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Review

Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application

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HIGHLIGHTS

- Guidelines on the most appropriate pretreatments for the main biogas feedstocks.
- Sludge pretreatment with steam explosion is most recommended, already at full-scale.
- Fatty residues saponification is preferred, with animal by-products sterilization.
- For lignocellulosic biomass alkali or biological pretreatments are most promising.
- Microalgae thermal pretreatment seems most promising so far.

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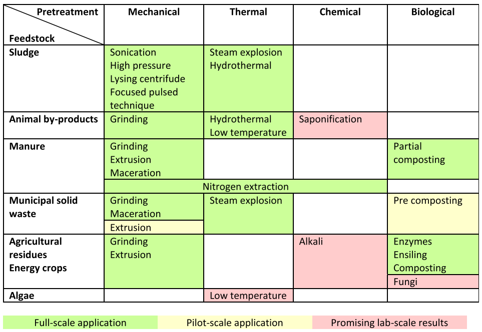
GRAPHICAL ABSTRACT



Pretreatment / Feedstock	Mechanical	Thermal	Chemical	Biological
Sludge	🟩 Sonication High pressure Lysing centrifude Focused pulsed technique	🟩 Steam explosion Hydrothermal		
Animal by-products	🟩 Grinding	🟩 Hydrothermal Low temperature	🟥 Saponification	
Manure	🟩 Grinding Extrusion Maceration			🟩 Partial composting
	🟩 Nitrogen extraction			
Municipal solid waste	🟩 Grinding Maceration 🟨 Extrusion	🟩 Steam explosion		🟨 Pre composting
Agricultural residues Energy crops	🟩 Grinding Extrusion		🟥 Alkali	🟩 Enzymes Ensiling Composting 🟥 Fungi
Algae		🟥 Low temperature		

🟩 Full-scale application 🟨 Pilot-scale application 🟥 Promising lab-scale results

ABSTRACT

When properly designed, pretreatments may enhance the methane potential and/or anaerobic digestion rate, improving digester performance. This paper aims at providing some guidelines on the most appropriate pretreatments for the main feedstocks of biogas plants. Waste activated sludge was firstly investigated and implemented at full-scale, its thermal pretreatment with steam explosion being most recommended as it increases the methane potential and digestion rate, ensures sludge sanitation and the heat needed is produced on-site. Regarding fatty residues, saponification is preferred for enhancing their solubilisation and bioavailability. In the case of animal by-products, this pretreatment can be optimised to ensure sterilisation, solubilisation and to reduce inhibition linked to long chain fatty acids. With regards to lignocellulosic biomass, the first goal should be delignification, followed by hemicellulose and cellulose hydrolysis, alkali or biological (fungi) pretreatments being most promising. As far as microalgae are concerned, thermal pretreatment seems the most promising technique so far.

1. Introduction

The Web of Science® shows an increasing number of published papers per year with "anaerobic digestion" and "pretreatment" as

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Table 1
Web of Science® bibliometric study with the topics "pretreatment" and "anaerobic digestion" and "feedstock" (April 2015).

Feedstock	Number of papers		
	Per year	Till April 2015	In 2014
Sludge	>10 since 2002 >100 since 2010	1075	186
Slaughterhouse or fatty waste	>10 since 2008	214	43
Manure	>10 since 2009	200	42
Lignocellulosic biomass	>10 since 2010	215	64
Food waste	>10 since 2011	155	42
Algae	>10 since 2013	91	39

topics; over 100 published papers per year since 2009, and up to 170 papers in 2012, 220 in 2013 and 305 in 2014 (Table 1). When properly designed, pretreatments may improve the methane potential and/or anaerobic digestion (AD) rate, enhancing digester performance. However, in most of the literature the impact of feedstock pretreatment on the anaerobic digestion is studied in batch biochemical methane potential (BMP) tests. Although this methodology is useful and effective for the determination of optimal pretreatment conditions, it is risky to extrapolate results to full-scale continuous plants, unless a complex modeling procedure is used (Souza et al., 2013a). Pretreatments may increase the anaerobic digestion rate and extent, the impact on kinetics being most of the time more severe than on methane yield. BMP tests are designed to assess the ultimate methane yield of an anaerobic digestion process, and are also used to assess degradation rates. However, the test conditions are quite different from those prevailing in continuous full-scale biogas plants, in which digestion rates play an important role and the impact of pretreatments is highly dependent on anaerobic digestion parameters such as the hydraulic retention time (HRT). In fact, the impact of pretreatment is more pronounced on the digestion rates for low HRT AD processes and on the methane yield for high HRT AD processes.

This review, which cannot be exhaustive given the number of published papers on each feedstock, focuses in priority on full- and pilot-scale results and on lab-scale continuous processes, except for the dry AD, which is often carried out in batch mode. The purpose of this paper is to give guidelines and present a rationale for the selection of pretreatment techniques for the main types of waste used in biogas plants. In particular, it considers feedstocks which are hardly or slowly biodegradable, for which pretreatments can significantly improve their conversion into biogas. The following sections deal with: sewage sludge, animal by-products (including slaughterhouse and fatty wastes), lignocellulosic biomass (including municipal solid wastes (MSW) and food wastes), and algae (third generation biomass).

2. Sewage sludge

Sewage sludge was the first biogas feedstock on which pretreatments were investigated (Table 2). Initially, pretreatments aimed at reducing the amount of sludge to dispose rather than improving the methane yield. To date, sludge is still the most studied feedstock as far as pretreatment for anaerobic digestion is concerned. Because waste activated sludge (WAS) is less biodegradable than primary sludge, pretreatment mainly concerns this sludge type. WAS is composed of flocs comprised of microbial biomass, exopolymeric substances (EPS, mainly proteins and carbohydrates) and compounds which are not degraded during the activated sludge process. The objective of WAS pretreatment is to improve the anaerobic digestion rate by enhancing the rate limiting step of the process (hydrolysis) and to improve the anaerobic digestion extent by rendering some recalcitrant compounds biodegradable or by improving the bioavailability of some compounds. This is achieved by destabilization of floc structures and above all, by sludge cell lysis and solubilisation of intracellular matter.

Numerous WAS pretreatment technologies have been studied, including biological (enzymatic, low temperature thermal pretreatment), thermal (conventional heating or steam injection), mechanical (sonication, grinding, high pressure, lysing centrifugation or microwave irradiation), chemical (ozonation and other advanced oxidation processes, acid or alkali pretreatment) and electrical methods. This section focuses on those pretreatments which seem the most promising and that have been implemented in full-scale digesters.

2.1. Thermal pretreatment

2.1.1. Steam explosion and hydrothermal pretreatment

Several research studies showed that thermal pretreatment is efficient at enhancing both the extent and rate of sludge anaerobic digestion. There is a consensus on the optimal treatment temperature, which should range from 160 to 190 °C, and on treatment duration, which is generally around 20–30 min, although duration has little impact as compared to temperature. However, the application of higher temperature leads to the formation of recalcitrant compounds. The increase in methane potential depends on the nature of the sludge: sludge from extended aeration processes, with low initial biodegradability, undergoes the highest methane production enhancement and published papers (Table 2) report a range of methane production enhancement in continuous digesters (17–82%). Other positive consequences include sanitation, dewaterability improvement and decrease in viscosity, easing transportation.

A key point defining the relevance of thermal pretreatment is the energy balance. Heat integration in the whole plant is essential (Perez-Elvira and Fdz-Polanco, 2012). For example, heat from pretreated sludge can be recovered to heat the digester (Zabranska et al., 2006) or to preheat influent sludge. In addition, the initial solids concentration of sludge is a key parameter. In the case of combined heat and power (CHP) generation from biogas, heat is often produced in excess and thermal treatment, using the heat released from biogas combustion is more energy effective than technologies that make use of electrical power (e.g. microwave heating) (Carrere et al., 2010).

Full-scale applications of sludge thermal hydrolysis were implemented already in 1995 (Kepp et al., 2000). The most referred commercial processes are: THP from Cambi, with more than 30 operating facilities; and Biothelys® or Exelys® from Veolia, with around 10 facilities constructed so far. Other processes such as Lysotherm® (SH+E group-UK), TurboTec® (Sustec-Netherlands), Thermal Pressure Hydrolysis (TPH-Thöni-Austria) have recently become available. As an example, in Davyhulme utility in Manchester UK, a four lines Cambi process allowed to treat 91,000 tTS/d and led to 35% increase in VS removal and superior cake hygienic quality (Kepp et al., 2000; Liao et al., 2014).

2.1.2. Low temperature or biological pretreatment

Thermal pretreatment has also been applied at low temperatures ranging from 50 to 70 °C, where biological mechanisms may also be involved (Table 2). Duration is longer, ranging from about 10 h to a few days (Ferrer et al., 2009). This pretreatment can take advantage of sludge endogenous enzymes (temperature phased anaerobic digestion (TPAD), which uses a first-stage AD at either thermophilic or hyper-thermophilic conditions), or employ microaerobic mechanisms (Souza et al., 2013a). While a 12 h microaerobic pretreatment led to 23% increase in methane potential (Carvajal et al., 2013), a 24 h hyperthermophilic microaerobic pretreatment led to some carbon oxidation in the

Table 2
Effect of pretreatments on sludge anaerobic digestion in continuous reactors.

Feedstock	Process conditions		Gas production increase (Gas yield)	Scale (Volume)	References
	Pretreatment	Anaerobic digestion			
Thermal					
Mixed sludge, only WAS pretreated	Continuous thermal 170 °C, HRT: 40 min, 7.6 bar followed by steam explosion	CSTR 35 °C HRT: 10 and 20 d Control reactor HRT: 20 d	+17% at 20 d HRT + 82% at 10 d HRT	Pilot-scale (300 L)	Souza et al. (2013b)
Mixed sludge	Steam explosion: 165–180 °C 30–60 min	CSTR HRT: 17 d	+20%	Full-scale 90,000 PE	Kepp et al. (2000)
Mixed sludge	Steam explosion: 140 °C 1 min 0.6 MPa	Two-stage 55–53 °C	+18%	Full-scale 100,000 PE	Zabranska et al. (2006)
Mixed sludge	Low temperature: 70 °C 9–48 h	CSTR 55 °C HRT: 10 d	+20%	Lab-scale (5 L)	Ferrer et al. (2009)
WAS	Microaeration: 55 °C 12 h	CSTR 35 °C HRT: 13–20 d OLR = 3.8–6.1 kg COD/m^3 d	+10–24%	Lab-scale (30 L)	Souza et al. (2013a)
WAS	Microaeration: 65 °C 1 day	CSTR 35 °C HRT: 21 and 42 d	No CH_4 increase, but 30% COD removal increase	Lab-scale	Dumas et al. (2010)
Mechanical					
Mixed sludge (62% WAS)	Sonication: 25% of WAS	Egg-shape digester HRT: 18 d	+30%	Full-scale 330,000 PE	Neis et al. (2008)
Mixed sludge (66% WAS) (1.5% VSS)	Sonication: 20 kHz 13.7 W/cm^2 Sludge flow: 8.33 m^3/s HRT: 1.5 s	Egg-shape digester 29–33 °C HRT: 22.5 d	+45%	Full-scale (5000 m^3)	Xie et al. (2007)
Mixed sludge (50% WAS)	Sonication: 5 ultrasonic horns	CSTR HRT: 24 d	+50%	Full-scale (4507 m^3)	Hogan et al. (2004)
Sewage sludge	Lysing centrifuge: 39 m^3/h 3140 rpm	CSTR Mesophilic HRT: 40 d	+26%	Full-scale (2 * 4400 m^3)	Zabranska et al. (2006)
Sewage sludge	Lysing centrifuge: 12 m^3/h 2250 rpm	CSTR Mesophilic HRT:35 d	+15%	Full-scale (2 * 1800 m^3)	
Sewage sludge	Lysing centrifuge: 200 m^3/h	CSTR 38 °C HRT: 40 d	+26%	Full-scale (20,000 m^3)	
Mixed sludge (32% WAS)	High pressure: 830 bar with NaOH addition only WAS pretreatment 8000 L_{WAS}/h	CSTR Mesophilic	14% increase VS removal	Full-scale	Stephenson et al. (2007)
Thickened mixed sludge	Focused pulsed technique: Opencel 20–30 kV Few msec Treatment of 85% sludge flow	CSTR Mesophilic HRT: 30–35 d	+40% (8% increase VS removal and ORL increase)	Full-scale (3300 m^3)	Rittmann et al. (2008)

aerobic stage and no increase in methane production, although it enhanced sludge biodegradability (Dumas et al., 2010). The company Monsal proposes a biological pretreatment using endogenous enzymes (Enhanced Enzymatic Hydrolysis).

2.2. Mechanical pretreatment

2.2.1. Sonication

Sonication has been extensively studied as WAS pretreatment (Pilli et al., 2011). Most studies deal with low frequency sonication (<40 kHz, 20 kHz in most cases). BMP enhancement has been shown to linearly correlate to the chemical oxygen demand (COD) solubilisation (Pilli et al., 2011). However, according to Kim et al. (2013) the yield of conversion of solubilised matter into methane decreases when sonication time increases. In consequence, too high sonication time or applied energy can lead to a reduction of sludge methane potential. In addition, a specific energy threshold cell disruption and sludge solubilisation is often reported. This specific energy threshold ranges from 1000 to 16,000 kJ/kg TS and depends on the TS concentration of the sludge. Furthermore, an optimum sludge concentration (around 20–30 g/L) has been reported for sludge pretreatment. Indeed, higher solid content in the liquid produces more cavitation sites and more hydro-mechanical shear forces due to implosion of more formed bubbles, while beyond the optimum concentration, the homogeneous distribution of acoustic waves is disrupted by absorption effects (Pilli et al., 2011). Sonication has also an impact on sludge dewaterability, which increases by applying high energy sonication, but decreases by low energy sonication. Finally, the application of low energy sonication is beneficial for the mitigation of bulking and foaming problems (Carrere et al., 2010). Lab-scale studies show a wide range of sonication impacts on the anaerobic digestion: from 10% to 40% enhancement of biogas production in continuous processes and from 20% to 140% enhancement of BMP (Carrere et al., 2010; Pilli et al., 2011).

Sonication pretreatment has been widely implemented in full-scale sludge anaerobic digesters (Table 2). The most referred processes concern Sonico Ltd. UK, Ultra WAVES GmbH and IWE Tec GmbH (Dr. Heildcher GmbH) (Perez-Elvira et al., 2009). In order to improve the efficiency, only a fraction of thickened WAS is generally sonicated. For example, 25% of WAS is pretreated in Bamberg's WWTP (Neis et al., 2008). Barber (2005) reviewed the performance of 7 full-scale plants equipped with sonication pretreatment and reported 25–50% increase of the digestion rate, allowing an increase of the organic loading rate (OLR) from 20% to 50% or a decrease of the HRT. However, according to Perez-Elvira et al. (2009), the energy applied ranges from 4 to 40 kJ/L and is far lower than that employed at lab-scale (200–900 kJ/L).

2.2.2. Lysing centrifuge

A research group has proposed to equip a classical sludge thickening centrifuge with a disintegration device mounted at the outlet of thickened sludge. The energy requirement for this pretreatment is thus low. It has been implemented in several full-scale anaerobic digesters, e.g. Liberec in Czech Republic, Furstenfeldbruck and Aachen-Soers in Germany (Table 2), leading to 15–26% increase in biogas production (Zabranska et al., 2006).

2.2.3. High pressure

Several high-pressure technologies have been applied to sewage sludge. In high-pressure homogenisers (up to 900 bar), sludge goes through a pressure valve and is rapidly depressurized. By applying this technology the digester HRT can be reduced and biogas production increased, but sludge dewaterability is decreased (Carrere et al., 2010). Various commercial devices are available: the Crown™ process, which operates at 12 bar; the Cellruptor™

process, where sludge is compressed at a pressure higher than 1 bar caused by the diffusion of a gas across cell walls followed by a rapid, non-equilibrium decompression; and the Microsludge™ process (Table 2), which combines high pressure (830 bar) and alkaline hydrolysis (Stephenson et al., 2007).

2.2.4. Pulsed power or electroporation

Sludge is subjected to high voltage, up to 10 kV, in pulse periods of few milliseconds by immersion of electrodes into the sludge stream. Sludge flocs and microbial cells are thus disrupted, leading to the release of soluble organic compounds, and enhancing sludge AD. The full-scale application of the OpenCel™ process on a fraction (up to 55%) of thickened mixed sludge led to an increase of volatile solids degradation (from 52% to 56%) and a 40% increase in biogas production, which was also due to a higher organic loading rate (Rittmann et al., 2008).

3. Animal by-products

Animal by-products (ABP) are characterized by a high organic content, mainly composed of proteins and fats, with different amounts of carbohydrates and inorganic compounds, depending on the waste management and sorting technologies used (Rodriguez-Abalde et al., 2011). They represent interesting AD substrates due to their high theoretical methane potential. However, efficient methane recovery is not easy to achieve because of the relatively slow hydrolysis rates related to the physical mass transfer from the solid to the liquid phase; and also because of inhibitory processes related to the accumulation of ammonia from protein decomposition and long chain fatty acids (LCFA) from lipids hydrolysis. In addition, lipids are insoluble, less dense than water and slowly biodegradable. Thus, the application of a pretreatment to disintegrate and hydrolyse complex compounds and to promote the solubilisation of lipids may accelerate the anaerobic digestion of ABP. Pretreatment technologies applied on ABP have been alkaline, thermal, thermo-chemical, enzymes and bacterial products, and ultrasounds (Battimelli et al., 2010; Cavaleiro et al., 2013; Hejnfelt and Angelidaki, 2009; Li et al., 2013; Luste and Luostarinen, 2010; Rodriguez-Abalde et al., 2011). All these studies are based on COD solubilisation and BMP tests, although some of them also looked at LCFA and/or volatile fatty acids (VFA) concentrations to evaluate the effect of pretreatment (Battimelli et al., 2010; Cavaleiro et al., 2013).

An additional reason for the application of pretreatments on ABP is sanitation. Since 2002, the management of ABP in the European Union is regulated by stringent environmental legislations in order to protect public and animal health. Particularly, pasteurisation (60 min at 70 °C) and sterilisation (20 min at 133 °C and 3 bar) were authorised for low risk materials, category III and category II respectively.

3.1. Thermal pretreatment

There are only a few studies on the effect of thermal pretreatments on slaughterhouse waste, including categories II and III of ABP in continuous anaerobic digesters, and they have divergent results (Table 3). Luste and Luostarinen (2010) evaluated the pasteurisation effect on the codigestion of slaughterhouse waste with sewage sludge and reported an increase in substrate solubilisation and biodegradability. Indeed, 10% and 24% higher methane production was obtained with the hygienised substrate as compared to the untreated one substrate at HRT of 25 and 20 days, respectively. Edstrom et al. (2003) also reported that pasteurisation of ABP led to a 4-fold increase in the biogas yield (from 0.31 to 1.14 m^3_{biogas}/kgVS). They suggested that this improvement was due to an increased accessibility of lipids to microorganisms. Moreover, stable processes at OLR exceeding 2.5 gVS/L d and HRT below 40 days were obtained in a pilot-scale digester (26 m^3) operating for more than 1.5 years. These results were used to design the first full-scale biogas plant (Linköping Biogas AB) using ABP in Sweden.

However, the methane production from pretreated substrates is not always higher than that obtained from untreated wastes, and in some cases it even decreases. Cuetos et al. (2010) reported a lower biogas production (2.9 L/ d) during the codigestion of slaughterhouse waste with the organic fraction of municipal solid waste (OFMSW), after a sterilisation process. These results were confirmed by other studies using BMP tests (Cavaleiro et al., 2013; Hejnfelt and Angelidaki, 2009; Luste et al., 2009). This reduced methane production was explained by the inhibitory effect of LCFA and ammonia, produced during the respective hydrolysis of lipids and proteins (Palatsi et al., 2012). System instability was usually associated with an increase in the OLR of digesters treating hygienised slaughterhouse waste (Bayr et al., 2012; Cuetos et al., 2010; Escudero et al., 2014). The negative effect of pretreatment was also related to the occurrence of Maillard reactions and the formation of recalcitrant compounds (Cuetos et al., 2010).

Pasteurisation has been implemented in a full-scale biogas plant, operating since 1996 and owned by Uppsala Vatten och Avfall AB in Uppsala, Sweden. By digesting 25,200 tons of a mixture composed by the OFMSW (about 82 wt%), food waste (about 3 wt%) and slaughterhouse waste (about 15 wt%) under thermophilic conditions, the plant produced 4.4 NMm^3 of biogas. Pasteurisation using steam produced by a biogas boiler is the sanitation method currently applied. The heat demand of this pretreatment corresponds to 9% of the produced biogas (Grim et al., 2015).

3.2. Thermo-chemical pretreatment

Saponification aims at minimising the sanitary risk of categories II and III of ABP; it also fulfills the requirements prescribed by ABP Regulation. Saponification consists of the reaction between a lipid and an alkali, resulting in the production of LCFA salts (soaps) and glycerol release. The conversion of lipids and free LCFA constituting insoluble fat, oil and grease wastes into soluble soaps improves contact between the substrate and microorganisms, thereby enhancing their anaerobic biodegradability. The saponification pretreatment was optimised (NaOH (50% w/w), 0.156 mol/g_{VS}, at 60 °C, 120 °C and 150 °C for 3 h) in order to improve the biodegradation of organic matter in batch-fed digesters (Battimelli et al., 2010). The alkali dose was adjusted to ensure an excess of hydroxide without exceeding the toxicity limit of the cation (3.5–5.5 g Na^+/L) (Chen et al., 2008). The reaction at 120 °C achieved the best anaerobic digestion performance in terms of specific gas production and equivalent degraded load. Affes et al. (2013) measured a lipid hydrolysis efficiency of 89% by applying the saponification pretreatment (NaOH, 0.04 mol/g COD, 70 °C, 60 min) to flesh fat carcass, while Cavaleiro et al. (2013) achieved a lower fat hydrolysis efficiency (52–54%) by pretreating meat processing waste with soda (0.3 g/gTS) at 55 °C for 24 h. However, this thermo-chemical pretreatment at moderate temperature did not alter the LCFA composition (Affes et al., 2013; Battimelli et al., 2010). Saponification is thus a promising pretreatment procedure for enhancing the hydrolysis step and initial degradation rate, reducing the digestion time (Table 3).

However, these advantages could be limited by an excessive accumulation of LCFA, eventually causing process inhibition and failure. To cope with these limitations, a novel reactor system configuration that integrates saponification and digested solids recirculation to the anaerobic digestion process was tested (Affes et al., 2013). The feasibility of this system configuration for solid

Table 3
Effect of pretreatments on animal by-products anaerobic digestion in continuous reactors.

Feedstock	Process conditions		Gas production increase (Gas yield)	Scale (Volume)	References
	Pretreatment	Anaerobic digestion			
Thermal					
Poultry ABP (entrails, content of the stomach, intestines)	Sterilisation: 20 min 133 °C >3 bar particle < 3 mm	CSTR 34 ± 1 °C HRT: 36 d OLR: 1.2–2.6 kg VS/m^3 d	Mono-digestion: −9% (2.9 L/d)	Lab-scale (3 L)	Cuetos et al. (2010)
ABP from meat-processing industry And sewage sludge	Pasteurisation: 70 °C 60 min	35 °C HRT: 25–20 d and 14 d OLR: 1.8–4 kg VS/m^3 d	+10% at 25 d HRT +24% at 20 d HRT −14% at 14 d HRT	Lab-scale (4 L)	Luste and Luostarinen (2010)
Rendering and slaughterhouse wastes (stomach contents, intestines of swine and bovine, without rumen and reticulum)	Sterilisation of the rendering wastes 133 °C 20 min 3 bar	CSTR 35 °C/55 °C HRT: 50 d Mesophilic: OLR: 0.5–1.5 kg VS/m^3 d Thermophilic: OLR: 1.5–2.5 kg VS/m^3 d	Mesophilic: 720 L CH_4/kg VS Thermophilic: 766 L NH_3, VFA, LCFA accumulation	Lab-scale (10 L)	Bayr et al. (2012)
Cat. 3 beef ABP	Pasteurisation: 2 h 70 °C (oven)	CSTR 35 °C HRT: 105–160 d OLR: 0.3–1.6 gVS/L d	Biogas: 2 L/L d (1.07 L/gVS) Lower HRT system instability	Lab-scale (8 L)	Escudero et al. (2014)
ABP, food waste, liquid manure	Pasteurisation: 1 h 70 °C	CSTR 37 °C Batch-fed digestion OLR: 2 gVS/L d	4-fold increase in biogas yield (1.14 L/g VS)	Lab-scale (3 L)	Edstrom et al. (2003)
ABP (19–38% dry matter) + food waste + liquid manure	Pasteurisation: 1 h 70 °C	CSTR (37 °C) OLR up to 5 gVS/L d at lab-scale OLR up to 3.2 gVS/L d at pilot-scale	Stable processes at OLR > 2.5 gVS/L d HRT < 40 d	Lab-scale (30 L) Pilot-scale (26 m^3)	
OFMSW (82 wt%), food waste (3 wt%), slaughterhouse waste (3 wt%)	Pasteurisation: 70 °C 1 h Integrated thermophilic sanitation (ITS): 52 °C 10 h	CSRT 52 °C HRT: 35 d OLR: 3 g VS/ L d	Heat demand: Pasteurisation: 1.9 ± 0.3 MJ/ kg VS 9% of biogas energy production ITS = 1.0 MJ/kg VS 5% biogas energy production	Full-scale (Uppsala, Sweden)	Grim et al. (2015)
OFMSW (82 wt%), food waste (3 wt%), slaughterhouse waste (15 wt%)	Pasteurisation: 72–74 °C 60 min (water bath)	CSTR 52 °C HRT: 35 d OLR: 3 g VS/L d	13 L_{biogas}/d No effect of pasteurisation	Lab-scale (5 L)	
Thermo-chemical					
Aeroflotation grease Flesh fat from cattle carcass	Saponification: 60–120–150 °C 3 h NaOH (50% w/w) 0.16 g/g VS	CSTR Batch-fed reactor 35 °C 1–5.3 g COD/L	Biodegradation improvement Bioavailability increase	Lab-scale (5 L)	Battimelli et al. (2010)
Flesh fat from cattle carcass	Saponification: 70 °C 1 h NaOH (32% w/w) 0.04 mol NaOH/ g COD	CSTR 35 °C Acclimation period HRT: 33 d OLR: 2.2 gCOD/L d Solids recirculation (20% of the outflow (w/w))	Lipid hydrolysis efficiency: 89% Increased bioavailability of solid fatty waste	Lab-scale (5 L)	Affes et al. (2013)

slaughterhouse fatty waste was evidenced in lab-scale reactors, reaching organic matter removal efficiencies higher than 90%. Both strategies acted synergistically: saponification promoted the emulsification and bioavailability of solid fatty residues, while solids recirculation resulted in substrate dilution and lower biomass washout risk, thus promoting the enrichment and adaptation of active biomass. Further research is needed in order to optimise the saponification pretreatment to ensure both sterilisation and solubilisation in the same process and to integrate it in pilot- and full-scale plants, minimising the cost and the toxicity effect of the reagent.

4. Lignocellulosic biomass

Lignocellulosic biomass is composed of three main fractions: cellulose, hemicelluloses and lignin. Cellulose consists of a polymer of D-glucose subunits and contains parts with an organized crystalline structure and parts with a poorly organized amorphous structure. Cellulose strains form the so-called cellulose fibrils or cellulose bundles. Hemicelluloses consist of heteropolymers of xylose, mannose, galactose, rhamnose, arabinose, glucose and uronic acids. They have amorphous structures and are more readily hydrolyzed than cellulose. Lignin consists of hydrophobic heteropolymers of three phenylpropane alcohols: p-coumaryl (H), coniferyl (G) and sinapyl (S). It presents an amorphous structure and gives the plant resistance against microbial attack. Lignin polymers are covalently bound to cell wall polysaccharides through lignin–carbohydrate complexes, which represent a limiting factor in the biodegradation of holocelluloses (cellulose and hemicelluloses) (Monlau et al., 2013).

A model was developed to predict the biochemical methane potential of lignocellulosic feedstocks, as a function of their compositional and structural features (Monlau et al., 2012). The most important parameter was shown to be the lignin content (which was negatively correlated to the BMP), followed by the soluble sugars content (positively correlated), the proteins content (positively correlated), the crystalline cellulose content (negatively correlated) and the amorphous holocelluloses (amorphous cellulose and hemicellulose) content (positively correlated). Thus, lignocellulosic biomass pretreatment objectives should be feedstock delignification, sugars solubilisation and cellulose crystallinity reduction, delignification being the main objective for the enhancement of methane production (Monlau et al., 2012). However, in full-scale plants, the primary objective of pretreatment is to ease feedstock management (i.e. storage), digester feeding, and avoid any floating layer in the digester.

Pretreatments of lignocellulosic biomass including energy crops, manure, crop residues as well as municipal solid wastes, have been extensively investigated at lab-scale. However, physico-chemical pretreatments, the most studied at bench-scale, have hardly been applied in full-scale plants. On the contrary, some biological and mechanical pretreatments are currently used in full-scale biogas plants.

4.1. Thermal pretreatment

Steam explosion is among the most widely applied thermal pretreatment methods for enhancing the methane production from lignocellulosic biomass. In this method, high-pressure saturated steam is applied for a few minutes on the properly milled lignocellulosic biomass, and then pressure is swiftly reduced, subjecting the biomass to an explosive decompression. Due to the high temperatures and pressures imposed, degradation of hemicellulose and sometimes lignin transformation occur, enhancing cellulose hydrolysis and increasing the biogas yield. However, when steam explosion is carried out under very severe conditions, lower enzymatic digestibility has been reported, which is generally attributed to the release of furans (furfural from xylose degradation and 5-hydroxymethylfurfural (5-HMF) from glucose degradation) and phenolics (from lignin degradation). Although the anaerobic digestion has been shown to be less sensitive to these inhibitors than other biological processes such as dark fermentation or enzymatic hydrolysis, too high concentrations of such compounds may have an inhibitory effect on methanogens, leading to a decreased gas production (Monlau et al., 2014). The degree of de-polymerization and formation of inhibitory compounds depends significantly on the severity of applied pretreatment conditions. Therefore, suitable steam-explosion pretreatment conditions should be selected to reduce or even avoid the formation of inhibitors.

Steam explosion of different lignocellulosic feedstocks has been thoroughly studied and applied at lab-scale. Forgacs et al. (2012) studied the codigestion of steam-exploded citrus waste with municipal solid wastes, in continuous reactors and found a methane production of 0.56 m^3 CH_4/kg VS d. When untreated citrus waste was used as a cosubstrate, the process failed, indicating the crucial role of pretreatment on the overall process. In the same study, it was shown that the process was economically viable and could be easily applied to upgrade the performance of an existing biogas plant. The performance of continuous AD processes from steam exploded lignocellulosic feedstocks is summarised in Table 4.

Even if this pretreatment is considered to be cost-effective, as a relatively low level of energy is required, no full-scale applications have been reported but for municipal solid wastes, mainly codigested with sewage sludge. For example, in the Ecopro plant (Verdal-Norway), the Cambi process is applied prior to the codigestion of 17,500 t/year of source-separated household waste, 12,500 t/year of sewage sludge and 5000 t/year of animal by-products (Sargalski, 2008).

4.2. Thermo-chemical pretreatment

The combination of steam explosion with chemical agents, such as acids or bases has also been tested and shown positive results. By combining wet oxidation with steam-explosion, a novel pretreatment process so-called wet explosion was developed, which has been applied for enhancing the methane production from manure (Ahring et al., 2015). During chemical or physico-chemical pretreatments lignocellulosic biomass is exposed to chemicals such as acids or alkali, at ambient or higher temperatures.

Acid pretreatment may be performed with acids such as H_2SO_4, H_3PO_4, HNO_3 and HCl. The main reaction that occurs is the hydrolysis of hemicellulose, especially xylan, since glucomannan is more stable. Under such conditions, furfural and HMF generation can occur, and further production of formic and levulinic acids can also be observed. Lignin is hardly solubilised, but it is disrupted to a high degree, increasing cellulose susceptibility to enzymes. On the other hand, alkaline pretreatments which involve alkaline solutions, such as NaOH, $Ca(OH)_2$ or ammonia, are more effective at lignin breakdown, causing depolymerization and cleavage of lignin-carbohydrate linkages. They also enhance hemicellulose solubilisation into its oligomers, though to a less extent than acid pretreatments. The cellulose structure is affected to a lesser degree.

Overall, alkaline pretreatments may be the most suitable for enhancing the anaerobic digestion process. The beneficial impact of alkaline pretreatment (10% NaOH at 40 °C for 24 h) on the methane from ensiled sorghum forage in continuous anaerobic digesters operated at an OLR of 1 g VS/L d and a HRT of 21 days was shown by Sambusiti et al. (2013) (Table 4). Interestingly, higher stability of the reactor fed by pretreated sorghum as compared to the control reactor fed by untreated sorghum was observed. This was due to the higher alkalinity of the system, preventing pH drops and destabilisation of the anaerobic digestion process (Sambusiti et al., 2013).

Despite these benefits, the presence of sodium might be detrimental for digestate land application. In addition, there is a threshold of sodium ion concentrations (around 3 g/L), above which inhibition or toxicity of methanogens may occur (Antonopoulou and Lyberatos, 2013). Alternatively, other alkali chemicals such as lime and ammonium hydroxide could be used. In particular, aqueous ammonia soaking (AAS) presents certain advantages, since ammonia is relatively safe to handle, non-corrosive, can be easily recovered and presents a high selectivity towards lignin reactions, while preserving carbohydrates (Antonopoulou et al., 2015), but full- or pilot-scale applications are still pending.

The NiX™ (nitrogen extraction) process has been applied at full-scale for manure, mainly for poultry manure which is rich in nitrogen. This technology combines high pressure and high temperature cooking with lime addition, which results in both ammonia stripping and an increase of the methane potential of manure (Fink, 2013).

4.3. Biological pretreatment

4.3.1. Enzymatic pretreatment

Enzymatic pretreatments have been investigated at lab-scale and their effect has been generally assessed by BMP tests. Investigated enzymes include: cellulases, cellobiases, endoglucanase, xylanases, pectinases, ligninolytic enzymes such as laccases, manganese and versatile peroxidases, as well as α-amylases and proteases in the case of municipal solid wastes. Enzymes are used to increase the biogas yield, but also to decrease the viscosity of the feedstock or the fermentation media.

Enzymes can be applied as pretreatment or rather added to a 1-stage digester or to the acidogenic reactor of a 2-stage process (Romano et al., 2009). Continuous lab-scale anaerobic digestion

Table 4
Effect of pretreatments on lignocellulosic biomass anaerobic digestion in continuous reactors.

Feedstock	Process conditions		Gas production Increase (Gas yield)	Scale (Volume)	References
	Pretreatment	Anaerobic digestion			
Thermo-chemical					
Steam exploded citrus waste, municipal solid wastes	Steam explosion: 150 °C 20 min 60 bar pressure (steam)	Semi-continuous, 55 °C HRT: 21 d	0.56 m^3 CH_4/kg VS d	Lab-scale (5 L)	Forgacs et al. (2012)
Manure	Wet explosion: 170 °C 25 min O_2 0.4 MPa	CSTR HRT: 10 d	+357% (320 ± 36 L CH_4/kgVS d)	Lab-scale (30 L)	Ahring et al. (2015)
Ensiled sorghum forage	Alkaline: 40 °C 24 h 10 g NaOH/100 g TS	CSTR 35 °C HRT: 21 d	+25%	Lab-scale (1.5 L)	Sambusiti et al. (2013)
Sunflower stalks	Alkaline: 55 °C 24 h 4 g NaOH/100 g TS	CSTR 35 °C HRT: 21 d	+26% (191 ± 3 mL CH_4 /g VS)	Lab-scale (1.5 L)	Monlau et al. (2015)
Hydrolysate of *Agave tequilana* bagasse	Acid: 123.6 °C 2.1 h 1.4%w/w	ASBR, 32 °C, HRT: 21 d	0.26 L CH_4/g COD or 0.3 L CH_4/L/d	Lab-scale (3.6 L)	(Arreola-Vargas et al., 2015)
Biological					
Maize silage Maize and sorghum silage Maize and rye silage	Enzyme addition in the digester 100 ppm (TS)	HRT: 63 d OLR: 5–5.8 kgVS/m^3 d		Full-scale (2000 m^3)	(Schimpf et al., 2013)
Organic fraction of municipal solid waste (30 mm average particle size)	Composting: Room temperature 24 h Inoculation with mature compost 2.5% (v/v)	CSTR 55 °C HRT: 15 d 30% TS	+73%	Lab-scale (5 L)	Fernandez-Guelfo et al. (2011)
Organic fraction of municipal solid waste	Pre-composting (short auto-heating in less than 1 week after sorting)	Agitated 55 °C HRT: 6 d OLR: 17.8 kgVS/m^3 d 16–22% TS	−11%	Pilot-scale (3 m^3)	Mata-Alvarez et al. (1993)
		Agitated 55 °C HRT: 12 d OLR: 9.7 kgVS/m^3 d 16–22% TS	+32%		
Mechanical					
Organic fraction of municipal solid waste diluted with sludge	Extrusion of OFMSW, grate with 8 mm holes	CSTR 40 °C OLR: 4.3 g VS/ L d	800 L_{biogas}/kg VS	Pilot-scale (1000 L)	Novarino and Zanetti (2012)
Liquid manure (39%), horse manure (20%), grain silage (11%), maize silage (11%), solid manure (8%), grass silage (7%), crushed grain (4%)	Cross-flow grinder (Bio-QZ, MeWa, Gechingen) 15 s 65% chamber filling	CSTR 40 °C HRT: 79 ± 16 d OLR: 2.9 ± 0.5 kg VS/m^3 d	+26.5%	Full-scale (800 m^3)	Monch-Tegeder et al. (2014)

of several enzyme pretreated feedstocks showed little increase (up to 13%), no impact or even a decrease (up to 10%) in biogas production. When enzymatic hydrolysis is applied upstream anaerobic digestion, there are strong risks that released sugars are consumed by endogenous microorganisms. A sterilisation step may thus be required to eliminate endogenous microorganisms, but this additional sterilisation step might be too costly within the biogas plant context. In full-scale plants, enzymes are rather directly introduced inside the digester. Schimpf et al. (2013) reported no or low (up to +4.7%) impact of enzyme addition on the biogas yield (Table 4). It is worth noting that the addition of the same enzyme to lab-scale batch anaerobic tests had led to higher biogas yield increase (from 6% to 15%, depending on the feedstock) (Schimpf et al., 2013). Most of the enzymes used to improve lignocellulosic feedstocks anaerobic digestion are produced from fungi, mainly *Aspergillus* and *Trichoderma* genus (Schimpf et al., 2013). Pretreatment with fungi presents some advantages over enzymatic pretreatment, especially the reduction of the number of steps of the treatment process by avoiding enzyme production and recovery steps (Rouches et al., submitted).

4.3.2. Fungi pretreatment

Fungi pretreatments are particularly interesting because lignocellulosic biomass can be degraded by white-, brown- and soft-rot fungi. Among them, white-rot fungi are the most investigated because of their capacity to delignify biomass (Rouches et al., submitted). Fungi pretreatments are carried out as aerobic solid-state fermentation processes, requiring low reactor volumes and amounts of water. For this reason, fungi solid-state fermentation should be preferred upstream solid state anaerobic digestion. Several lab-scale studies investigated the impact of fungi pretreatment on various biomasses methane potential. The results generally show a significant increase in specific methane potential (referred to pretreated solids), up to 50% and even higher in the case of feedstocks with very low initial biodegradability. Nevertheless, organic matter losses (generally around 10–20%) are scarcely mentioned and may lead to lower methane potential increase (referred to initial solids). For this reason, fungi selection and their solid-state fermentation on biomass should be optimised in order to maximise lignin degradation while mitigating carbohydrate consumption.

4.3.3. Two-stage anaerobic digestion

In two-stage anaerobic digestion processes, the first hydrolysis/acidogenic step can be considered as a pretreatment for biogas production. Two-stage processes are generally reported to be more stable, to require less reactor volume, to improve the methane production, leading to higher energy recovery from the feedstock by hydrogen recovery in the first stage. These systems may be preferred for easily biodegradable wastes such as food wastes or vegetable wastes. Some authors have improved the hydrolysis step by supplying small amounts of oxygen (microaeration) along with a cellulolytic consortium (Table 4) (Zhang et al., 2013). Zhu et al. (2009) showed that sufficient microaeration of flowers and vegetables wastes promoted the hydrolysis of easily biodegradable carbohydrates and proteins, but insufficient microaeration led to unstable and decreased performance.

4.3.4. Composting

Composting may be used as a pretreatment for batch dry anaerobic digestion, with the main objective of increasing the feedstock temperature through auto-heating, thus reducing heat requirements for anaerobic digestion start-up. But composting also leads to organic matter degradation. Comparing anaerobic digestion of fresh and composted municipal solid wastes at low HRT (6 and 12 days), Mata-Alvarez et al. (1993) showed that composting removes the easily degradable fraction of MSW causing a worse digester performance at the lowest HRT (Table 4). Additionally, the highest biogas production from composted MSW at the highest HRT showed depolymerization of a complex organic fraction that became degradable in the investigated conditions.

4.3.5. Ensiling

Ensiling is the most common process used for farm-scale storage of energy crops such as maize, grass or sorghum. Chopped biomass undergoes anaerobic lactic fermentation. According to Williams and Shinners (2014), sorghum ensiling makes around 98% of cellulose and hemicellulose recovery possible. In another study, matter losses up to 13% were measured, but no losses in methane production were observed after one-year ensiling, considering the increase in methane yield and dry matter loss (Herrmann et al., 2012). On the contrary, Pakarinen et al. (2011) measured an increase (up to 50% for hemp), a decrease (up to 34% for faba beans) or no significant change (for maize) of the methane potential of ensiled biomass in comparison to the fresh feedstock.

4.4. Mechanical pretreatment

The impact of size reduction on lignocellulosic biomass AD has been widely investigated through BMP tests, but very few studies concern continuous lab-scale digesters. Generally, grinding does not affect the methane yield, but it leads to higher digestion rates (Table 4). In contrast, shredding or milling of feedstocks is required to introduce them into full-scale digesters. Extrapolation of lab-scale results is not evident and published full-scale data are very scarce, in spite of the high number of available technologies. Kratky and Jirout (2011) published a review on biomass size reduction machines, and concluded that colloid mills and extruders are only suitable for comminuting wet materials, with moisture contents over 15–20%, whereas hammers and especially knife mills are only suitable for comminuting dry biomass with moisture contents up to 10–15%. However, dry fractionation through milling has a high energy requirement, when compared to other pretreatment technologies (Barakat et al., 2013). Extrusion, which combines thermal and mechanical pretreatments, has been applied at both lab and full-scale (Montgomery and Bochmann, 2014).

5. Algae

Most research on biogas production from algal biomass has been focused on microalgae. The main characteristics influencing microalgae anaerobic biodegradability are the macromolecular composition (lipids, proteins and carbohydrates) and the cell wall structure. Microalgae cell wall is mostly composed of organic compounds with slow biodegradability and/or low bioavailability. This resilient cell wall hinders the methane yield, since organic matter retained in the cytoplasm is not easily accessible to anaerobic microorganisms (Gonzalez-Fernandez et al., 2011). In eukaryotic microalgae, the cell wall is generally composed of a microfibrillar layer of cellulose, which may be surrounded by an amorphous layer. Outside the outer amorphous layer a laminated polysaccharide cover may be present. Its composition can be more or less complex, containing: 25–30% cellulose, 15–25% hemicellulose, 35% pectin and 5–10% glycoproteins (Gonzalez-Fernandez et al., 2011). The cell wall structure depends on the microalgae species. Some species are naked, lacking a cell wall (e.g. *Dunaliella salina*), or have a glycoprotein cell wall (e.g. *Clamydomonas* sp., *Euglena* sp. and *Tetraselmis* sp.). In these cases, anaerobic digestion has a higher rate. However, most microalgae have a polysaccharide-based cell wall, with multilayers of cellulose and hemicellulose (e.g. *Chlorella* sp. and *Nannochloropsis* sp.) and recalcitrant compounds, such as sporopollenin and polyterpene, which hampers the anaerobic digestion process (Gonzalez-Fernandez et al., 2011). In order to increase both the rate and extent of biogas production, pretreatment methods may be used (Passos et al., 2014a). In this way, particulate biomass is solubilised, enhancing the biodegradability and bioavailability of organic molecules to anaerobic microorganisms. To date, thermal, mechanical, biological and chemical pretreatments have been applied to improve microalgae anaerobic digestion. Even though most studies have been carried out in batch reactors; some long-term studies in continuous digesters have already shown promising results (Table 5). However, the relation between solubilisation and methane yield increase, and the effect of pretreatment on microalgae cell structure is still not clear. What is known is that the pretreatment effectiveness is strongly related to the applied conditions and to the algae species.

5.1. Thermal pretreatment

Thermal pretreatments have been the most studied for increasing microalgae methane yield. Temperatures from 55 to 170 °C have been applied prior to batch and continuous reactors. This technique may be sub-divided into three categories: low temperature (<100 °C), hydrothermal (>100 °C) and steam explosion (140–170 °C and 4–6 bars).

5.1.1. Low temperature pretreatment

The main advantage of applying temperatures below 100 °C is the low energy demand for biomass heating. In fact, energy requirements may be fulfilled using waste heat from cogeneration engines fueled by biogas. Pretreatment performance may be influenced by both temperature and exposure time. However, the first study on microalgae low temperature pretreatment already mentioned that temperature was the dominant factor affecting the anaerobic biodegradability in respect to exposure time and biomass concentration, explaining 50% of the pretreatment effectiveness in a model analysis (Chen and Oswald, 1998). A mixture of *Pediastrum* sp., *Micractinium* sp. and *Scenedesmus* sp. biomass was digested at 16–20 °C after thermal pretreatment at 60 °C, which changed microalgal biomass from green to brown, achieved 11% COD solubilisation over an exposure time of 3.7 h, and 32% increase in the methane yield (0.136 L CH_4/g VS) (Kinnunen et al., 2014). Continuous reactors operated at 20 days HRT were used to study the anaerobic digestion performance of microalgal biomass grown in raceway ponds for wastewater treatment. Biomass methane yield was increased by 70% when thermal pretreatment at 75 and 95 °C was applied to the mixed community of green microalgae and diatoms over an exposure time of 10 h. This study showed how pretreatment effectiveness depended on the microalgae species investigated. For instance, *Stigeoclonium* sp. was hardly digested without pretreatment, while it was damaged and partly disrupted after thermal pretreatment, and degraded after

Table 5
Effect of pretreatments on microalgae anaerobic digestion in continuous reactors.

Feedstock	Process conditions		Gas production Increase (Gas yield)	Scale (Volume)	References
	Pretreatment	Anaerobic digestion			
Thermal					
Scenedesmus sp. and *Chlorella* sp.	Low temperature: 100 °C 8 h	CSTR Mesophilic HRT: 28 d	33% (0.270 L CH_4/g VS)	Lab-scale (5 L)	Chen and Oswald (1998)
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Low temperature: 75 and 95 °C 10 h	CSTR Mesophilic HRT: 20 d OLR: 0.70 g VS/L d	70% (0.180 L CH_4/g VS)	Lab-scale (2 L)	Passos and Ferrer (2014)
Pediastrum sp., *Micractinium* sp. and *Scenedesmus* sp.	Low temperature: 60 °C 2, 4, 6 h	CSTR 20 °C HRT: 14–16 d OLR: 1.0 g VS/L d	32% (0.136 L CH_4/g VS)	Pilot-scale (20 L)	Kinnunen et al. (2014)
Nannochloropsis salina	Hydrothermal: 100–120 °C 2 h	CSTR Mesophilic HRT: 120 d OLR: 1.96 g VS/L d	108% (0.130 L CH_4/g VS)	Pilot-scale (22 L)	Schwede et al. (2013)
Oocystis biomass	Hydrothermal: 130 °C 15 min	CSTR Mesophilic HRT: 20 d OLR: 0.70 g VS/L d	42% (0.120 L CH_4/g VS)	Lab-scale (2 L)	Passos and Ferrer (2015)
Mechanical					
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Microwave: 70 MJ/kg VS 26 g TS/L	CSTR Mesophilic	60% (0.272 L CH_4/g VS)	Lab-scale (2 L)	Passos et al. (2014b)
Biological					
Chlorella vulgaris	Enzymatic: Protease 0.585 UA 65 g TS/L	CSTR Mesophilic HRT: 20 d OLR: 1.50 g COD/L d	260% (0.128 L CH_4/g COD)	Lab-scale (1 L)	Mahdy et al. (2015)

anaerobic digestion. On the other hand, the diatom *Nitzschia* sp. was not digested even after pretreatment (Passos and Ferrer, 2014).

5.1.2. Hydrothermal pretreatment

Hydrothermal pretreatment takes place at temperatures above 100 °C, under pressure. After pretreatment, accumulated pressure is gradually released until it reaches ambient conditions. It has similar effect as the low temperature pretreatment, but with shorter exposure times. Hydrothermal pretreatment at 100–120 °C for 2 h was applied to *Nannochloropsis salina*, increasing the methane yield from 0.13 to 0.27 L CH_4/g VS (108% increase) in continuous anaerobic reactors. Transmission electronic microscope (TEM) images indicated that microalgae cells were partly damaged after pretreatment; in fact the outer sublayer of the cell wall was still intact, but both inner sublayers were cleaved (Schwede et al., 2013). Similarly, *Oocystis* sp. biomass grown in wastewater treatment raceway ponds was pretreated at 130 °C for 15 min and led to 42% methane yield increase in continuous reactors operating at 20 days HRT (Passos and Ferrer, 2015). In this study, microscopic images showed how the outer layer of microalgae cells was disrupted, enhancing anaerobic digestion performance.

5.1.3. Steam explosion

For thermal pretreatment with steam explosion, biomass is placed in a vessel and steam is applied at high temperature (~160 °C) and pressure (~6 bars) for a few minutes (10–30 min); afterwards, steam is flashed and biomass is quickly cooled down in another vessel. The sudden pressure drop leads to cell wall rupture and biomass disintegration, and is known as steam explosion. In the case of microalgae, this pretreatment is yet to be investigated in continuous reactors. In batch tests, this technique applied at 140–180 °C and 3–10 bars was effective at enhancing organic matter solubilisation and methane yield (40–80% increase) (Mendez et al., 2014).

5.2. Mechanical pretreatments

Mechanical pretreatments act by directly breaking cells through a physical force. Mechanical methods are less dependent on microalgae species and less likely to contaminate the final product, in comparison with chemical pretreatments. However, the main disadvantage is high electricity consumption. The most common methods are ultrasound and microwave pretreatments.

5.2.1. Sonication

Ultrasound pretreatment of microalgae has only been studied in batch tests. Nevertheless, results have shown how this technique promotes microalgae cell wall disruption and organic matter solubilisation, although they depend on the microalgae species and pretreatment conditions, namely the applied specific energy. For instance, with a specific energy of 76.5 MJ/kg TS the methane yield of *Scenedesmus* sp. increased by 14%, whereas it increased by 75–88% with a specific energy of 100–130 MJ/kg TS (Gonzalez-Fernandez et al., 2012). Furthermore, the comparison of several physical pretreatments on microalgal biomass grown in wastewater treatment raceway ponds showed how thermal and microwave pretreatments outperformed sonication at 70 MJ/kg TS (Passos et al., 2015).

5.2.2. Microwave irradiation

Similar to sonication, experimental results on microwave pretreatment indicated that pretreatment effect on biomass solubilisation and methane increased with the applied specific energy, regardless of the output power and exposure time. In continuous reactors operating at 20 days HRT, the methane yield was 60% higher after microwave pretreatment (0.27 L CH_4/g VS) as compared to the control (0.17 L CH_4/g VS). Furthermore, optic microscope and TEM images revealed that cell organelles were damaged beyond repair, which possibly improved the anaerobic biodegradability (Passos et al., 2014b).

5.3. Biological pretreatment

Biological methods are a promising alternative to energy-consuming pretreatments. Moreover, enzymatic pretreatment does not involve inhibitory compounds. Hydrolytic enzymes convert compounds of the microalgae cell wall, such as cellulose and hemicellulose, to compounds with lower molecular weight, which are more readily available for anaerobic bacteria. The most important parameters influencing the pretreatment effect are the enzyme dose, temperature and pH, which are usually set within the optimal activity range of each enzyme, along with the exposure time. To date, literature in this field is very scarce. The sole study carried out in continuous reactors evaluated the effect of protease on *Chlorella vulgaris*. The authors reported 45% COD solubilisation, 77% nitrogen mineralisation and 2.8-fold methane yield increase in a continuous stirred tank rector (CSTR) operated at 20 days HRT compared to untreated microalgae (Mahdy et al., 2015). However, batch tests showed that the effect of an enzyme mix was better than that of single enzymes, which was attributed to a chain behavior, where the hydrolysis of one compound enhanced the bioavailability of another one that could then be hydrolysed (Ehimen et al., 2013). This should be further evaluated in continuous reactors.

5.4. Chemical and thermo-chemical pretreatment

Chemical pretreatments have been by far less investigated than thermal and mechanical ones. Acid and alkali reagents are commonly used to solubilise polymers, favoring the availability of organic compounds for enzymatic attack. However, some solubilised compounds might induce the formation of potentially toxic by-products for methanogens. So far, there are no studies using chemical methods prior to microalgae anaerobic digestion in continuous reactors. Regarding batch tests, mostly alkali pretreatments have been applied to microalgae, often combined with heat. For instance, BMP tests under different NaOH concentrations (from 0 to 21 g/L) showed that alkali pretreatment was ineffective for several microalgae species (*Chlorella* sp., *Nannochloropsis* sp., *Thalassiosira weissflogii*, *Tetraselmis* sp., and *Pavlova_cf* sp.), while thermochemical pretreatment at 121 °C for 30 min increased the methane yield of *Chlorella* sp. and *Nannochloropsis* sp. by 30–40% (Bohutskyi et al., 2014).

6. Comparison of pretreatments and feedstocks

Pretreatments may be beneficial to improve the methane potential and/or the digestion rate of a wide range of feedstocks. However, pretreatment techniques must be economically feasible and environmental friendly, so they should have low energy, chemicals and water requirements. Bearing in mind that all pretreatments have strengths and weaknesses, the most appropriate technique will depend on the characteristics of each feedstock (Table 6).

Thermal pretreatments involve heat and also electricity if dewatering is required, as for steam explosion. Nevertheless, they can lead to positive energy balances due to the increased biogas production, and the heat needed is produced on-site through biogas combustion. Indeed, these processes have been implemented at full-scale for sewage sludge, municipal solid wastes and animal by-products, providing also sanitation. Mechanical pretreatments are quite diverse, but in general they are less sensitive to substrate

Table 6
Comparison of pretreatment methods for improving biogas production.

Pretreatment	Control parameters	Increase of biogas production	Strengths	Weaknesses	State of the art
Thermal					
Low temperature	Temperature Exposure time	√√	Low energy demand Scalability Sanitation	High exposure time	Promising for algae
Hydrothermal	Temperature Exposure time	√√	Scalability Sanitation	High heat demand Risk of recalcitrant compounds formation	Full-scale applications for animal by-products sanitation
Steam explosion	Temperature Exposure time Pressure	√√√	Scalability Sanitation	High heat and electricity demand Dewatered biomass Risk of recalcitrant compounds formation	Full-scale applications for sludge, municipal solid wastes
Mechanical					
Ultrasound	Power Exposure time	√√	Particle size reduction Scalability No risk of recalcitrant compounds formation	High electricity demand	Full-scale applications for sludge
Microwave	Power Exposure time	√√	Particle size reduction No risk of recalcitrant compounds formation	High electricity demand Scalability	
High pressure	Pressure	√			Full-scale applications for sludge
Grinding/ Maceration/ Pulping	Power Exposure time		Ease feedstock management No risk of recalcitrant compounds formation	High electricity demand	Full-scale application for lignocellulosic biomass
Chemical and thermochemical					
Chemical	Chemical dose Exposure time	√	Low energy demand	Chemical contamination Risk of inhibitors formation Cost	
Thermo-chemical	Chemical dose Exposure time Temperature	√√	Lower energy demand than thermal alone	Chemical contamination Risk of inhibitors formation Cost	Promising for lignocellulosic biomass and animal by-products
Biological					
Enzymatic	Enzyme dose Temperature pH Exposure time	√	Low energy demand Scalability	Enzyme-substrate specificity Cost	Addition in full-scale plants
Fungi	Fungi strains Exposure time	√	Low energy demand Scalability	Carbon losses High exposure time	Promising for lignocellulosic biomass
Composting/aerobic	Exposure time	√	Low energy demand Scalability	Carbon losses	Full-scale application in manure dry anaerobic digestion
Ensiling	Exposure time		Low energy demand Scalability		Storage method for energy crops

specificities than other methods, and there is no inherent risk of recalcitrant compounds or inhibitors formation. However, they all involve high electricity consumption. Full-scale devices are already used for sewage sludge and lignocellulosic biomass, although eventually only part of the flow rate may be treated in the inlet or in a recirculation loop. Chemical and thermochemical pretreatments may lead to a moderate increase in biogas production, but they involve chemical contamination and risk of recalcitrant compounds formation, in addition to the chemical cost. For these reasons they are not preferred when other alternatives are readily available. Nonetheless, the thermochemical one has shown promising results at lab-scale for lignocellulosic biomass and animal by-products, providing also sanitation. Biological pretreatments are among the least energy consuming, while the may lead to a moderate increase in biogas production. They involve the activity of specific enzymes, fungi and/or bacteria, requiring relatively long exposure times, with the risk of carbon loss. Enzymes may be costly, but implementation in full-scale facilities seems quite straightforward. Composting and ensiling have been implemented at full-scale for manure and energy crops, respectively.

From the feedstock point of view, the most appropriate pretreatment will depend on its nature, composition and structure. Sewage sludge is the most common feedstock in full-scale pretreatment plants, probably because such processes allow both to increase biogas production and to decrease sludge amounts and its management costs. Waste activated sludge is composed of bacteria cells, so methods easing cell disruption are recommended, such as thermal pretreatment with steam explosion, or mechanical pretreatments as sonication and high pressure. Animal by-products require pretreatments able of both physical size reduction and chemical hydrolysis to maximise biogas production. To date, thermal pretreatment and saponification seem particularly appropriate due to the high content of fat and to the simultaneous sanitation. Regarding lignocellulosic biomass, including agricultural residues, energy crops and manure, delignification followed by hemicelluloses and celluloses hydrolysis enhancement should be the goal of pretreatments. The combination of size reduction with delignification by alkali pretreatment or ligninolytic fungi pretreatment during storage seem promising alternatives. Among alkali, aqueous ammonia soaking leads to digestates free of added minerals, which is a great advantage when used as fertilizer. In the case of microalgae, thermal pretreatment seems to be the most promising so far. It should be noted that thermal, mechanical and chemical pretreatments have long been investigated, while biological techniques have received less attention and therefore research efforts should move towards these relatively novel and promising alternatives.

7. Conclusions

When properly designed, pretreatments may enhance the methane potential and/or anaerobic digestion rate of particulate feedstocks. Sewage sludge pretreatment has been implemented at full-scale, particularly the thermal pretreatment with steam explosion which increases the methane potential and digestion rate, ensures sludge sanitation and consumes on-site produced heat. Regarding fatty residues, saponification is preferred for enhancing their solubilisation and bioavailability; which can be optimised for sterilising animal by-products. Lignocellulosic biomass requires first delignification, followed by hemicellulose and cellulose hydrolysis, being alkali or biological (fungi) pretreatments most promising. In the case of microalgae, thermal pretreatment seems the most promising technique so far.

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