria hyperborea* biomass was digested with semi-continuous feeding at mesophilic condition and compared with other substrates, such as cattle manure and *Ascophyllum nodosum* biomass. Cattle manure at higher loading rates than those used for *Laminaria* sp. digestion showed a methane production below the algal substrate. *Ascophyllum nodosum* showed the lowest methane production even at the highest loading rate [91].

Table 1
Methane production at different OLR and algal species.

6. AD Inhibition in Algal Substrate

6.1 Ammonia

Ammonia inhibition during AD can be triggered by several factors. Chen et al [92] enumerate factors such as ammonia concentrations, pH, temperature, presence of other ions and

acclimation. In aqueous solution the principal forms of inorganic ammonia nitrogen are the ammonium ion (NH_4^+) and free ammonia (NH_3) [92]. The NH_4 concentration up to 1500 mg L^{-1} have no inhibitory effects on the methanogenesis, but above this threshold it may lead to severe toxicity [26]. At the same time, NH_3 has been recognized to play a major role in ammonia inhibition. A value of 80 mg N L^{-1} of NH_3 has been found to be the minimum inhibitory level. In general, a wide range of inhibiting ammonia concentrations has been identified, spanning from 1.7 to 14 g L^{-1} [92]. Such large concentration interval is related to the nature and kind of fermenting substrates and inocula, environmental conditions and acclimation periods. In addition, it has been demonstrated that the NH_3 fraction increases with temperature and pH [93]. An increase of ammonia, due to high pH values, causes process instability resulting in VFAs accumulation, this leads to a decrease in pH and a consequent declining concentration of NH_3. The interaction between NH_3, VFAs and pH determines, as denominated by Chen et al [92], an "inhibited steady state", where the process is running stably but with a lower methane yield. The authors highlighted that generally higher NH_3 concentrations at thermophilic conditions leads to more easily inhibited state than at mesophilic temperatures.

In thermophilic condition, a methane yield decrease up to 25% was observed when the ammonia concentration was increased to 4g N L^{-1} or more [93]. On the other hand, when ammonia was introduced gradually, the process was unaffected up to 3 g N L^{-1} and only slightly affected at 4 g N L^{-1}, with signs of recovery after 1 Retention Time, likely due to the adaptation of the microorganisms. This phenomenon has been defined under the name of acclimation.

Ammonia inhibition may not occur when digesting macroalgae due to the high dilution factor used in the digester and/or nature of co-substrates. Studies on nitrogen content of *Ulva* sp., *Enteromorpha* sp., *Gracilaria* sp., and *Gracilaria vermiculophylla*, suggested that nitrogen content between 3.5% and 8.7% may lead to methanogenesis inhibition [26]. In this case, an efficient dilution permitted to maintain ammonium levels between 53 and 827 mg NH^+ - N L^{-1}, and at the same time the pH within ideal values.

An investigation on a pilot-scale plant using *Laminaria* sp. and *Ulva* sp. mixed with milk as fermentation materials, reported an ammonium ion concentration approximately of 1200 mg L^{-1}, not high enough to prevent methane fermentation. In this case, the use of milk in co-digestion caused an ammonium ion concentration that inhibited methane production. Similarly to [26], a later water dilution prevented ammonia inhibition [73]. Ammonia concentrations in *Saccorhiza polyschides*, *Ulva* sp., *Laminaria digitata*, *Fucus serratus* and *Saccharina latissima* were studied by [94] when co-digesting with bovine slurry, and in *Ulva* sp. codigestion with pig slurry by [95]. The authors reported ammonia levels of about 94-350 and 68 mg L^{-1} respectively, with no inhibition taking place, confirming results reported by [26]. From the reported cases, as macroalgae present high levels of nitrogen, inhibition caused by ammonia accumulation can occur but can be prevented by adjusting the amount of diluting water.

Also microalgal substrates present high nitrogen content, possibly leading to ammonia inhibition. Codigestion of swine manure and microalgal biomass caused ammonium concentration to increase up to 1.1 g L^{-1}, while producing the highest methane yield [96]. In this case ammonia concentration threshold for hampering methanogenic bacteria activity was considered far above the values achieved. Alzate et al. [97] studied the AD of three microalgae mixtures. The authors found the highest ammonium concentration at almost 1500 mg L^{-1}, without registering any inhibitory phenomenon. In addition, it was observed that the amount of ammonium released per gram of VS added or eliminated was mostly affected by microalgae sp.

6.2 Volatile Fatty Acids (VFA)

Analysing the different levels of VFAs in the digestate could aid in predicting the digester's performance and help to identify underlying process problems, such as overloading [85]. The inhibition level of VFAs for AD has been reported to be above 6000 mg L^{-1} [98]. The digestion of *Laminaria* sp. and *Ulva* sp. mixed with milk presented acetic and propionic acids concentrations in the prefermentation phase ranged from 2000 to 6000 ppm and from 1500 to 3000 ppm, respectively [73]. During the methane fermentation phase, organic acids were consumed and were stable at low concentrations, thus no inhibitory phenomena due to the accumulation of acids observed in the prefermentation were detected. Codigestion of ground *Ulva* sp. with manure also presented low volatile fatty acids concentrations as reported by [15]. Another investigation resulted in VFAs accumulation when digesting a mixture of brown and red macroalgae. In this case, a water dilution of the reactor content improved the VFA production and the release of soluble organics, by decreasing the concentration of VFAs [99]. Also codigestion of *Ulva* sp. with pig slurry resulted in high levels of VFAs, with a maximumof 3.2 g L. These levels were not toxic as long as the buffering capacity was sufficient to maintain the pH value in the system [95]. In fact, when the buffer capacity is not able to prevent the pH drop, a consequent inhibition of methanogenic bacteria takes place [48]. In some microalgae species the OLR was found to be a crucial factor in determining excessive VFAs accumulation [88]. When digesting cyanobacteria and *Chlorella* sp., VFA concentrations increased with rising loading rates. Above 7 mL L^{-1} day^{-1}, a decrease in methane production was observed, having VFAs reached inhibitory levels. The pH value in the reactors did not fall below 6.5, when reactor's instability and low methane production rates were occurring. It oscillated around 7.0 even during VFAs accumulation and reduction in COD removal efficiency. The reason of this was identified in a possible deactivation of methanogenic bacteria at the bottom of the reactor by the author (presence of pockets of clog feedstock), that created a zone with reduced methanogenic activity, so that the digester became unstable. Although such explanation might be valid, it would have been beneficial to investigate the presence of ammonia-related inhibitory phenomena in order to reject the possibility of an inhibited steady state, as by [92]. In a study on digestion of *Chlorella vulgaris*, the OLR was kept at a safe level of 1 g COD day^{-1} L^{-1} which maintained the VFAs and NH_3 concentrations far below inhibitory levels, as mentioned in Section 5.1 [87]. This indicates that VFA accumulation, as response to free ammonia toxicity, did not occur. When digesting post transesterified microalgae residues (*Chlorella* sp.), substrate concentrations > 40 kg VS/m^3 digester were found to increase VFAs concentration, leading to lower CH_4 production [85]. The high VFA concentrations were >5000 mg L^{-1}, regardless of the substrate C/N ratios and HRT used. Furthermore, a reduction in HRT significantly increased the VFA accumulation, indicating a comparably faster acid formation process in relation to the CH_4 forming phase. According to this result, the authors suggested that the methanogenic process could be the rate limiting step for the AD of *Chlorella* residues. However, it has to be noted that the use of longer HRT may solve this inconvenience.

6.3 Hydrogen Sulfide (H_2S)

H_2S production during AD may reduce the methane yield by competition between methanogens and sulfate-reducing bacteria [92, 100]. The inhibitory sulfide levels reported in the literature range from 100 to 800 mg L^{-1} for dissolved sulfide or approximately 50–400 mg L^{-1} for undissociated H_2S [92].

Digestion studies on *Saccorhiza polyschides*, *Ulva* sp., *Laminaria digitata*, *Fucus serratus* and *Saccharina latissima* inoculated with bovine slurry registered H_2S values above 200 mg L^{-1}. Despite such high concentrations, no inhibition of methane production was detected [94]. Similarly, when using fresh algae mix and sediments [48], the methanogens activity was not hindered, likely due to the pre-existing adaptation of microbial community. More than 200

mg L^{-1} of H_2S was detected during digestion of *Laminaria digitata* [100]. Also in this case, even though H_2S reached high concentration, no inhibitory effects were observed. The co-digestion of *Ulva* sp. with pig slurry showed 99 mg S L^{-1} of dissolved H_2S for a pH value of 7.6. This concentration was expected to inhibit methane production. However, such inhibition did not occur, and it was suggested that the sludge acclimatised to this significant amount of H_2S.

Microalgae hardly contain sulphureted amino acids, and for this reason their digestion releases a lower amount of hydrogen sulfide compared to other types of organic substrates [10]. However, some authors [101] suggested focusing future investigations on combustion and purification characteristics of biogas from microalgae. In fact, it has to be noticed that high concentrations of H_2S are problematic for further use of biogas, due to its corrosive properties for pipes and cogeneration engines. The maximum concentration of H_2S specified by co-generator manufacturers is about 150 mg/m^3 (around 100 mg L^{-1}). Thus, in the case of macroalgae, treatments are needed not only after digestion in order to reduce H_2S content, but also during digestion with the aim of limiting the production of H_2S [95].

7. Pretreatment

In AD, the hydrolysis phase is identified as the rate-limiting step. In order to improve the hydrolysis rate, a substrate pretreatment can be necessary. The pretreatment phase has been extensively discussed for lignocellulose biomass conversion [102]. A multitude of different pretreatment technologies have been suggested. They can be classified into biological, physical, chemical and physico-chemical, according to the different forces or energy consumed in the pretreatment process [103]. In general, physical pretreatments accomplish the task of breaking down the crystalline structure of cellulose, solubilising hemicellulose or lignin and altering lignin morphology. This causes an increase of the specific surface area and thus an increased access for degrading enzymes and enhanced hydrolysis. The result is either an increased in final methane yield or a more rapid biogas production at an initial stage, although this may still result in the same final methane yield [102, 104]. Table 2 provides a brief summary of the main results obtained. It is noteworthy to observe that the lignocellulosics pretreatment permits to achieve high methane yields as well as improvement's margin. Despite this, industrial applications are still unviable due to the high costs involved. Such costs are believed to be lower in the case of macro and microalgae as they do not need harsh pretreatments. A recent study has concluded that experimental and implementation works should focus on technologies for pretreatment and conditioning of algae biomass as they have a direct impact on methane fermentation process [31]. Figures 1-2 provide a qualitative overview of the main pretreatments applied both in macroalgae and microalgae. The bubble radius is related to the methane yields produced (Fig 1) and its percentage of improvement when a pretreatment is applied (Fig 2).

Table 2
Methane production and pretreatment improvement for lignocellulosic biomass.

Fig. 1. Methane production (mL g^{-1} VS) at different pretreatments.

Fig. 2. Percentages of improvement (• positive, ○ negative) at different pretreatments.

7.1 *Macroalgae pretreatment*

The extent of methane production oscillates between 100 and 330 mL g^{-1} VS. From Table 3, it can be seen that the best improvement (+68%) is achieved when using mechanical

maceration. The mechanical pretreatment seems to affect positively the methane production of macroalgae, even though the result may be dependent on the species used. Indeed, the main effect is to reduce the particle size of the substrate making the complex organic matter more available to the attack of the hydrolytic enzymes. Methane yields from macerated *Ulva lactuca*, *Gracilaria vermiculophylla* and *Chaetomorpha linum* rose up to 68%, 11% and 17%, respectively. The authors suggested that the reason of such different results between macroalgae was due to the composition of the species [16]. Bruhn et al. [4] studied the effect of several physical pretreatments on *Ulva* sp. Washing resulted to have no effect on methane yield as well as drying. Maceration of washed algae caused a moderate increase, while the best result was achieved on unwashed and macerated substrates with a significant boost. Much poorer improvements were obtained when applying thermal treatments. A negative effect was observed at 110 °C, while a low increase was achieved at 130 °C.
In another study, washing *Ulva* sp. biomass slowed down the beginning of the digestion and decreased the methane yield. The authors attributed this to a change in osmotic pressure, caused by the washing. This would determine a loss of some soluble metabolites, consequently decreasing the methane production. On the other hand, grinding improved the methane yield, but a latent phase with accumulation of VFA was observed [15].
Studies by Otsuka et al. [29] confirmed some of the above mentioned results achieved by Bruhn et al. [4]. For harvested sea lettuce biomass, it was observed that untreated, washed-only and ground-only feedstocks had almost the same effect on final biogas production. When the pretreatment included washing and grinding, the improvement in methane yield was consistent.
The effect of particle size reduction via mechanical pretreatment was also examined by Tedesco et al. on a mixture of *Laminaria digitata, hyperborea* and *saccharina* biomass [105, 106]. Depending on the seasonal variation of the plant's inner fermentable sugars, such as mannitol and laminarin, extra biogas and methane yield was achieved by pretreating the mentioned substrates prior to incubation. The extent of such enhancement resulted to be also strictly correlated to the particle size achieved by the comminution step. *Laminaria saccharina* was also treated with steam explosion in another work [27]. The best result was achieved when steam explosion was applied as pretreatment. The authors concluded that despite the methane yield improvement, the effects were not significant enough to justify such a harsh pretreatment. Steam explosion is indeed more suitable and beneficial on more recalcitrant substrates, i.e. lignocelluloses. It is likely that the effect of thermal pretreatment depends on the macroalgal species. In fact, considering *Saccharina latissima* [27], the main storage carbohydrates such as mannitol and laminarin are easily digested [107], thus the main effect of thermal pretreatment might have been an increase of alginate digestibility which degrades relatively slowly under anaerobic conditions [108]. This would explain the increase of methane yield due to the thermal pretreatment applied. On the contrary, for other species such as *Ulva* sp. [4] and *Palmaria palmata* [109], it has been shown that thermal pretreatment at high temperature (higher than 100°C) has a negative effect on methane production. Jard et al. [109] showed that the more the temperature increased, the higher the acidification (pH = 4.8 after 180°C treatment, pH = 4.2 after 200°C treatment). The pH values are too low to permit an efficient AD. In fact, methanogenesis occurs efficiently at pH 6.5 – 8.2 [110], while hydrolysis and acidogenesis occur at pH 5.5 and 6.5 [111], respectively. The main reason is that high temperature pretreatment leads to high solubilisation yields as well as the formation of refractory compounds (pseudo-lignin) which have been demonstrated to hamper the AD [112, 113].
Hydrothermal depolymerization followed by enzymatic hydrolysis was studied as pretreatment before methane fermentation of a macroalgal mixture (90% Pilayella, 8% Ectocarpus, some traces of the genus Enteromarpha) [114]. The hydrothermal depolymerization was carried out at 200°C under a pressure of 1.7 MPa for 120 minutes in a

muffle furnace. Subsequently, an enzymatic multicomplex of Cellulast 1.5 L, Novozym 188, and Hemicellulase was added to the mixture. Dry matter decreased by 32% on average, while without addition of enzymes the reduction was only 15%. The content of methane improved from 71.8 to 73.2%, with a biogas increase up to 64% (0.054 dm^3 g^{-1} of substrate) compared with depolymerized mixture without enzymes addition. The main effect of the enzymatic hydrolysis was to release a considerable quantity of carbohydrates to the filtered sample, which became more available and rapidly consumed during methanogenesis.
VFAs are important intermediates in the AD of biomass. It was observed as alkaline and thermal pretreatments affected VFA productivity in *Laminaria japonica* fermentation. At low substrate concentration, the pretreatment effect was minimal. The increase of *Laminaria japonica* concentration up to 50 g L^{-1}, led to a rise of VFAs concentration from 11.4 g L^{-1} to 15.2 g L^{-1} with an alkaline pretreatment (0.5 N NaOH) and to 13.5 g L^{-1} with a heat pretreatment (autoclaved at 120°C for 20 min). Besides, pretreatment also improved the fermentation rate [28]. It would be interesting to investigate the effect of such pretreatments also on the methanogenic phase.

Table 3
Methane production and pretreatment improvement for macroalgal biomass.

7.2 *Microalgae pretreatment*

Microalgae cell walls could prevent enzymes from digestion of microalgal biomass, thereby creating resistant to hydrolysis. Pretreatment can be applied in order to facilitate the hydrolysis of this recalcitrant portion and thus increase the methane yield [88]. Some data indicate that the presence and composition of the cell wall is the main reason for the differences observed in the cell disruption and subsequent biogas production. Gunnison and Alexander [77] investigated the resistance of certain algae to microbial decomposition using *Pediastrum duplex*, *Staurastrum* sp., and *Fischerella muscicola* as test organisms. Little proteins or lipids but considerable carbohydrates were found in the walls of the refractory organisms, but resistance was not correlated with the presence of a unique sugar monomer. It was suggested by the authors that resistance to degradation resulted from the presence of sporopollenin in *P. duplex*, a lignin-like material in *Staurastrum* sp., and possibly heteropolysaccharides in *F. muscicola.*
In theory, strains with no cell wall or a protein-based cell wall should be preferred because disruptive, and consequently energy consuming pretreatments can be avoided. However, it cannot be excluded the possibility that even microalgae with no rigid cell wall could be bad substrates for fermentative biogas production [101]. For instance, during AD of *Chlorella vulgaris* it was found that 50% of the biomass did not undergo AD, even under long retention times. This indicates the necessity of further research on pretreatment performance [87]. In fact, the use of ultrasound has been proven to be successful at improving the disintegration and anaerobic biodegradability of *Chlorella vulgaris* by Park et al. [115]. In details, ultrasonic pretreatment in the range of 5-200 J mL^{-1} was applied to microalgal biomass waste, which was then used in batch digesters. This technique was successful and showed higher soluble COD at higher applied energy inputs.

Ultrasound and thermal pretreatment effects were studied and compared by González-Fernández et al. on *Scenedesmus* biomass [116]. Ultrasound was applied at 20 Hz with an Es (energy level) of 128.9 MJ kg^{-1}, and proved to be effective at disrupting cell walls. The result was a 3.1-fold organic matter solubilization and an approximately 2-fold methane production in comparison with untreated biomass. The highest anaerobic biodegradability reached was 44%, which was about 2-fold compared with the untreated. Also thermal pretreatment at 80°C caused cell wall disruption and improved anaerobic biodegradability by 1.6-fold

compared to untreated biomass. The authors highlighted that since sonication caused a temperature increase in samples to as high as 85°C, it is likely that thermal effects accounted for much of the observed changes in the biomass. It was concluded that the higher energy requirement of sonication might not justify the use of this approach over thermal treatment. The thermochemical pretreatment efficiency was also investigated in algal fermentation by [117]. The results indicated that pretreatment best efficiency was attained with a temperature of 100°C for 8 h at concentration 3.7% solids and without NaOH. Compared with untreated algae, pretreatment improved the efficiency of methane fermentation at a maximum of 33%. High pressure thermal hydrolysis and lipid extraction were performed on a mixed culture enriched with *Scenedesmus* sp. microalgae prior to AD. The high pressure thermal hydrolysis treatment increased the methane yield by 81% compared with raw algae and by 58% with respect to the algal residue from lipid extraction. When combining lipids extraction with high pressure thermal hydrolysis the result was a cumulative methane yield [118].

In *Nannochloropis salina*, cell disruption by heating, microwave and French press showed a considerable increase in specific biogas production [119]. Ultrasonic and freezing treatments were also tested, and were found to have a negative effect on biogas yield compared with the untreated despite presenting higher %s of VS reduction. Hence, the VS reduction does not represent a comprehensive index of all mechanisms involved. Other indicators revealing the development of inhibitory phenomena should be monitored across the digestion.

The AD of three different blends of microalgae mixtures was evaluated considering three different pretreatments such as thermal, ultrasound and biological. The biological pretreatment was followed by negligible enhancements on CH_4 productivity, while the highest increases were achieved with thermal hydrolysis. The optimum temperature of this pretreatment was strictly correlated to the microalgae species. *Microspora* sp. gave the highest methane yield at the minimum temperature tested of 110°C. The ultrasound pretreatment brought increases in CH_4 productivity ranging from 6% to 24% at 10,000 kJ kg^{-1} TS, without further increases at higher energy inputs. Also for this pretreatment, the best result was obtained for *Microspora* sp. [97]. Thermal pretreatment effect at two temperatures (70 and 90°C) on *Scenedesmus* biomass was studied by [120]. While raw and pretreated at 70°C microalgae attained 22-24% anaerobic biodegradability, microalgae pretreated at 90°C achieved anaerobic biodegradability of 48%. The methane yield increased by 2.2-fold with regard to untreated microalgae substrate. In general, thermal pretreatment affects positively the methane production by microalgae. It is likely that a rise of temperature up to 100°C, either by heating or other means (sonication, microwave), can enhance the biodegradability potential of this kind of biomass. A positive effect of increased temperature on AD of microalgae was first observed by Golueke et al. [121]. The main effect of temperature is to increase soluble COD, VFA – COD and NH_4^+ concentration and to promote the cell wall disruption. In particular, González-Fernández et al. [120], demonstrated that anaerobic biodegradability enhancement in *Scenedesmus* biomass taking place at 90°C was not only due to soluble COD released but also to the cell wall breakage rendering the substrate more accessible for anaerobic degradation.

Enzymatic pretreatment effect on biogas production was studied on outdoor cultivated *Rhizoclonium* biomass in thermophilic conditions by [122]. The results showed that the application of a combined biomass blending (<0.1 mm length) and an enzymatic pre-treatment enhanced the methane yields compared to a mechanical size reduction method alone. The enzymatic mixture was composed of α-amylase, cellulase, lipase, protease and xylanase. Only 39-60% of the theoretical achievable CH_4 yield was however obtained by the authors, suggesting possibilities of further improvement.

The benefits of drying microalgal biomass were investigated by [101]. According to the authors, drying microalgae has a negative effect on biogas fermentation and it should be avoided. The reasons of such result may lay, as explained by the authors, in the loss

ofvolatileorganic compounds and/or a decreased accessibility of the dried organic compounds for the bacterial biocenosis within the fermenter sludge.

Table 4
Methane production and pretreatment improvement for microalgal biomass.

8. Conclusions

As algal biomass is subjected to seasonal variation, a series of solutions can be implemented in order to address the feedstock supply and composition variability. A drying step can be used to store the biomass as well as allowing higher digester OLR. However, it is necessary to consider how such phase impacts on the overall cost of the process and on the AD performance. Furthermore, co-digestion with other substrates and cultivation of algae under specific conditions such as high incoming irradiance and nitrogen starvation would result into improved nutrients balance within the digesters.

Some inhibition phenomena such as those caused by ammonia and VFA concentrations can be prevented by using an appropriate water dilution factor as well as the co-digestion with other substrates. Besides, it is important to study the interaction amongst pH, VFA and NH_3, in order to identify possible inhibition states. An efficient control of the buffering capacity of the system can prevent such types of inhibition. As high levels of H_2S are present in macroalgae-derived biogas, it would be beneficial for the entire system to apply a purification treatment not only following, but also during digestion.

Depending on the type of pretreatment and algal species, an evident enhancement in methane yield can be achieved. A harsh pretreatment is not necessary on algal biomass differently from lignocellulosic feedstocks due to negligible lignin content. For this reason, physical pretreatments have been preferred up to date, due to their simplicity and effectiveness. However, this review concludes that pretreatment of algal biomass has not been fully investigated up to date. Consequently, it would be beneficial to investigate the effects of different pretreatments under optimal AD parameters. Also the combination of different kinds of pretreatment seems to be an interesting route to be explored.

This study underlined the obstacles related to the exploitation of macroalgae and microalgae as feedstock for biogas production, reporting the main solutions presented in the literature so far. The potential of algal biomass for bioenergy production has been widely recognised, however few studies are available on the economic feasibility of their exploitation.

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Table 1
Methane production at different OLR and algal species.

Algae	Temp (°C)	HRT (Days)	Drying	OLR	Methane	Ref
Ulva	35	15	-	1.7 g VS L^{-1} d^{-1}	203 mL g^{-1} VS^{-1}	[15]
Laminaria hyperborea	35	24	-	1.65 g VS L^{-1} d^{-1}	280 mL g^{-1} VS^{-1}	[91]
Ascophyllum nodosum	35	24	-	1.75 g VS L^{-1} d^{-1}	110 mL g^{-1} VS^{-1}	[91]
Ulva lactuca	53	15	YES	4.4 g VS L^{-1} d^{-1}	16 mL g^{-1} $feed^{-1}$	[16]
Ulva lactuca	50	22	-	0.3 g VS L^{-1} d^{-1}	157.6 mL g^{-1} VS^{-1}	[90]
Laminaria sp.	50	22	-	1.2 g VS L^{-1} d^{-1}	185.7 mL g^{-1} VS^{-1}	[90]
Laminaria sp.	35	22	-	1.2 g VS L^{-1} d^{-1}	139 mL g^{-1} VS^{-1}	[90]
Chlorella vulgaris	35	28	-	1 g COD day^{-1} L^{-1}	174 mL L^{-1} day^{-1}	[87]
Chlorella sp.	25	3	-	7 g VS	100 mL $L^{-1}day^{-1}$	[88]

Table 2
Methane production and pretreatment improvement for lignocellulosic biomass.

Feedstock	AD Process	T (°C)	Pretreatment	Methane	Improvement	Ref
Ley crop silage	Batch	37	Ground	180 mL g^{-1} VS	+59%	[105]
Straw	Batch	35	Extruded	370 mL g^{-1} VS	+11%	[106]
Grass	Batch	35	Extruded	200 mL g^{-1} VS	+9%	[106]
Wheat straw	Batch	35	Microwave 150°C	344 mL g^{-1} VS	+28%	[107]
Barley straw	Batch	40	Thermal 90°C 30 min	340 mL g^{-1} VS	+42%	[108]
	Batch	40	Thermal 120°C 30 min	338 mL g^{-1} VS	+41%	[108]
	Batch	40	Mechanical (particle size 5 cm)	286 mL g^{-1} VS	+19%	[108]
	Batch	40	Mechanical (particle size 2 cm)	339 mL g^{-1} VS	+41%	[108]
	Batch	40	Mechanical (particle size 0.5 cm)	370 mL g^{-1} VS	+54%	[108]
Wheat straw	Batch	40	Thermal 90°C, 30 min	295 mL g^{-1} VS	+62%	[108]
	Batch	40	Thermal 120°C, 30 min	299 mL g^{-1} VS	+64%	[108]
	Batch	40	Mechanical (particle size 5 cm)	285 mL g^{-1} VS	+57%	[108]
	Batch	40	Mechanical (particle size 0.2 cm)	334 mL g^{-1} VS	+84%	[108]
Rice straw	Batch	40	Thermal 90°C, 30 min	207 mL g^{-1} VS	+5%	[108]
	Batch	40	Thermal 120°C, 30 min	261 mL g^{-1} VS	+33%	[108]
	Batch	40	Mechanical (particle size 5 cm)	203 mL g^{-1} VS	+3%	[108]
Maize stalk	Batch	40	Thermal 120°C, 30 min	267 mL g^{-1} VS	+9%	[108]
	Batch	40	Mechanical (particle size 2 cm)	254 mL g^{-1} VS	+3%	[108]
	Batch	40	Mechanical (particle size 0.2 cm)	272 mL g^{-1} VS	+11%	[108]
Sunflower Oil Cake	Batch	35	Ultrasonic	220 mL g^{-1} COD_{added}	+54%	[109]
Cassava residues	Batch	55	Biological	260 mL g^{-1} VS	+97%	[110]
Wheat Grass	Batch	50	Enzymatic	N.A.	Negligible	[111]
Paper tube residuals	Batch	55	Steam Explosion and Chemical	493 mL g^{-1} VS	+107%	[112]

Table 3
Methane production and pretreatment improvement for macroalgal biomass.

Feedstock	AD	Process	T(°C)	Pretreatment	Methane	Improvement	Ref
Saccharina latissima	Batch	-	37	Steam explosion at 130°C, 10 min	268 mL g^{-1} VS	+20%	[27]
Laminaria digitata+L. hyperborea+L. Saccharina	Batch	-	50	Beating	425 mL g^{-1} TS	+53%	[105]
Ulva lactuca	Batch	-	55	Unwashed, macerated	271 mL g^{-1} VS	+56%	[4]
	Batch	-	55	Washed, macerated	200 mL g^{-1} VS	+17%	[4]
	Batch	-	55	Washed, 130°C/20 min	187 mL g^{-1} VS	+7%	[4]
	Batch	-	55	Washed, 110°C/20 min	157 mL g^{-1} VS	-10%	[4]
	Batch	-	37	Unwashed, roughly chopped	162 mL g^{-1} VS	-7%	[4]
	Batch	-	55	Dried, ground	176 mL g^{-1} VS	+1%	[4]
Gracilaria vermiculophylla	Batch	-	53	Washed, Macerated	147 mL g^{-1} VS	+11%	[16]
Ulva lactuca	Batch	-	53	Washed, Macerated	255 mL g^{-1} VS	+68%	[16]
Chaetomorpha linum	Batch	-	53	Washed, Macerated	195 mL g^{-1} VS	+17%	[16]
Saccharina latissima	Batch	-	53	Washed, Macerated	333 mL g^{-1} VS	-2%	[16]
Ulva lactuca	Lab-scale CSTR	Cattle manure	53	Dried, ground	15-16 ml g $feed^{-1}$	N.A.	[16]
Ulva sp.	Batch	Sewage sludge	35	Washed	126 mL g^{-1} VS	0%	[29]
	Batch	Sewage sludge	35	Ground	126 mL g^{-1} VS	0%	[29]
	Batch	Sewage sludge	35	Washed, ground	180 mL g^{-1} VS	+30%	[29]
Ulva sp.	Batch	-	35	Unwashed	110 mL g^{-1} VS	N.A.	[15]
	Batch	-	35	Washed	94 mL g^{-1} VS	-14%	[15]
	Batch	-	35	Dried	145 mL g^{-1} VS	+32%	[15]
	Batch	-	35	Dried, ground	177 mL g^{-1} VS	+60%	[15]
	CSTR	Bovine manure	35	Ground	203 mL g^{-1} VS	N.A.	[15]
Palmaria palmata	Batch	Sludge	35	NaOH, thermal pretreatment at 20°C/ 30 min	365 mL g^{-1} VS	+19%	[109]

Table 4
Methane production and pretreatment improvement for microalgal biomass.

Feedstock	AD Process	Co-digestion	T (°C)	Pretreatment	Methane	Improvement	Ref
Pilayella, Ectocarpus, traces Enteromarpha	Continuous	-	35	Hydrothermal depolymerization + enzymatic hydrolysis	0.054 dm^3/g substrate	+64% biogas	[114]
Chlorella vulgaris	Batch	Sewage sludge	35	Ultrasonic	N.A.	+90% biogas	[115]
Scenedesmus	Batch	-	35	Ultrasonic	153.5 mL g^{-1} COD	+100%	[116]
	Batch	-	35	Thermal at 80°C	128.7 mL g^{-1} COD	+60%	[116]
Scenedesmus	Batch	-	38	High pressure thermal hydrolysis + lipid extraction	380 mL g^{-1} VS	+110%	[118]
	Batch	-	38	High pressure thermal hydrolysis	320 mL g^{-1} VS	+81%	[118]
	Batch	-	38	Lipid extraction	240 mL g^{-1} VS	+33%	[118]
Nannochloropis salina	Batch	-	38	Thermal	549 mL g^{-1} VS	+58%	[119]
	Batch	-	38	Microwave	487 mL g^{-1} VS	+40%	[119]
	Batch	-	38	French press	460 mL g^{-1} VS	+33%	[119]
	Batch	-	38	Frozen	233 mL g^{-1} VS	-33%	[119]
	Batch	-	38	Ultrasonic	247 mL g^{-1} VS	-29%	[119]
Chlamydomonas, Scenedesmus, Nannocloropsis	Batch	-	35	Thermal	398 mL g^{-1} VS	+46%	[97]
				Ultrasound	310 mL g^{-1} VS	+14%	[97]
				Biological		Negligible	[97]
Acutodesmus obliquus, Oocystis sp., Phormidium and Nitzschia sp.	Batch	-	35	Thermal	307 mL g^{-1} VS	+55%	[97]
				Ultrasound	223 mL g^{-1} VS	+13%	[97]
				Biological	N.A.	Negligible	[97]
Microspora	Batch	-	35	Thermal 110°C	413 mL g^{-1} VS	+62%	[97]
				Ultrasound	314 mL g^{-1} VS	+24%	[97]
				Biological	N.A.	Negligible	[97]
Scenedesmus	Batch	-	35	Thermal 90°C	170 mL g^{-1}COD	+124%	[120]

Rhizoclonium	Batch	-	53	Blending + Enzymatic	145 mL CH_4 g^{-1} TS	+20%	[122]
Chlamydomonas reinhardtii	Batch	-	38	Drying	N.A.	-20%	[101]
Chlorella	Batch	-	38	Drying	N.A.	-23%	[101]

Fig. 1. Methane production (mL g^{-1} VS) at different pretreatments.

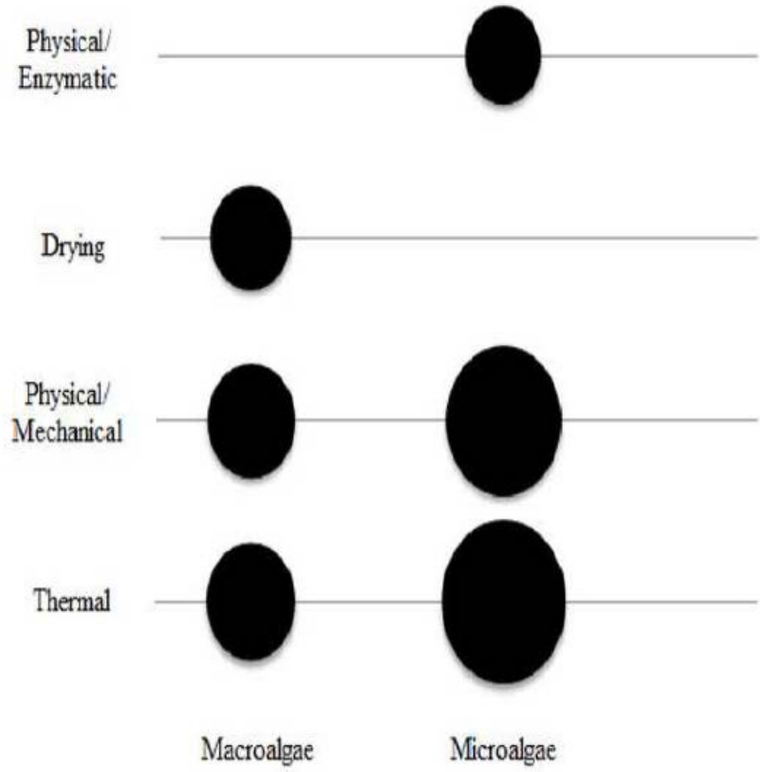

Fig. 2. Percentages of improvement (• positive, ○ negative) at different pretreatments.