Pretreatment of feedstock for enhanced biogas production

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1 Introduction

Anaerobic digestion (AD) is a well-established process for renewable energy production in which biomass (also referred to here as substrate or feedstock) is broken down and converted to biogas (a mixture of methane, carbon dioxide and traces of other gases) by microorganisms.

Commonly used substrates for biogas production include industrial waste such as dairy waste, agricultural waste such as fodder residue and manure, and energy crops such as maize (corn). The ability to make biogas out of many different substrates is one of the main advantages of anaerobic digestion over other processes like ethanol production. However, some substrates can be very slow to break down (so that biogas is produced) because:

- *they contain chemicals that inhibit the growth and activity of the microorganisms,*
- *they create physical problems like floating, foaming or clumping, and block impellors and pipes in biogas plants, or*
- *their molecular structure is poorly accessible to microorganisms and their enzymes (for instance because of their highly crystalline structure or low surface area).*

Sometimes all these problems occur at once. Pretreatment can be used to overcome some of these problems. This brochure mainly focuses on substrates with poorly accessible molecular structures (i.e. lignocellulosic substrates), which include many agricultural residues such as maize leaves, some industrial residues such as brewers' spent grains, and some energy crops such as switchgrass. Some emerging biogas substrates also come under this category, such as oil palm empty fruit bunches (EFB). Pretreatment technologies that are used for other substrates such as sewage sludge, but not for lignocellulose, are covered briefly in section 8.

In biogas substrates, the main sources of methane are sugars and other small molecules. In plants (lignocellulosic substrates) these small molecules come from the breakdown of starch, cellulose and hemicellulose. While starch (α-1-4 linked D-glucose) is relatively easy and quick to break down biologically, cellulose (β-1-4 linked D-glucose) and hemicellulose (a polymer of various sugars and uronic acids) are used to maintain the structure of the plant, and are, by necessity, difficult and slow to break down. The breakdown of cellulose and hemicellulose is further complicated by the bonds between different cellulose chains (termed cellulose crystallinity) and by the presence of lignin, another polymer which slows down the breakdown process (see Figure 1). It is generally believed that lignin cannot be degraded by anaerobic bacteria, although this has been challenged (DeAngelis et al., 2011), and may even inhibit the degradation of other substances like cellulose. Pectin also affects breakdown, binding cellulose fibrils together and binding plant cells together (Carpita and Gibeaut, 1993). Breaking down this lignocellulose complex is the key to biogas production (Noike et al., 1985).

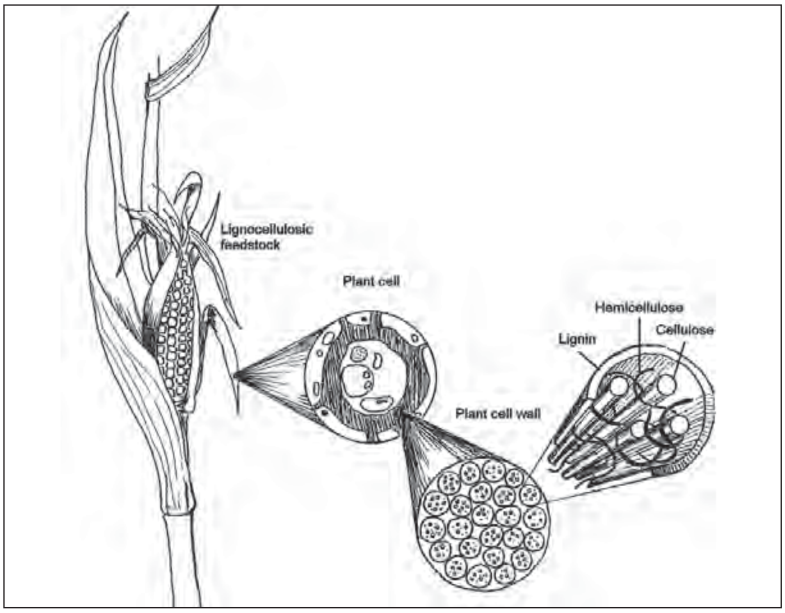

Figure 1: Structure of the lignocellulose complex in plant cell walls

2 Methods used to assess pretreatment

Various pretreatment technologies have been developed in recent years to increase the availability for AD of sugars and other small molecules in biogas substrates, particularly in lignocellulosic material. These pretreatment technologies aim to:

- *make AD faster,*
- *potentially increase biogas yield,*
- *make use of new and/or locally available substrates, and*
- *prevent processing problems such as high electricity requirements for mixing or the formation of floating layers.*

Many of these technologies have been developed by the wastewater treatment or bioethanol industries.

The aim of this brochure is to describe different pretreatment technologies and to discuss their positive and negative aspects with respect to different substrates for AD. Due to the wide range of different technologies and information from different providers, this brochure does not give detailed information about specific costs.

There are many different types of pretreatment, and they can be divided up into the principles by which they function (Table 1).

Before discussing the different pretreatment methods it is important to note how these pretreatment methods are assessed. There are different ways to study the effect of substrate pretreatment on AD (see Figure 2), from laboratory-scale experiments to trials at full-scale biogas plants. A lot of information can be obtained from lab-scale experiments but to prove that a pretreatment method is effective under real conditions, it must be tested at full-scale biogas plants. This is mainly because the equipment used for pretreatment at large scale is not the same as the equipment used at lab scale. Another factor is that reported methane yields may be theoretical values obtained from chemical analysis or batch tests, and methane yields under real conditions could be different because factors like altered pH and accumulation of toxic compounds are not taken into account with these methods.

In general, differences in the microbial community in different biogas plants and inocula can mean differences in reported biogas yield results. Differences in the same substrate cultivated or produced under different conditions can also cause differences in reported biogas yields.

Table 1: Overview of different pretreatment principles and techniques

Principle	Technique
Physical	Mechanical
	Thermal
	Ultrasound
	Electrochemical
Chemical	Alkali
	Acid
	Oxidative
Biological	Microbiological
	Enzymatic
Combined processes	Steam explosion
	Extrusion
	Thermochemical

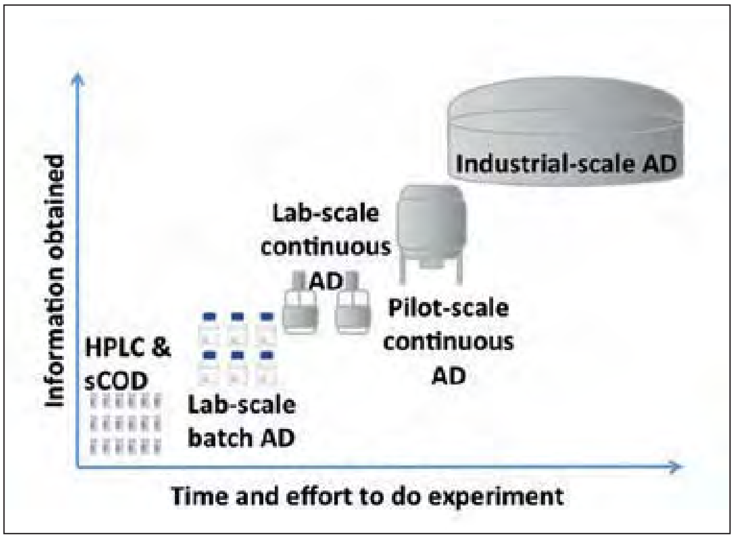

Figure 2: Overview of the experiments commonly used to compare biogas production from pretreated and untreated substrates

The most informative test is full-scale anaerobic digestion, but even at full scale it is difficult to assess whether or not a pretreatment method increases biogas yield as there can be significant variation between different biogas plants.

It is important to note that information for most pretreatment technologies is obtained from laboratory-scale studies or from claims by the company selling the technology.

It will be stated throughout this brochure if pretreatment technologies have been studied at large scale, at small scale in continuous or batch digestion, or only with high-performance liquid chromatography (HPLC) or soluble chemical oxygen demand (sCOD) analysis.

Box 1: Chemical analysis

The fastest methods to study the effect of pretreatment are analytical chemistry methods including high-performance liquid chromatography (HPLC), structural carbohydrate determination, and soluble chemical oxygen demand (sCOD). These methods determine how much the lignocellulose has broken down on a chemical level. These values can then be used to calculate theoretical methane yields. However, greater lignocellulose breakdown does not necessarily translate into greater biogas production because substances that inhibit methane production can also be produced during pretreatment. Theoretical methane yields calculated from sCOD or HPLC values must be viewed with caution.

Box 2: Batch test

A very common technique used to investigate pretreatment is the biomethane potential test (standard method under development by IWA, currently many different methods, e.g. DIN 2006; ISO 1995) also called a BMP test, a batch test or cumulative biomethane production test. This method gives information about the amount of biogas produced and its production rate. However, this method can be interpreted differently, depending on the duration of the batch test (as visible in Figure 3) and the inoculum used.

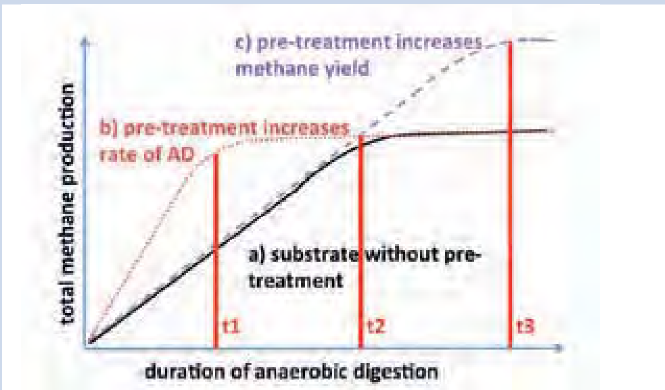

Figure 3: A pretreatment method can increase the rate of anaerobic digestion (pretreatment b) or can increase the methane yield (pretreatment c). Both effects will improve the running of a biogas plant. However, depending when a BMP test is ended, different interpretations are possible (t1: pretreatment b doubles the methane yield; t2: none of the pretreatment methods increase methane yield; t3: pretreatment c increases the methane yield by 25% but pretreatment b has no effect).

Box 3: Continuous AD

Batch AD does not always correlate with continuous AD, because during continuous AD, microorganisms have more time to adapt to new substrates or inhibitors and inhibitors have more time to accumulate from bacteriostatic to toxic levels. For more information about the long-term effects of pretreatment on AD, laboratory-scale and pilot-scale continuous AD (e.g. VDI 2006) can be carried out. However, not all laboratories are equipped with larger digesters and these experiments are time-consuming.

3 Mechanical pretreatment

Mechanical pretreatment is carried out by mills and either makes the pieces of substrate smaller or squeezes them to break open the cellular structure, increasing the specific surface area of the biomass. This gives greater possibility for enzymatic attack, which is particularly important for lignocellulosic substrates. Particle size reduction not only increases the rate of enzymatic degradation, it can also reduce viscosity in digesters (thus making mixing easier) and can reduce the problems of floating layers. All particle size reduction is helpful, but a particle size of 1 to 2 mm has been recommended for effective hydrolysis of lignocellulose (Schell & Harwood, 1994). A major disadvantage of mechanical pretreatment is that mills can be damaged by inert materials in the substrate such as stones or pieces of metal, and equipment repairs can be very expensive.

Scientific literature divides mills into hammer or knife mills, depending on whether they grind or cut the substrate (Figure 4), but in practice many industrial-scale mills work by a combination of cutting and grinding. Literature also limits classic shredders and hammer mills to biomass with a moisture content of under 15% (Kratky and Jirout, 2011; Taherzadeh and Karimi, 2008), but many industrial shredders use substrates with much higher moisture contents. There is a significant difference between lab-scale research and industrial-scale mills. Most published research into the effect of milling on biogas production has been carried out at lab scale with batch AD tests. However, large-scale mills are already used in many biogas plants treating wastes, typically with the aim of reducing processing problems with very fibrous, bulky or inhomogeneous substrates. Extruders, which combine mechanical and thermal pretreatment, can be found in section 6.2.

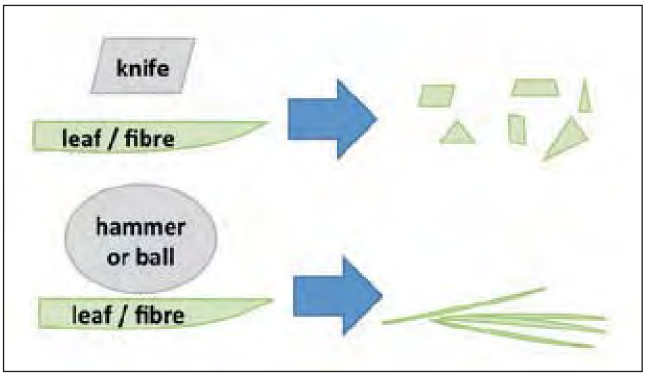

Figure 4: Difference between knife and hammer milling. Knife milling slices the fibres and typically produces small pieces (similar to chopping with a knife). Hammer milling grinds the fibres and typically produces long thin fibres (similar to a mortar and pestle).

3.1 Knife mills and shredders

Knife mills or shredders cut or shred the substrate. Figure 5 shows an example of an industrial-scale shredder.

Menind and Normak (2010) used dried hay from different sources (including from a nature reserve where the hay was harvested once a year) and milled with a laboratory knife mill. They found an approximately 10% higher gas yield was achieved after knife milling hay to 0.5 mm compared to 20 to 30 mm. Another study showed that knife-milling sisal fibres from 100 to 2 mm achieved an approximately 20 to 25% higher gas yield (Mshandete et al., 2006). Both of these results were obtained from batch tests at laboratory scale.

The energy demand for knife milling increases with higher moisture content, larger initial particle size and smaller final particle size. Knife mills are typically not suitable for substrates containing stones or metal that might damage the knife blades.

Figure 5: Example of a shredder called CRAMBO by Komptech GmbH, Austria (image from Komptech).

3.2 Hammer mills and other systems

Hammer mills have an energy demand of roughly 2 to 5 times that of knife mills (Kratky and Jirout, 2011) but are relatively easy and cheap to operate, and are less easily damaged by stones etc. Figure 6 shows an example of a hammer mill used at industrial scale, and Figure 7 shows an industrial cross-flow shredder, which has a cutting and grinding effect.

A study by Menardo et al. (2012) showed that for some substrates, such as barley and wheat straw, mechanical pretreatment increased methane yield, but not for maize stalks or rice straw. They calculated that for wheat and barley straw, the energy gained from the increased methane yield justified the energy used during milling. They made this calculation using literature values for the electricity demand of large-scale straw shredders or straw hummer grinders and using the increased methane yield in their lab-scale knife mill experiments.

More tests at full scale are required to determine whether or not the electricity input for milling is justified by the electricity saved by improved mixing. However, this milling pretreatment is recommended for very bulky substrates to ease processing.

Other mill types, such as ball mills, are currently not commonly used at industrial scale for biomass. Colloidal mills are also not currently used for biomass. These mills might in the future find uses with emerging substrates like algae.

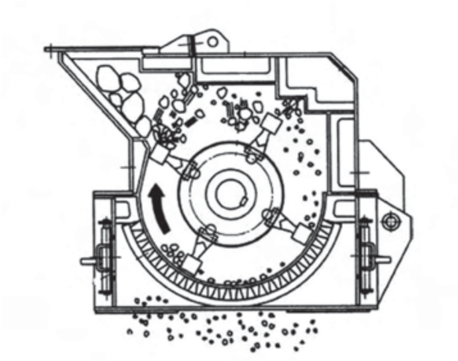

Figure 6: Schematic diagram of a hammer mill (Kratky and Jirout, 2011). Biomass is fed in above and hammers rotate and grind the substrate. Ground particles fall out at the bottom.

Figure 7: Example of a mechanical pretreatment unit called "Querstromzerspaner" (sometimes called cross-flow shredder) by MeWa Recycling Maschinen und Anlagenbau GmbH, Germany (a). Inside the chamber of the machine, a thick metal chain (b) spins around and the impact of the chain on the fibres causes them to break (cutting and grinding). This unit is installed at an agricultural biogas plant using grass silage as a main substrate. Unmilled (left), partially milled (middle) and fully milled grass silage (right) (images a and c by Siegfried Legath, image b from ANDRITZ Mewa).

4 Thermal pretreatment

In pure thermal pretreatment, the substrate is heated (typically 125 to 190 °C) under pressure and held at that temperature for up to one hour. In the laboratory, this can be carried out with pressure cookers, autoclaves or microwave heaters. Dry substrates need additional water before thermal treatment. The presence of heat and water disrupts the hydrogen bonds that hold together crystalline cellulose and the lignocellulose complexes, causing the biomass to swell (Garrote et al., 1999). Thermal pretreatment is often carried out with chemicals or in combination with mechanical shearing (see section 6).

One example of thermal pretreatment at large scale is TDH (from the German "Thermo-Druck-Hydrolyse" sometimes known as "thermal hydrolysis" in English, see Figure 8) developed at ATZ Entwicklungszentrum in Germany. In this process, substrates such as kitchen waste are diluted to about 10 to 15% dry matter and interfering material like plastics are skimmed off the surface (Schieder et al., 2000). If the substrate is bulky, it is crushed and then placed in the TDH reactor. The reactor is put under a pressure of 20 to 30 bar, and at a temperature of 170 to 200 °C for 20 minutes. Heat is recycled in this process as it can be recovered from the material leaving the reactor and also from the exhaust gas of the process. The company claims increased biogas yields of 20 to 30% for energy crops at large scale, and significantly shorter residence times (Dinglreiter, 2007).

Figure 8: TDH unit for thermal pretreatment from ATZ (photo and unit by ATZ, Germany).

Thermal pretreatment is only effective up to a certain temperature (see Box 4). The maximum temperature varies with different substrates and using batch AD tests has been found to be 175 °C for sludge (52% increase in methane production) (Distefano and Ambulkar, 2006), 190 °C for crops (Dinglreiter, 2007), and 160 °C for brewers' spent grains (Bochmann et al., 2010). However, these values are dependent on pretreatment retention time.

Thermal pretreatment is also possible with microwaves, although for lignocellulosic substrates the microwaves are used to heat a surrounding water bath and are not used to heat the lignocellulose directly. To our knowledge, microwave pretreatment has not been carried out at large scale, presumably due to high costs.

Overall, thermal pretreatment is less effective than thermochemical pretreatment (section 6.3) but it has the advantage that chemicals do not need to be bought or taken into account during the subsequent AD phase. Another advantage is for kitchen waste in countries that require all kitchen waste to be sanitised with heat. The conditions inside the TDH reactor ensure that the material is sanitised (Schieder et al., 2000). Thermal pretreatment is particularly well suited to locations where there is a supply of waste heat, for example from a nearby factory or power plant.

Box 4: Inhibitory products formed during thermal pretreatment

Many studies (Bochmann et al., 2010; Distefano and Ambulkar, 2006; Zhang et al., 2011) show that thermal (including thermochemical or thermomechanical) pretreatment only increases biogas yield up to a certain temperature, above which biogas production decreases. Therefore, the trick with all pretreatment involving high temperatures is to find the optimum conditions that break down the substrate.

At very high temperatures, certain dark-coloured xylose and lignin breakdown products are formed. These compounds include heterocyclic and phenolic compounds (such as furfural). Although it is known that these compounds are toxic to yeasts, it is not entirely clear if they are toxic to all AD microorganisms or if they are simply very difficult to degrade anaerobically. There is evidence to suggest that they inhibit AD microorganisms (Boopathy, 2009), but there is also evidence to suggest that some AD microorganisms can break down these compounds (Barakat et al., 2012).

5 Chemical pretreatment

Chemical pretreatment has been investigated using a range of different chemicals, mainly acids and bases of different strengths under different conditions. The use of temperature and chemicals together is described in section 6.3. To our knowledge, chemical pretreatment is not currently carried out at large scale for biogas production, although it is in widespread use for ethanol production.

5.1 Alkali pretreatment

As previously mentioned, lignocellulosic materials are resistant to hydrolysis due to their structure and composition. Alkali addition causes swelling of lignocelluloses (Kong et al., 1992) and partial lignin solubilisation. Alkali treatment can be carried out with different alkalis, commonly using lime or sodium hydroxide (NaOH).

There have been several reports of alkali treatment being effective for AD. He et al. (2008) showed a significant increase in biogas yield in batch tests using rice straw pretreated with 6% solid NaOH for 3 weeks at ambient temperature. Liew et al. (2011) carried out simultaneous solid-state pretreatment and methanisation using 3.5% NaOH on fallen leaves, and showed that the methane yield increased by 20% during batch tests. These studies demonstrated that alkali pretreatment can increase gas yield from lignocellulose-rich substrates. It is important to note that alkali pretreated substrates have high pH values. These experiments were carried out using small-scale batch tests, but during continuous fermentation, alkali pretreatment leads to salt build up and increased pH. The high salt concentration and the resulting effect on the ammonium-ammonia balance inhibits methanisation (Chen et al., 2008). The pH increase might be beneficial for substrates with low pH or high lipid content (e.g. as demonstrated by Beccari et al. (2001) with olive oil mill effluent and $Ca(OH)_2$).

In general, this pretreatment technology is economically unattractive due to the high costs of alkalis (Chang et al. 1997), but it may be useful for acidic and lignin-rich substrates that could otherwise not be anaerobically digested.

5.2 Acid pretreatment

Unlike alkali pretreatment, acid pretreatment does not disrupt lignin but is thought to work by breaking down hemicellulose and disrupting ether bonds between lignin and hemicellulose (Knappert et al., 1981). Acid pretreatment is typically used in combination with heat, so this is discussed in section 6.3.

5.3 Oxidative pretreatment

Oxidative pretreatment with hydrogen peroxide or ozone affects lignocellulose in a similar way to alkaline pretreatment as it can also break down lignin. Song et al. (2012) recently looked at the effect of hydrogen peroxide and ammonium pretreatment on biogas production from rice straw and found that it more than doubled the biogas production. Pretreatment was carried out at room temperature for a long time (7 days) with concentrations of chemical up to 4% w/w. One possible disadvantage is that introducing more oxygen into the system increases the proportion of CO_2 in the biogas produced. To our knowledge, this pretreatment is also not carried out at large scale, presumably partly due to high costs.

6 Combined processes

Combined processes cannot be categorised as mechanical, thermal or chemical pretreatment because they use a combination of mechanisms. They are typically more effective than the processes using one mechanism, but they are often more complex.

6.1 Steam explosion

Steam explosion makes substrates more digestible by a combination of heating and sudden pressure change. The substrate is heated up in a closed system to a temperature of typically 160 to 220 °C, causing a rise in pressure. After a retention time of around 5 to 60 minutes, the pressure is released abruptly. This sudden drop in pressure causes intracellular water to evaporate very rapidly causing a phenomenon known as steam explosion or phase explosion. These forces rupture cells and their surrounding fibre. Figure 9 shows such a unit for pilot scale tests. The difference between steam explosion and other thermal pretreatments is explained in Box 5.

Bauer et al. (2009) carried out steam explosion of straw and then measured biogas yield in batch tests. They found up to 20% more methane yield from steam-exploded straw than from untreated straw in batch tests. However, Vivekanand et al. (2012) showed that methane yields from steam exploded rape straw were similar to untreated rape straw, although there was an increase in the rate of biogas production (also important, see Figure 3).

Box 5: Is it called thermal hydrolysis, steam explosion or extrusion?

There is some confusion about the difference between thermal (pressure) hydrolysis, steam explosion and extrusion, because all three processes use heat, steam and pressure. Extrusion (see section 6.2) always includes a screw conveyor that has a strong grinding effect on the substrate and also leads to high pressure and temperature being reached in the extruder. Steam explosion (see section 6.1) does not include powerful grinding equipment but involves a sudden pressure drop that causes steam to form very quickly. This steam formation is so quick that it is classified as a phase explosion (in contrast to a chemical explosion). Thermal (pressure) hydrolysis can use steam and pressure but does not involve a steam explosion in the physical sense. Thermal hydrolysis may also include a screw conveyor, but its primary function is to convey a substrate along a tube, not to generate high pressure and heat, and it does not have a strong grinding effect. These terms are occasionally used incorrectly, even in scientific literature and by equipment manufacturers.

One study has been done with continuous AD and steam-exploded straw. Risberg et al. (2013) codigested steam-exploded wheat straw and manure and compared it to codigestion with untreated straw and manure. They found no significant difference between steam-exploded and untreated straw in terms of process stability and methane yields. They also found that the inoculum they recovered from their continuous AD reactors was less effective at breaking down cellulose than the original inoculum that they used (from a biogas plant using organic waste and grass silage). They suggest this could be partially due to the accumulation of inhibitors (generated during the pretreatment).

One of the disadvantages of steam explosion is that, like thermal pretreatment, the long retention times and high temperatures can actually decrease the methane yield. Another negative aspect is that only limited recovery of heat is possible from this pretreatment.

One of the advantages of steam explosion is that it may allow new substrates like straw to be used for biogas production.

Figure 9: Pilot-scale steam explosion unit by CAMBI, Norway (photo by University of Natural Resources and Life Siences, Vienna, Division of Agricultural Engineering).

6.2 Extrusion

Extrusion is a process adapted from other industries such as the plastic-processing industry, where material is subjected to high shear, temperature and pressure. In an industrial extruder, the material is fed into the extruder and conveyed by screw along a tube, where it is exposed to high pressure, temperature and shear forces. In the plastic-processing industry, the material is subsequently pushed out of a hole of a specific shape to form the final product, which could be a pipe or a sheet. Biogas substrates in extruders are subjected to the same forces, causing tough fibres to break. The sudden drop in pressure as the substrate leaves the extruder might also help substrate breakdown. The difference between extrusion and other thermal pretreatments is explained in Box 5.

Depending on the final consistency required, the substrate can be placed under a pressure of up to 300 bar at temperatures from 60 to 300 °C (60 to 70 °C generated by friction, higher temperatures if a heater is used). Extrusion effectively breaks open the cell structure of biomass which results in faster methane production, which in turn facilitates higher organic loading rates (Lehmann, 2011). The appearance of unextruded and extruded wheat straw is shown in Figure 10.

Hjorth et al. (2011) investigated the effect of extrusion on batch methane yield of the following substrates: straw, fresh (unensiled) grass, solid fraction of manure from screw press, solid fraction of manure after flocculation, and deep litter from cattle. They showed that biogas production from extruded material was faster than from untreated material, most significantly for straw.

Approximately 10 to 15 kW of power is needed for extrusion per tonne of substrate. This is a similar value to the parasitic electrical demand of a continuous stirred-tank reactor (CSTR) digesting slurry (Murphy & McCarthy 2005). The high electricity cost is a disadvantage of this process.

A major problem with extrusion pretreatment technology is the screws, which have to be changed after a few months due to abrasion. As with other mechanical pretreatment technologies, stones or metallic materials in the substrates severely reduce the life time of the screws. This has a negative impact on the economics of the extrusion process.

Figure 10: Unextruded (left) and extruded (right) wheat straw (photo by Ludek Kamarad).

6.3 Thermochemical pretreatment

Different kinds of bases and acids can be used in thermochemical pretreatment (ammonia or solvents can also be used, in theory). Thermochemical pretreatment at temperatures from 60 to 220 °C have been investigated. As with other pretreatments involving heat, temperatures of more than around 160 °C, particularly in combination with acids, show a drop in methane production, depending on input material (Delgenès et al., 2000; Distefano and Ambulkar, 2006; Penaud et al., 1999).

Acid pretreatment of cassava with heating was investigated by Zhang et al. (2011). A 57% higher gas yield was found during batch AD for pretreated cassava, when compared to untreated cassava, using 160 °C, 3% H_2SO_4 and 20 minutes retention time.

The influence of thermal, chemical and thermochemical pretreatment on dewatered pig manure was analysed by Rafique et al. (2010). High concentrations of lime (5%) showed maximum enhancement of gas yield at 70 °C, much better than lime alone or heat alone. An increase of 78 % biogas was observed during batch AD tests.

Monlau et al. (2012) compared the effect of different chemicals and temperatures on methane yields from sunflower stalks. They used batch tests and found that pretreatment with heat alone was not very effective, but that pretreatment with H_2O_2 or NaOH (4 g / 100 g total solids) did increase methane yield by about one third at 55 °C (rather than 30 or 80 °C). They found that this pretreatment solubilised lignin. Pretreatment with HCl at 170 °C increased methane yield by around 20% and solubilised hemicellulose but not lignin.

Thermochemical pretreatment with acids below 160 °C could be very useful for recalcitrant, lignocellulose-rich substrates, provided the energy needed can be offset by the energy gained. Thermochemical pretreatment with alkali at around 50 °C could be useful for lignin or phenol-rich substrates. Although thermochemical pretreatment has been tried at pilot scale several times, to our knowledge there is currently no example of large-scale thermochemical pretreatment of substrates for biogas production.

7 Biological pretreatment

Silage making (ensiling) is sometimes referred to as a pretreatment technology, but it has a limited effect on AD. Ensiling is predominantly carried out for storage reasons and not to increase the rate of biogas production. Although some studies show that ensiling increases the methane yield from certain crops (Pakarinen et al. 2011), other research (Kreuger et al., 2011) has shown that this is due to a very widespread and underreported calculation error and that ensiling actually has a minimal effect on methane yield. For this reason, ensiling will not be addressed further in this brochure.

The general advantages of biological pretreatment over chemical or thermal pretreatment is that biological pretreatment can take place at low temperature without using chemicals. One disadvantage is that it can be slower than non-biological methods.

7.1 Anaerobic microbial pretreatment

Anaerobic microbial pretreatment, also known as pre-acidification, two-stage digestion or dark fermentation, is a simple kind of pretreatment technology in which the first steps of AD (hydrolysis and acid production) are separated from methane production. While the pH during methane production must be between 6.5 and 8, the pH value of the first digester (the pre-acidification step) should lie between 4 and 6, which inhibits methane production and causes volatile fatty acids to accumulate (Deublein and Steinhauser, 2010; Thauer, 1998).

Microbiological pretreatment can speed up the degradation rate of substrates in AD. In general, cellulose-degrading, hemicellulose-degrading and starch-degrading enzymes work best between pH 4 and 6 at temperatures from 30 to 50 °C, so the pre-acidification step increases the degradation rate by creating an optimal environment for these enzymes. For example, Liu et al. (2006) achieved an additional biogas yield of 21% using two-stage continuous AD of household waste at a hydraulic retention time (HRT) of approximately 30 days.

Another positive effect of this pretreatment method is on the methane concentration in the biogas. In addition to H_2 and volatile fatty acids, CO_2 is formed during the pre-acidification step. CO_2 can be present in three forms: at higher pH values it is present in the form of the carbonate ion CO_3^{2-}, at neutral pH as HCO^{3-} and in acidic environments as CO_2. Due to the low pH, most of the carbonate is in the form of CO_2, which is volatile and is released into the hydrolysis gas produced from the pre-acidification step. This means there is less CO_2 in the gas phase of the methanogenesis step, and therefore a higher CH_4 concentration is obtained. Nizami et al. (2012) produced biogas with 71% methane in a two-stage continuous AD system digesting grass silage, as compared with 52% methane content in a single-stage continuous AD system with the same grass silage.

Another advantage of two-stage digestion is that the microorganisms of the first stage are less sensitive to many chemicals (such as phenols, ammonia, etc) than the microorganisms of the second stage, and many inhibiting chemicals can be broken down in the first stage.

At large scale, pre-acidification systems are offered by several biogas plant providers, varying from continuous to batch pre-acidification systems (see Figure 11). Fresh substrate is fed into a CSTR, and material is removed daily to feed a second CSTR. Plug-flow reactors are also available, and have the advantage that a specific retention time can be guaranteed, unlike in a CSTR. Leach-bed reactors have also been investigated (Lehtomäki et al., 2008; Nizami et al., 2011), where the solid waste (such as grass silage or organic household waste) is pre-acidified and only the leachate (hydrolysis juice) is fed into the anaerobic digester. The remaining solid fraction must then be disposed of, for example by composting.


Figure 11: Two stage digestion from AAT GmbH, Austria, and enbasys/BDI, Austria.

Overall, two-stage digestion is useful for a range of different substrates and higher investment costs for an additional reactor are typically offset by faster digestion rates (due to optimised pH and temperature for the hydrolytic enzymes) and the added stability of feeding with a constant pH. In addition, higher gas methane yields might lead to lower gas upgrading costs. This reactor set-up is used at full scale but is not yet very common.

7.2 Aerobic microbial pretreatment

Aerobic microbial pretreatment can be carried out with naturally occurring mixed cultures. The concept behind this pretreatment is that some aerobic organisms produce cellulose, hemicellulose and/or lignin degrading enzymes rapidly and in large amounts, and these solubilise the substrate. As with anaerobic microbial pretreatment, the pH and temperature in the pretreatment reactor is well-suited to hydrolysis enzymes, and the microorganisms present can break down chemicals that might otherwise inhibit methanogenesis in the anaerobic digester.

Mshandete et al. (2005) used aerobic pretreatment to treat sisal fibres. The pretreatment was carried out in flasks (aerated by shaking) using aerobic sludge as an aerobic and then anaerobic inoculum. To prevent acidification during pretreatment, they added sodium bicarbonate as a buffer. Their batch tests showed that methane yields could be increased 26% using aerobic pretreatment for 9 hours, although 6 and 12 hours also showed good results. Longer pretreatment led to aerobic breakdown of the substrate into CO_2 and, as a result, pretreatment for 48 or 72 hours decreased methane yield.

Although the process described by Mshandete et al. (2005) has not been carried out at large scale, one example of an integrated aerobic-anaerobic pretreatment process at large scale is the ISKA® Percolation system used in the Global Renewables UR-3R Process® (Global Renewables, 2014). In this process, the organic fraction of municipal solid waste is fed into an aerated leach bed reactor. The percolate (hydrolysis juice) is collected and fed into an anaerobic digester. The remaining undegraded solid fraction can be disposed of, for example by composting. This process is similar to the anaerobic leach bed system described by Lehtomäki et al. (2008) and Nizami et al. (2011), but with aerobic conditions in the leach bed reactor.

The major advantage of these leach bed reactors (aerobic or anaerobic) is that there are no processing problems due to fibres or large chunks in the anaerobic digester. Although the anaerobic digester can be fed continuously with these set-ups, the disadvantage is that the leach bed reactors need to be emptied (not usually a continuous process) and the solid fractions needs to be disposed of. There have been no studies comparing anaerobic microbial pretreatment to aerobic microbial pretreatment. In general, the advantage of an aerobic process is that it is considerably faster, but the disadvantage is that a lot of the organic matter that could be degraded to methane is instead degraded to CO_2 if the pretreatment phase is too long. Although anaerobic processes are slower, more of the organic matter enters the anaerobic digester. It is possible to combine the two processes, for example with microaeration in an anaerobic pretreatment reactor, which has been reported to increase methane yields significantly (Jagadabhi et al., 2010; Jenicek et al., 2008; Johansen and Bakke, 2006).

7.3 Fungal pretreatment

Many fungi, particularly white-rot fungi, are known for their ability to remove environmental pollutants from solid and liquid waste (Barr and Aust, 1994; Reddy, 1995). In the context of anaerobic digestion, these pollutants could either inhibit anaerobic digestion or cause problems during digestate use. Fungal pretreatment has been investigated as a method to remove phenolic toxins from wastewater before anaerobic digestion (Dhouib et al., 2006; Hodgson et al., 1998). It has also been used to detoxify coffee cherry husks for anaerobic digestion (Jayachandra et al., 2011). There are currently several publications on solid waste detoxification with white rot fungi for use as animal feed or simply for safe disposal, and it is likely that in the future there will be more research on solid waste detoxification before anaerobic digestion.

Treatment of straw with white-rot fungi that degrade lignin has also been investigated as a pretreatment for anaerobic digestion (Ghosh and Bhattacharyya, 1999), but most research has been aimed at producing animal feed (Moyson and Verachtert, 1991) and bioethanol (Salvachúa et al., 2011). There has also been some research on fungal pretreatment of waste to increase biogas yields (Wagner et al., 2013). It is not clear what effect fungal pretreatment has on biogas yields, because although white-rot fungi can delignify substrates, they also remove some of the organic matter than could be used for anaerobic digestion. To our knowledge, fungal pretreatment has not been carried out at large scale.

7.4 Enzyme addition

Enzymes that break down biomass are already present in anaerobic digesters as they are produced by the microorganisms of AD. To enhance this breakdown, a mixture of enzymes can be added, and may include cellulose-, hemicellulose-, pectin- and starch-degrading enzymes. Enzyme additives can be applied in three different ways: by direct addition to a single-stage anaerobic digester, by addition to the hydrolysis and acidification vessel (first stage) of a two-stage system (see section 7.1 and 7.2), or by addition to a dedicated enzymatic pretreatment vessel.

The addition of enzymes to AD has been analysed in many different studies. There is some evidence to suggest that enzymes added directly to the biogas reactors have no significant effect (Rintala and Ahring, 1994) and are degraded quickly after addition (Binner et al., 2011). Several batch AD studies have indicated that the addition of enzymes to the first stage of a two-stage anaerobic digestion process leads to slightly higher substrate solubilisation (leading to higher biogas yield), such as with cellulases on grass (Romano et al., 2009) or with cellulosic enzyme cocktails on wheat straw (Quéméneur et al., 2012). Some studies showed that enzymatic pretreatment in a dedicated vessel leads to higher substrate solubilisation or biogas yields in batch

AD tests, for example with pectinase on hemp (Pakarinen et al. 2012), pectinase on switchgrass (Frigon et al. 2012), or various agricultural residues with a cellulolytic enzyme cocktail (Suárez Quiñones et al., 2012). Small increases in biogas yield were seen with continuous anaerobic digestion of different agricultural residues pretreated in a dedicated vessel (Suárez Quiñones et al., 2011).

A study by a Swiss (Warthmann et al., 2012) group looked at the effect of 25 different commercially available enzyme preparations including enzyme mixtures marketed to biogas plants as well as pure enzymes normally marketed to other industries. They found that the effect of enzymatic pretreatment on biogas yield from sludge and manure was minimal and speculated that this was because the enzymes were being degraded by the native microorganisms. Some of the enzyme products increased the biogas yield by around 10% in grass silage and green waste silage in batch tests. These enzymes also increased methane concentration in the gas produced in the first week. However, the authors note that the enzyme dosage was so high that it is unlikely to be economically feasible.

Enzyme products for biogas plants are offered by several different companies, but some enzymes have a relatively high price for a limited increase in biogas yield.

Figure 12: Ultrasound substrate treatment unit (Ultrawaves GmbH, Germany)

8 Pretreatment of other substrates

Some pretreatment technologies are available that are not primarily aimed at lignocellulosic material but are effective for other substrates such as sewage sludge.

8.1 Sanitation

Some substrates – such as animal by-products that fall into category II and III of European regulations (1774/2002/EC and 1069/2009/EC) – require hygienisation (1 h at 70 °C) or sterilisation (20 min at 133 °C) before anaerobic digestion. Thermal pretreatment systems such as TDH (see section 4) can also be used for these substrates, and may increase the rate of anaerobic degradation as well as meeting the legal requirements.

8.2 Ultrasound treatment

Ultrasound treatment can be used as pretreatment for sludge or to treat the liquid effluent from anaerobic digesters, for example to aid solid-liquid separation. Ultrasound frequencies (over 20 kHz) cause cavities to form and then implode, producing shockwaves in a process called cavitation. These forces cause the disruption of microbial cell walls in the liquid. In general, this technology is used for treatment of sewage sludge. Ultrasound has been found to only disintegrate microbiological biomass and not lignocellulosic material (Onyeche et al., 2002), although there is some evidence that it improves accessibility to cellulose (Zhang et al., 2013). The destruction of cells sets hydrolytic enzymes free and helps to increase the hydrolysis rate of biomass (Klingspor & Sörensen 2012). Detailed information of the influence on specific chemical bonds has not been published.

Overall, this is a simple technology with low costs that can have a positive influence on sludge-fed biogas plants that are not operating optimally.

8.3 Electrokinetic disintegration

Electric fields are used for a variety of processes in modern biotechnology. Electrokinetic disintegration is mainly used for sewage sludge treatment, where the main inhibiting factor for good AD is the presence of aggregated clumps of microorganisms (flocs) and particles in sludge. The application of an electrical field to sewage sludge disrupts these ionic bonds and breaks the flocs apart (Tyagi and Lo, 2011). It is also likely that electric fields disrupt microbial cells by changing the charge of the cell membranes. It is not clear what effect, if any, this treatment has on lignocellulosic material. The German companies Südchemie and Vogelsang make electrokinetic disintegration devices where the sludge is fed through a section of pipe with an electrode inside that applies a voltage of typically around 30 kV (range between 10 to 100 kV) (Hugo Vogelsang Maschinenbau Gmbh., 2011; Südchemie, 2011). Figure 13 shows such a unit. The companies claim an increased biogas yield from sewage sludge of around 20 % (Südchemie, 2011). Vogelsang claims the device can increase biogas production from agricultural residues (Hugo Vogelsang Maschinenbau Gmbh., 2011), but a study by the Bavarian State Research Center for Agriculture, LfL, showed no significant increase in biogas production from agricultural residues (Lehner et al., 2009). Like ultrasound treatment, electrokinetic disintegration may be better suited to treating the liquid effluent from anaerobic digesters, or to pretreat substrates similar to sewage sludge.

Figure 13: Electrokinetic disintegration unit (photo and unit by Atres, Germany)

9 Advantages and disadvantages

No single pretreatment technology is suitable for all anaerobic digestion systems and substrates. The different pretreatment technologies described above may be better suited to a particular reactor design or size of reactor, as well as the political drivers or economic situation of the region. Table 2 gives an overview of the advantages and disadvantages of the different pretreatment technologies.

Aside from these general advantages and disadvantages, the choice of pretreatment method is strongly dependent on substrate composition. The greatest challenge for pretreatment of biogas substrates is combining the right substrate composition with the right pretreatment technology to increase the bioavailability of the substrate. For example, substrates with very high dry matter content are better suited to milling or extrusion, provided they contain no stones or metal fragments. Substrates with high lignin contents are better suited to alkali pretreatment, provided chemicals are available at low cost and inhibition can be prevented, for example by dilution of inhibitors with untreated substrates. Table 3 gives an overview of the influence of different pretreatment technologies on lignocellulose.

The most important factors for selecting a pretreatment technology are the energy balance and costs. In most cases, pretreatments with a low energy demand have a lower impact on the rate of degradation and corresponding biogas yield compared to pretreatments with high energy input. The wrong choice of pretreatment can make a process uneconomical. As high investment costs are often needed, a correspondingly high increase in gas yield or gas production rate is necessary to make the process financially feasible.

Future prospects for pretreatment technologies

Many principles of pretreatment were and continue to be developed for other purposes, such as ethanol production from lignocellulosic feedstocks. The influence of pretreatment technologies on AD has only been investigated in recent years and there is still a need to optimise these technologies for the biogas sector.

In addition to the primary advantages of pretreatment – increased rate of AD and increased gas yields – there are a range of potential secondary advantages such

Table 2: Advantages and disadvantages of different pretreatment technologies (adapted from Taherzadeh et al. 2008; Hendriks and Zeeman 2009)

Process	Advantages	Disadvantages
Milling	• increases surface area • makes substrate easier to handle • often improves fluidity in digester	• increased energy demand • high maintenance costs / sensitive to stones etc.
Hot water (TDH)	• increases the enzyme accessibility	• high heat demand • only effective up to certain temperature
Alkali	• breaks down lignin	• high alkali concentration in digester • high cost of chemical
Microbial	• low energy consumption	• slow • no lignin breakdown
Enzymatic	• low energy consumption	• continuous addition required • high cost of enzymes
Steam explosion	• breaks down lignin and solubilises hemicellulose	• high heat and electricity demand • only effective up to certain temperature
Extrusion	• increases surface area	• increased energy demand • high maintenance costs / sensitive to stones etc.
Acid	• solubilises hemicellulose	• high cost of acid • corrosion problems • formation of inhibitors, particularly with heat

Table 3: The influence of different pretreatment methods on the breakdown of lignocellulose (adapted from Taherzadeh et al. 2008; Hendriks and Zeeman 2009).
A plus symbol (+) indicates that the pretreatment method has this effect, a minus symbol (-) indicates that it has no effect, and no symbol means it is unclear if there is an effect or not.

Pretreatment method	Cellulose decrystallisation	Hemicellulose degradation	Lignin degradation	Increasing specific surface
Biological				+
Milling	+			+
Steam explosion		+	+	+
Concentrated acid		+	+	+
Diluted acid		+		+
Alkali		-	+	+
Extrusion				+

as smaller volumes of digestate and lower methane emissions from the digestate leading to reduced greenhouse gas emissions. The major secondary effect – if energy crops are used – is the fact that less substrate (i.e. less land) is needed to achieve the same energy production. Further research is needed to determine the extent of these secondary effects.

The investment costs for pretreatment of recalcitrant substrates are high at the moment due to high expenditure in process engineering. However, if with further development these costs are decreased to an affordable level, new non-food or non-feed substrates will be made economically available for biogas production.

Research and development is also needed in the field of reactor design. Currently the CSTR is widespread in biogas sector. It is well-suited to traditional substrates like manure, sludge and some easily digestible substrates. Current pretreatment systems are useful to transform a very fibrous substrate into something resembling manure or maize. This allows lignocellulosic substrates to be used in existing reactors. However, although many companies still sell CSTRs for lignocellulosic substrates, there is much evidence that a different reactor design may be more suitable for lignocellulosic substrates. If the substrate has a very high solid content, dry digester types could be used. For wetter lignocellulosic substrates, a leach bed reactor or a percolator could be used, combined with a high-rate reactor like an upflow anaerobic sludge blanket (UASB) reactor, an anaerobic filter or a hybrid reactor to digest the leachate. If hydrolysis of the fibres is separated from the methane generation from the leachate, then enzymatic, chemical, thermal, or thermochemical pretreatment could be carried out in the same reactor as the biological pretreatment. It is likely that further research will focus on whole-process engineering, where the pretreatment is integrated into the digester, rather than viewing pretreatment as something separate.

In summary, many pretreatment methods are expensive or have a high energy demand and their efficiency is often difficult to prove. However, some pretreatment methods can sometimes stabilise biogas plants that have stability problems (for example by adjusting the pH) and can potentially make new substrates available for anaerobic digestion. Pretreatment is particularly well suited for substrates where the degradable biomass is sterically not available for enzyme attack. Pretreatment is typically not necessary for substrates with high degradation rates. Finally, it should be noted that pretreating all substrates with one technology is not realistic.

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11 Glossary

Anaerobic digestion (AD)

The bacterial degradation of organic substances under exclusion of oxygen. The degradation process is also called biomethanation and delivers biogas, which typically contains around 50 to 70% methane, 20 to 45 % carbon dioxide and some trace gases.

Batch test

A useful laboratory experiment to determine how much methane it is biologically possible to obtain from a substrate.

Chemical oxygen demand (COD)

The amount of organic compounds in a substrate, in other words, the theoretical amount of material that can be converted into biogas. COD also refers to the test used to indirectly determine this value.

Continuously stirred-tank reactor (CSTR)

In the case of AD, this is an anaerobic digester with mixers or impellers where material is fed in and removed so as to maintain a steady-state breakdown reaction inside the tank.

Digestate

The material that is discharged from the digester vessels at the end of the digestion period. It mainly contains material that is difficult to digest including lignin, minerals and remnants of bacteria. Typically the nutrients of the feedstock are conserved in the digestate and as such it is a good fertiliser.

Dry matter (DM) or total solids (TS)

Residual substance after complete elimination (drying) of water, usually given in percent of fresh material.

Fermentation (digestion)

Anaerobic metabolic processes caused by microbial enzymatic activities.

Hydraulic retention time (HRT)

Mean statistical retention time of substrates in a bioreactor.

Lignocellulosic biomass

Plant-derived material where the majority of the mass is made of the structural cell-wall components cellulose, hemicellulose and lignin. Cellulose and hemicellulose are made up of long chains of sugars, and lignin is made up of various cross-linked compounds and contains many aromatic rings. Lignin, cellulose and hemicellulose are bound together in a complex that gives plant material mechanical strength, and is difficult to break down.

Organic dry matter (ODM) or volatile solids (VS)

Total amount of organic matter in a substance.

Retention time (RT)

See HRT.

Substrate

Any substance used for a biological transformation, in this case anaerobic digestion and typically organic waste or energy crop. In this context, the substrate is sometimes referred to as feedstock or biomass.

Yield of biogas

Amount of biogas per unit substrate, typically in Nm^3/kg VS.

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Task 37 – Energy from Biogas

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