Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment

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List of abbreviations:

AFEX: Ammonia fiber explosion
ARP : Ammonia recycle percolation
CrI : Crystallinity index
DM : Dry matter
DP: Degree of polymerization
FPU : Filter paper unit
LCC: Lignin-carbohydrate complex
LHW : Liquid hot water
Lignin G : coniferyl alcohol
Lignin H : p-coumaryl alcohol
Lignin S : sinapyl alcohol
Mw : Molecular weigh
SA : Surface area
VFA : Volatile fatty acids
VS : Volatile solids

Abstract:

Production of energy from lignocellulosic biomass or residues is receiving ever-increasing interest. Among the different processes, dark fermentation for producing biohydrogen and anaerobic digestion for producing biomethane present considerable advantages. However, they are limited by the accessibility of holocelluloses which are embedded in the lignin network. This paper proposes a review of works on the conversion of biomass into biohydrogen and biomethane with the comprehensive description of i) biomass composition and features that may impact on its anaerobic conversion, ii) the impact of different kinds of pretreatment on these features and on the performance of biohydrogen and methane production.

KEYWORDS: Lignocellulosic biomass, anaerobic digestion, dark fermentation, pretreatments, biogas, hydrogen, methane, physicochemical properties

1. Introduction

The development of renewable sources of bioenergy has recently generated considerable interest due to the energy crisis and global warming.

The use of lignocellulosic biomass as a source of bioenergy is particularly interesting on account of its abundance, its renewability and the fact that it does not create competition for land used for food or feed production (Ohman et al., 2006; Kleinert & Barth, 2008). The use of lignocellulosic biomass for the production of the so-called second generation biofuels has recently been widely investigated for the production of liquid biofuels (Gnansounou,

2010; Hromadko et al., 2010; Sims et al., 2010; Sivakumar et al., 2010). The production of biomethane (Frigon & Guiot, 2010) and biohydrogen (Guo et al., 2010) from lignocellulosic biomass also needs to be considered. In fact, biomethane is a versatile energy source because it can be used to produce heat, electricity combined with heat (cogeneration), or biofuel. Compared to most liquid biofuels, biomethane has been shown to have a far better performance with regard to both agricultural land area efficiency and life cycle emissions (Borjesson & Mattiasson, 2008). Biohydrogen can be used in fuel cells to produce electricity or in internal combustion engines. The main advantages to the use of hydrogen as a biofuel are the absence of CO_2 emission, its high energy content and its combustion kinetics (Koroneos et al., 2004). An alternative utilization of hydrogen is as “hythane” gas, a mixture of hydrogen and methane. As a fuel in internal combustion engines, Hythane offers several advantages compared to pure methane. Hydrogen has a flame speed eight times higher than that of methane and the addition of hydrogen to methane decreases the air/fuel ratio, resulting in a more stable combustion than with methane while at the same time lowering emissions levels (Sierens & Rosseel, 2000).

Biohydrogen and biomethane can be produced by dark fermentation and anaerobic digestion respectively (Figure 1). Anaerobic digestion is a biological conversion process in which biomass is transformed into biogas, a mixture of methane and carbon dioxide. The process can also be oriented towards dark fermentation, H_2 producing instead of CH_4, by controlling such operational parameters in the reactor as pH and retention time and by inhibiting methanogenesis (Hawkes et al., 2007; Nath & Das, 2004). The residue of an anaerobic digestion process is known as the digestate, made up of stabilised organic materials which are enriched (Frigon & Guiot, 2010) in nitrogen and phosphorus. The digestate can thus be used as an environmentally-friendly fertiliser for growing biomass (Figure 1).

Lignocellulosic biomass consists of holocelluloses (cellulose, hemicellulose) and lignin fractions (Figure 1), which quantitatively and qualitatively vary according to the plant material (Aman, 1993). Holocelluloses, which are the major component of most lignocellulosic materials, have been shown to be anaerobically biodegradable in their pure form. But hemicelluloses and lignin are covalently bonded through lignin–carbohydrate complexes, as demonstrated several decades ago by Björkman (Björkmann, 1957). These covalent links are important in the biological roles of plant cell walls (mechanical resistance, protection against pathogens) and in the ability of lignocellulosic materials to be transformed in practical application (production of pulp or chemicals, biodegradation, bioethanol, biohydrogen, biogas, etc). Hemicelluloses serve as connections between the lignin and the cellulose fibers and give rigidity to the whole cellulose–hemicellulose–lignin network (Atalla et al., 1993; Laureano-Perez et al., 2005; Salmen & Olsson, 1998). However, lignin content and the lignin-carbohydrate matrix limit the digestibility of lignocellulosic biomass because lignin is a cross-linked network hydrophobic polymer (Monties & Fukushima, 2001; Watanabe et al., 2003) that remains insoluble in all solvents and is fairly resistant to anaerobic degradation. The presence of lignin is apparently the most important factor affecting the biodegradability of lignocellulosic materials although others factors such as the crystallinity of cellulose and accessible surface area may also play an important role (Chang & Holtzapple, 2000; Koullas et al., 1992; Laureano-Perez et al., 2005; Puri, 1984).

Figure 1*: Strategy of biohydrogen and biomethane production from lignocellulosic materials in integrated lignocellulosic biomass production.*

Hence, both yields and rates of biohydrogen and biomethane production from biomass can be enhanced by the pretreatment of lignocellulosic materials. Such pretreatment should make hollocelluloses more accessible to the microorganisms involved in the biological process. The objectives of such pretreatments are thus to dissolve lignin structure (delignification), reduce the degree of polymerization of cellulose and hemicelluloses, decrease the crystallinity of cellulose and increase the available surface area. Many types of pretreatment have been widely investigated for the production of second generation bioethanol and several review papers published (Demirbas, 2005; Galbe & Zacchi, 2007; Galbe & Zacchi, 2002; Kumar et al., 2009; Mosier et al., 2005; Sun & Cheng, 2002). These pretreatments are generally divided into four categories: physical (milling, irradiation...), chemical (alkali, acid, oxidizing agents and organic solvents), thermal (steam explosion, ammonia fiber explosion (AFEX), wet oxidation...), biological and enzymatic, or a combination of two of them. However, the choice of the pretreatment is closely related to the final product. Bioethanol production uses only cellulose and the objective of pretreatment is to separate lignin and hemicelluloses from cellulose in order to enhance enzymatic cellulose hydrolysis, whereas biohydrogen and biomethane production may use both cellulose and hemicelluloses. The application of pretreatments to improve the anaerobic digestion or dark fermentation of lignocellulosic biomass has been less well investigated than their use in bioethanol production. However, the anaerobic digestion of lignocellulosic materials has been shown to be limited by the biological hydrolysis step and the accessibility of biodegradable compounds (cellulose and hemicelluloses) (Pavlostathis & Giraldogomez, 1991). Thus to achieve high biodegradation yields, lignocellulose must first be pretreated.

The objective of this paper is to review previous work dealing with the production of biohydrogen and biomethane from lignocellulosic biomass, with special attention paid to the impact and mechanisms of pretreatment processes. First of all, the composition of

lignocellulosic material is described. In a second part, properties that can impact the biological conversion of biomass (degree of polymerization (DP), crystallinity, accessible surface area, lignin contents) are mentioned. Then, lignocellulosic pretreatments are detailed and their influences on the previously mentioned parameters are discussed. Finally, the association of biohydrogen and biomethane processes are presented, along with the effect of pretreatments on biohydrogen and biomethane production.

2. Composition of Lignocellulosic materials

The composition of biomass depends essentially on the nature of the feedstock (Mosier et al., 2005). Cellulose is most abundant, representing 30-70 % of lignocellulosic biomass while hemicelluloses and lignin represent, respectively, 15-30% and 10-25 % of the biomass (Table 1).

2.1. Cellulose

Cellulose consists of D-glucose subunits, linked by β-(1→4) glycosidic bonds (Fengel, 1992; Fengel & Wegener, 1984). The cellulose in a plant consists of parts with an organized crystalline structure and parts with a poorly organized amorphous structure. The cellulose strains are 'bundled' together and form so-called cellulose fibrils or cellulose bundles. These cellulose fibrils are mostly independent and weakly bound through hydrogen binding (Atalla & Vanderhart, 1984; Liang & Marchessault, 1959; Vanderhart & Atalla, 1984). Cellulose, insoluble in water and most organic solvents, is chiral and biodegradable. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. Many properties of cellulose depend on its chain length, crystallinity or degree of polymerization.

2.2. Hemicelluloses

Hemicelluloses can be any of the heteropolymers (matrix polysaccharides) present in almost all plant cell walls along with cellulose (scheme 2) (Aman, 1993). While cellulose is crystalline, strong and resistant to hydrolysis, hemicelluloses have a random, amorphous structure with little strength. Hemicellulose has a lower molecular weight than cellulose. It has branches with short lateral chains that consist of different sugar monomers and can include xylose, mannose, galactose, rhamnose and arabinose, which are polymers that can be easily hydrolysed (Ebringerová & Heinze, 2000; Fengel & Wegener, 1984; Kacurakova et al., 1999) by both dilute acid or a base as well as by numerous hemicellulase enzymes. Xylose is always the sugar monomer present in the largest amount, though uronic and ferulic acids also tend to be present (Table 1). The dominant component of hemicelluloses from hardwood and agricultural plants such as grasses and straw is xylan, while in softwoods glucomannan is dominant (Ebringerová & Heinze, 2000; Ebringerova et al., 1994; Fengel & Wegener, 1984; Sun et al., 1996). Hemicelluloses are embedded in the cell walls of plants, sometimes in chains that form a 'ground': they bind to cellulose with pectin to form a network of cross-linked fibers made up of hemicelluloses and lignin which are covalently linked through lignin–carbohydrate complexes (LCCs) and, as such, represent a limiting factor in the biodegradation of lignocellulosic materials (Watanabe et al., 2003). The hemicelluloses are the most thermal-chemically sensitive of lignocellulosic components (Levan & Winandy, 1990; Sweet & Winandy, 1999; Winandy et al., 1991). During thermal–chemical pretreatment, the side groups of hemicelluloses react first, followed by the hemicellulose backbone.

2.3. Lignin

Lignin is the third most abundant polymer in nature, after cellulose and hemicelluloses, and is present in cell walls. It is an amorphous heteropolymer consisting of three different phenylpropane alcohols: p-coumaryl (H), coniferyl (G) and sinapyl (S) (Figure 2). The nature and the quantity of lignin monomers (H, G, S) vary according to species, maturity and the space localization in the cell (Yoshizawa et al., 1993). For instance, an increase in lignin content from 3% to 7% was observed during the maturing of grass (Nizami et al., 2009). The biosynthesis process consists, in the main, of the coupling of radicals and creates a unique lignin polymer in each plant species (Figure 2) (Boerjan et al., 2003; Vanholme et al., 2010). There are three main groups of lignins: the lignins from softwoods (gymnosperms) contain mainly guaïacyl units, those from hardwoods (angiosperms) mainly guaïacyl and syringyl units, whereas the lignins from herbaceous plants (non-woody or gramineae) contain all three units (H, G, S) in significant amounts with different ratios (Billa & Monties, 1995; Boerjan et al., 2003; Lapierre et al., 1986; Nimz et al., 1981; Vanholme et al., 2010). They are held together by different kinds of linkage (Adler, 1977; Akiyama et al., 2002; Sarkanen, 1971; Sarkanen, 1971; Sarkanen & Hergert, 1971). These are classified as either carbon-carbon bonds (5-5, β-1, β-5, β-β) or diaryl-ether (4-O-5), both of which are "condensed". They are resistant to usual chemical degradation. Other connections described as "non-condensed" or "unstable" are of the type aryl-ether (α-O-4, β-O-4), the β-O-4 bonds being the most frequent in natural lignins. The main purpose of lignin is to give the plant structural rigidity, impermeability and resistance against microbial attack and oxidative stress. The amorphous heteropolymer is also insoluble in water and optically inactive, which makes the degradation of lignin very difficult (Akin, 2008; Fengel & Wegener, 1984; Grabber, 2005). Like hemicelluloses, lignin normally starts to dissolve in water at around 180°C under neutral conditions (Kubikova et al., 1996). The solubility of lignin in acid, neutral or alkaline

environments depends, however, on the precursor of the lignin (p-coumaryl, coniferyl, sinapyl alcohols or combinations of them) (Grabber, 2005).

***Figure 2**: Simplified scheme of the lignification, supramolecular organisation and composition of plant cell walls "lignocellulosic matrix". Monolignols oxidized by a peroxydase/H_2O_2 system form radicals. The coupling and oxidation of these radicals result in lignin polymer.*

3. Factors affecting accessibility and biodegradability of lignocellulosic materials

The mechanical, physical, chemical and biological properties of lignocellulosic materials are dependent not only on the chemical composition of the matrix but also on the organisation of their constituents and the interaction between them (Salmen & Olsson, 1998). The cell wall may be schematically viewed as cellulose microfibril bundles arranged in parallel in a matrix of amorphous hemicelluloses and lignin, as shown in Figure 1. A considerable amount of work has been carried out to try to determine the substrate characteristics which lead to a decrease in the rate of cellulose hydrolysis and, in many cases, incomplete hydrolysis of the lignocellulosic substrates (Hendriks & Zeeman, 2009; Koullas et al., 1992; Tarantili et al., 1996; Yoshida et al., 2008; Zhu et al., 2008). Most of this work has concerned bioethanol production and focused on the separation of cellulose from lignin and hemicelluloses in order to enhance enzymatic cellulose hydrolysis. However some studies provide insight useful in assessing or understanding the anaerobic biodegradability of lignocellulosic materials. Indeed, the anaerobic digestion of such materials has been shown to

be limited by the biological hydrolysis step as well as by the accessibility of biodegradable compounds (cellulose and hemicelluloses) (Pavlostathis & Giraldogomez, 1991). Thus, to achieve high biodegradation yields or high yields of polysaccharide monomers (glucose and xylose), lignocellulose must first be pretreated. Moreover, biological degradation (anaerobic digestion or enzymatic hydrolysis) can be affected by factors such as the degree of polymerization and crystallinity of the cellulose, the structure of hemicelluloses, structural surface area and pore volume, lignin composition and content and cross linking (LCCs).

3.1. Crystallinity and degree of polymerisation of cellulose

It has been suggested that during the enzymatic hydrolysis of cellulose the readily accessible regions (amorphous regions) are more efficiently hydrolyzed, resulting in an accumulation of crystalline cellulose (Hayashi et al., 2005). Other authors have suggested that the decreasing rate of cellulose degradation occurs as a result of structural transformations during the initial stages of hydrolysis with the result that the more resistant fraction remains unhydrolyzed (Gupta & Lee, 2009; Jeoh et al., 2007; Mansfield & Meder, 2003; Mooney et al., 1999) and as a consequence, cellulase digestibility of the treated biomass is limited by cellulose accessibility. Many properties of cellulose depend on its crystallinity (CrI), molecular weight (M_w), particle size, degree of polymerization (DP), surface area and solubility, all of which depend on the species, plant part, and plant maturity (Table 1). All these parameters have been shown to influence the enzymatic hydrolysis of cellulose. Some work has shown a good correlation between crystallinity and the rate of enzymatic hydrolysis of pure cellulose. However, with lignocellulosic materials this relationship is not so clear-cut, due to the more heterogeneous nature of this material and the contribution of other

components such as lignin and hemicelluloses. (Chang & Holtzapple, 2000; Ciolacu et al., 2008; Gupta & Lee, 2009; Koullas et al., 1992; Lee et al., 2010).

Table1*: Biochemical composition of different lignocellulosic biomass*

3.2. Physicochemical properties of hemicellulose

In contrast to cellulose, the effect of the physicochemical properties of hemicelluloses on the accessibility of lignocellulosic substrates and their biodegradability into biogas and bioethanol has not been studied. Yet, of the total mass in the residues of annual plants which can be fermented to biogas, hemicelluloses (C5-sugars) represent about 20-40%. Hemicelluloses serve as a connection between the lignin and the cellulose fibers and give rigidity to the whole cellulose–hemicellulose–lignin network (Salmen & Olsson, 1998; Watanabe et al., 2003). In general, the dominant hemicelluloses from all plant cell walls are xylans (Table 1). Xylans display significant variability in their structural characteristics. The structure of xylan is more complex than that of cellulose and has been fully described in several reviews on hemicelluloses in wood (Puls, 1997) and grass (Izydorczyk & Dexter, 2008; Izydorczyk & MacGregor, 2000; Puls, 1997; Roubroeks et al., 2000; Saake et al., 2001). Xylan structure depends on the degree of substitution of the xylose linear chains by arabinose, hydroxycinnamic and uronic acids and the molar mass. All these parameters depend on the species, plant part, and plant maturity (Table1) (Aman, 1993; Dervilly et al., 2000; Ebringerova et al., 1994; Izydorczyk, 2009; Saulnier et al., 1997; Saulnier et al., 1999). The type and the distribution of substitution determine the degree of solubility as well as the capacity to bind to the components of the plant cell wall. Ferulic and *p*-coumaric acids (Figure 3C) represent the major cross-linking phenolic acids in the cell walls of grasses (Russel et al.,

2000; Russell et al., 1999; Tuyet Lam et al., 1992; Wallace & Fry, 1994). The presence of ferulic and *p*-coumaric acids further increases the chemical and structural complexity of xylans and, as a consequence, impacts the enzymatic accessibility and biodegradability of hemicelluloses (Beaugrand et al., 2004; Beaugrand et al., 2004; Benamrouche et al., 2002; Bernard Vailhe et al., 2000; Faulds et al., 2006; Grabber, 2005). Considering the chemical and structural complexity of grass hemicelluloses, it is not surprising that nature has developed a complete arsenal of hemicellulose-hydrolyzing enzymes that, through their concerted action, bring about complete degradation of these polymers. The main depolymerising enzyme is xylanase whose action is complemented by that of arabino-hydrolyzing enzymes such as α-L-arabinofuranosidase, β-D-xylosidase, α-D-glucuronidase and β-feruloyl esterases. Generally, xylanase action is most efficient on β-(1,4) bonds linking non-branching xylose residues. Therefore, the specific structural features of xylan, such as the degree of branching and the oxidative coupling of xylan-xylan chains (ferulate dimers) and/or xylan-lignin *via* hydroxycinnamic acid, form a cohesive wall network that may limit enzyme access (Figure 3).

3.3. Surface area and pore volume

Others parameters such as pore volume and surface area have been shown to affect the biodegradability of lignocellulosic materials. Several groups show good correlation between pore volume, surface area and the enzymatic digestibility of lignocellulosic materials (Chang & Holtzapple, 2000; Koullas et al., 1992; Laureano-Perez et al., 2005; Park et al., 2007; Puri, 1984). Increasing the surface area of a substrate enhances its digestibility. But this may be influenced by the lignin content and distribution. Indeed, alkali washing of a steam-exploded

substrate resulted in decreased hydrolysis rates, despite an increase in pore volume and the reduced lignin content of the substrate (Mooney et al., 1998).

3.4. Lignin content and composition

One of the major roles of lignin is to maintain fiber integrity and the structural rigidity of the plant. Lignin is a polymer of phenylpropane units which form a three-dimensional network inside the cell wall. The major inter-unit linkage is an aryl-ether type β-O-4 link. The macromolecular structure of the lignin polymer depends on the β-O-4 linkage, monomer distribution (G, S and H) and molecular weight, all of which depend on the species, plant part, and plant maturity (Fukushima & Terashima, 1991; Vanholme et al., 2010). These different parameters modify the architecture and supramolecular organization of the cell wall and influence its accessibility and digestibility (Akin et al., 1995; Chang & Holtzapple, 2000; Jung & Engels, 2002; Laureano-Perez et al., 2005; Tian et al., 2010). The enzymatic degradability of cell walls in leaves and particularly in the stems of plants declines during maturing because of accumulation and progressive lignification of the primary and secondary cell walls of vascular and sclerenchyma tissues. Such reduced biodegradability is partly related to the increased lignin content of cell walls. However, variations in three-dimensional structure, the composition of lignin and its hydrophobicity, in encrustation, supramolecular organization and cross-linking to other matrix components have also been implicated (Boukari et al., 2009).

The distribution and composition of lignin are also very important for enzyme accessibility and the digestibility of biomass (Adler, 1977; Clark et al., 2009; Guo et al., 2010; Ntaikou et al., 2010; Yuan et al., 2008). For example, these factors have been cited as being responsible for the higher recalcitrance of softwood-derived substrates (Mooney et al., 1999;

Mooney et al., 1998). It has also been suggested that guaïacyl lignin restricts fiber swelling and, hence, enzyme accessibility more than syringyl lignin. G-type units are able to form C–C bonds, but this is not possible in S-type units as they have a C5 position substituted by a methoxy group (Figure 2). This structural variability of the lignin polymer can influence the reactivity of lignin molecules, for example in the course of depolymerization and repolymerization reactions during pretreatment. Depolymerization/repolymerization of lignin molecules is a very important parameter that impacts on the accessibility and biodegradability of lignocellulosic biomass. The original C–C bonds and C-C bonds formed by repolymerization are not cleaved during pretreatment of the lignocellulosic biomass (for example, in pulping wood) due to their higher stability. As a consequence, lignins whose composition is strictly of G and G-H units are expected to show higher molecular weight than those presenting a high level of S units. Under highly alkaline conditions, some α-hydroxyl groups form quinone methide (Figure 2) that reacts easily with other lignin fragments, giving alkali-stable methylene linkages (Ralph & Young, 1983; Sipilä, 1990; Sipilä & Brunow, 1991). This reaction, occurring especially during the Kraft process due to the more severe conditions used, causes the formation of species with a high molecular weight.

In an attempt to correlate substrate accessibility with the efficiency of enzymatic hydrolysis, various studies measured the initial enzyme adsorption capacity of different substrates and correlated this with the initial rates of hydrolysis. It was found that substrates containing little or no lignin showed good correlation between initial hydrolysis rates and adsorption capacity, while substrates with higher lignin content demonstrated a poor correlation (Chang & Holtzapple, 2000; Koullas et al., 1992).

3.5. Lignin-carbohydrates complex: LCC

Lignin is associated with other cell wall polysaccharides to form a lignin-carbohydrates complex (LCC). The first two components are hydrophilic whereas the latter is hydrophobic (Barakat et al., 2008; Monties & Fukushima, 2001). LCC are insoluble in water and partially soluble in organic solvents (Wong et al., 1996; Yaku et al., 1981). Such a complex structure makes lignocellulosic materials hard to biodegrade and difficult to use by microorganisms, resulting in low hydrolysis rates. Lignin does not exist in plant tissue as an independent polymer but is always associated with hemicelluloses, not only as physical mixtures, but through covalent bonds (LCC). The mechanism of formation of LCC can be divided into two main processes. The first is based on the oxidative coupling of phenolic compounds in plant cell walls (figure 3C). This process is restricted to herbaceous species. The second process, ubiquitous in all lignified cell walls (Terashima, 2001; Xi et al., 2000), involves the attack of nucleophilic groups (i.e. hydroxyl or carboxylic groups of hemicelluloses, phenols, water,…) generally on the α-carbon of transient quinone methide intermediate generated during the oxidative polymerization of lignin (Freudenberg & Neish, 1968) (Tanaka et al., 1979; Toikka & Brunow, 1999; Watanabe et al., 1986) (Figure 2).

Figure 3*: A) chemical structure of ferulic acid and p-coumaric acid and chemical structure of Lignin-Carbohydrates Complex (LCC) B) via glucuronic acid and arabinose (woody plant cell walls) and C) via phenolic acids and arabinose non-wood (grass cell walls).*

The cleaving of LCC bonds and the removal of lignin remain the major obstacles to the biodegradability of lignocellulosic biomass. If LCC bonds are cleaved, the cellulose/hemicelluloses become more accessible for enzymatic hydrolysis and fermentation. In herbaceous plants, hydroxycynamic acids (*p*-coumaric and ferulic acids) (Figure 3A) are attached to lignin and hemicelluloses *via* ester and ether bonds as bridges between lignin and hemicelluloses forming lignin/phenolics–carbohydrate complexes (Figure 3C) (Barakat et al.,

2007; Barakat et al., 2008; Sun et al., 2002; Wallace & Fry, 1994). However, lignin and carbohydrates in wood are attached to each other *via* benzyl-ether and benzyl-ester bonds (Figure 3B). The direct evidence for the existence of these LCC is obtained with the oxidative cleavage of benzyl-ether and benzyl-ester bonds, reduction, methylation analysis, chromatography, spectroscopy and electron microscopy. More detailed information on recent developments related to LCC in wood has been published by Koshijima and Watanabe (Watanabe et al., 2003).

The lignin–carbohydrate complexes from grasses are structurally different from those in wood and contain ferulic bridges between lignin and carbohydrates (xylans) *via* ester-linked ferulic acids (Grabber & Lu, 2007; Wallace & Fry, 1994; Wallace et al., 1995). Therefore, they are often referred to as "lignin/phenolic–carbohydrate" complexes (Fig 3C). Ferulic acid is attached to lignin with ether bonds and to carbohydrates with ester bonds (Das Nath et al., 1981; Quideau & Ralph, 1997; Takahashi & Koshijima, 1988; Wallace & Fry, 1994; Wallace et al., 1995). Ferulate dimers, already cross-linking polysaccharide chains, can also be incorporated into lignins *via* an oxidative mechanism (Figure 3C). It is generally accepted that the association of phenolic components with carbohydrates presents the greatest barrier to the utilization of carbohydrates. Whether this barrier is caused primarily by polyphenols (i.e. lignin), oligomeric phenols or monophenols is debatable and it may depend on the species, plant part, and plant maturity. Therefore, specific fractionation technology must be designed for each herbaceous crop, depending on its lignin structure.

4. Effects of pretreatment on structural features

Lignin in plant cell walls combines with holocelluloses to form carbohydrate complexes (LCC). These lignin-carbohydrate complexes make the plant cell wall resistant to microbial attack. Therefore, prior to anaerobic digestion, a pretreatment process which alters the structure and composition of the substrate may be useful to break up the lignocellulosic feedstock. The main objective of pretreatment in biohydrogen and biomethane production is delignification so as to make the holocelluloses more accessible to microbial attack. The crystallinity of cellulose is another important factor governing anaerobic digestion. Indeed, during pretreatment it is important to reduce the crystallinity of cellulose because the crystalline structure prevents penetration by micro-organisms. But effective pretreatment may also increase porosity, reduce the degree of polymerization of holocelluloses and be environmentally friendly. Finally, pretreatment has to be as cost-effective as possible because several economic models show that pretreatment is an essential operation requiring a dedicated unit in a lignocellulosic biorefinery, accounting for 16-19% of total cost equipment (Aden et al., 2002). In order to facilitate the production of methane and hydrogen from lignocellulosic substrates, several types of pretreatment can be carried out to accelerate the hydrolysis phase and to increase the availability of compounds. Pretreatment methods can be divided into different categories: mechanical, chemical, thermal, thermo-chemical and biological or various combinations of these.

4.1. Mechanical pretreatment

4.1.1 Mechanical comminution

Mechanical pretreatment methods include chipping, grinding, milling (e.g. two-roll, hammer, colloid and vibro ball). Such mechanical pretreatment leads to a reduction in the particulate size, usually to 10-30 mm after chipping and 0.2-2 mm after milling or grinding. Mechanical pretreatment transforms the biomass into a fine powder, thus increasing the

surface area of the cellulose and reducing the degree of crystallinity of celluloses as well as decreasing the degree of polymerisation of celluloses and hemicelluloses (Galbe & Zacchi, 2007; Palmowski & Muller, 2000; Taherzadeh & Karimi, 2008).

Gharpuray *et al.*(1983) investigated the effects of ball milling, fitz milling and roller milling on the structural features (crystallinity, surface area and lignin content) of wheat straw. Ball milling pretreatment was found to be effective in increasing the specific surface area (2.3 m^2/g compared to 0.64 m^2/g for the raw wheat straw) and decreasing the crystallinity index (23.7 compared to 69.6 for the raw wheat straw) (Gharpuray et al., 1983). Palmowski *et al.* (2003) have also studied the effect of comminution on different organic samples (apples, rice, sunflower seeds, hay and maple leaves). After comminution of these substrates, a release of soluble organic compounds occurred for two reasons: cells were destroyed through comminution; and/or the dissolution of organic components through newly-generated accessible surfaces (Palmowski & Muller, 2003). However, Bridgeman *et al.* (2007) showed that a big reduction in the size of switchgrass is undesirable as it causes significant carbohydrate losses which ultimately result in a small amount of reducing sugars. Moreover, this process is not cost-effective because it requires too much energy and it has been shown that greater amounts of energy are needed to reduce size when biomass has a higher moisture content (Yu et al., 2006).

4.1.2 Other types of mechanical pretreatment

Digestibility of lignocellulosic biomass can also be enhanced by use of high-energy radiation using γ-rays, electron beam or microwaves. Kumakura & Kaetsu (1983) investigated the effect of irradiation pretreatment on bagasse: after enzymatic hydrolysis, a double yield of glucose was observed for the pretreated sample. Cleavage of β-(1,4)-glycosidic bonds leading

to an increase in surface area and a reduction in crystallinity was observed after applying γ-rays to cellulose (Takacs et al., 2000).

Microwave pretreatment combined with acid pretreatment (HCl) was used on wheat straw and wheat bran (Fan et al., 2005; Pan et al., 2008). The total soluble sugar in the microwave-assisted acid-pretreated wheat bran increased from 0.086 g/g_{TS} to 0.461 g/g_{TS} at 9 min hydrolysis time. Generally, microwave irradiation can change the ultrastructure of cellulose, degrade hemicelluloses and increase the accessibility of the substrate (Zhu et al., 2005). However microwave pretreatment has several disadvantages, including high energy consumption, complicated operation procedures and strict monitoring of equipment (Pan et al., 2008).

4.2. Thermal pretreatment

4.2.1 Liquid hot water

During liquid hot water (LHW) treatment, the lignocellulosic substrate is heated to a high-temperature (200-230°C) for a few minutes. Water under high pressure can penetrate into the biomass, increasing surface area and hence the removal of hemicelluloses and lignin. Generally, all the hemicelluloses, 35-60% of the lignin and 4-22% of the cellulose are dissolved (Mosier et al., 2005; Wyman et al., 2005).

Three types of reactor can be used for liquid hot water pretreatment: co-current (biomass and water are heated together for a certain residence time), counter-current (water and lignocelluloses move in opposite directions), and flow-through (hot water passes over a stationary bed of lignocelluloses) (Liu & Wyman, 2005).

4.2.2 Steam explosion

During steam explosion, lignocellulosic biomass is heated rapidly to a high temperature (160-260°C) with sufficient pressure (7-50 bar) to enable water molecules to penetrate the substrate structure for a few minutes. The pressure is then suddenly released to allow the water molecules to escape in an explosive manner. This pretreatment opens up the plant cells, increases surface area and enhances the digestibility of biomass (Ballesteros et al., 2000). According to Ramos (2003), lignin is primarily degraded through the homolytic cleavage of β-O-4 ether and other acid-labile linkages, producing a series of cinnamyl alcohol derivatives and by-products of condensation.

One limitation of steam explosion is the incomplete disruption of the lignin-carbohydrate matrix (Kumar et al., 2009). Consequently, steam pretreatment can be improved by using an acid catalyst, such as H_2SO_4 or SO_2 (1-2% w/w), which increases the recovery of hemicellulose sugars (Galbe & Zacchi, 2007). SO_2 steam explosion at 190°C for 2 min was applied to wheat straw and spruce: solubilization of hemicelluloses was observed at, respectively, 46 % and 85% (Li & Chen, 2007).

4.2.3 Ammonia fiber explosion (AFEX)

Ammonia fiber explosion (AFEX) is a physicochemical pretreatment in which the biomass is exposed to liquid ammonia at a relatively high temperature (90-120°C) for a period of 30 min, followed by the suddently reduction of pressure. AFEX pretreatment reduces lignin content, increases surface area, and cellulose and hemicellulose are well preserved, showing little or no degradation (Moniruzzaman et al., 1997). AFEX was shown to be insufficiently effective for substrates with high lignin content such as aspen in the form of chips or wood (McMillan, 1994).

For example, Gupta & Lee. (2010) applied AFEX to switchgrass and observed 68%, 45% and 1% of solubilization of lignin, hemicellulose and cellulose, respectively. On the

other hand, Kumar & Wyman (2009) observed no solubilization (neither of lignin, hemicellulose nor cellulose) after treatment of poplar. However, the crystallinity index of corn stover was significantly reduced (from 50.3 to 36.3) whereas the crystallinity index of poplar did not change.

4.2.4 CO_2 explosion

In CO_2 explosion, the biomass is exposed to CO_2 at low temperatures (30-50°C) and high pressure (140-180 bar) for a short period of time, followed by a sudden drop in pressure. CO_2 explosion is similar to steam explosion and AFEX: carbon dioxide molecules are comparable in size to those of water and ammonia and are able to penetrate small pores accessible to water and ammonia molecules. With the explosive release of carbon dioxide pressure, disruption of the cellulosic structure increases the accessible surface area (Zheng et al., 1998). Moreover, an increase in pressure facilitates the faster penetration of carbon dioxide molecules into the crystalline structures and more glucose is produced in further biological hydrolyse of biomass. Once dissolved in water, carbon dioxide forms carbonic acid. Even though it is a weak acid, it should be helpful in hydrolysing hemicelluloses as well as cellulose (Zheng et al., 1998). CO_2 explosion is more cost-effective than steam explosion because the temperature required in the process is lower. It was also shown to be more cost-effective than ammonia explosion (Zheng et al., 1998). A further advantage of using CO_2 explosion and AFEX rather than steam explosion is that they both avoid xylose decomposition which produces furfural, an inhibitor of the biological process involved in bioethanol production (Dale & Moreira, 1982).

4.2.5 Wet oxidation

Wet oxidation pretreatment involves the treatment of the biomass with air or oxygen at temperatures above 120°C. It was presented in the early 1980s as an alternative to steam explosion. This process is an effective method for separating the cellulosic fraction from lignin and hemicelluloses. The hemicelluloses are cleaved to monomeric sugars, lignins undergo both cleavage and oxidation and cellulose is partly degraded (Saha, 2003; Schultz et al., 1984). Wet oxidation at 195°C for 15 min led to the solubilization of 95 % of hemicelluloses and 40-50% of lignin of sugarcane bagasse (Martin et al., 2007). Alkaline wet oxidation at 185°C for 5 min on the same substrate solubilized only 30% of hemicelluloses and 20% of lignin (Martin et al., 2007). The combination of wet oxidation with alkaline pretreatment permitted the reduction of temperature in the process and, consequently, avoided the formation of soluble compounds such as furfural (Ahring et al., 1996; Martin et al., 2007).

4.3. Chemical pretreatment

4.3.1 Acid pretreatment

Acid pretreatment is used for efficiently removing hemicelluloses by breaking ether bonds in lignin/phenolics-carbohydrates complexes without dissolving lignin (Knappert et al., 1981).

Concentrated acids (typically 72% H_2SO_4 or 42% HCl at low temperature) usually lead to the conversion of at least 90% of the potential glucan in the biomass into glucose (Xiao & Clarkson, 1997). However, concentrated acids are corrosive and toxic and to make the process economically feasible, need to be recovered after pretreatment (Sun & Cheng, 2002). Consequently, dilute acid pretreatment appears to be a more promising process and has been widely studied. Concentrations of 0.4-2% H_2SO_4 at temperatures between 160-220°C for a few minutes are typically employed (Willfor et al., 2005). Dilute sulfuric acid treatment has been used successfully to hydrolyze hemicelluloses to sugars with high yields, to change the

structure of the lignin and to increase the cellulosic surface area (Mosier et al., 2005; Wyman et al., 2005).

Sulfuric acid is the most widely used but other acids have been used, including hydrochloric, phosphoric, nitric and acetic acid associated at times with nitric acid (Xiao & Clarkson, 1997). Peracetic acid, which is also an oxidant (oxidation potential : 1.81 eV) was shown to lead to a drastic reduction in the crystallinity index; this was attributed to structural swelling and dissolution of the crystalline cellulose component (Gharpuray et al., 1983). The disadvantages of acid pretreatment are that it implies a corrosive reagent, with corresponding downstream neutralization, and special materials for reactor construction.

4.3.2 Alkaline pretreatment

Alkaline pretretament is used mainly for the cleavage of ester bonds in lignin/phenolics-carbohydrates complexes (Buranov & Mazza, 2008). It leads to the saponification of esters of the uronic bonds between hemicelluloses and lignin, swells the fibers and increases pore size, facilitating the diffusion of the hydrolytic enzymes (Datta, 1981).

Aqueous lime or soda pretreatment was shown to be effective at a lower temperature than that used in acid treatment but the time required is of the order of hours or days rather than the minutes or seconds needed for acid pretreatment. For example, the degradation of lignin, the cleavage of links between lignin and carbohydrates, the solubilization of lignin (14%) and the increase in the accessibility of holocelluloses were observed after lime pretreatment was applied to wheat straw at 85°C for 3h, (Chang et al., 1998).The destruction of the ester bond in lignin-carbohydrates complexes (LCCs) was observed during the NaOH pretreatment of rice straw. Pretreatment of miscanthus with 12% NaOH at 70°C for 4h led to

77% of delignification of the raw material and to 44 % hydrolysis of hemicellulose (de Vrije et al., 2002).

Ammonia can also be used in the ammonia recycle percolation (ARP) pretreatment. Aqueous ammonia passes through the biomass in a percolation reactor (packed-bed, flow-through type) at high temperatures (150-170°C). Under these conditions, ammonia reacts with lignin and not with cellulose. ARP is effective for the delignification of hardwood and agricultural residues but less effective for softwood (Mosier et al., 2005). For example, in corn stover, ARP removed 75-85% of the total lignin and solubilized 50-60% of hemicelluloses, but retained more than 92% of the cellulose content (Kim & Lee, 2005). In the case of herbaceous biomass, Iyer et al (1996) observed 60-80% delignification of a mixture of corn cobs and stover and 65-85% delignification of switchgrass. Moreover, a pretreatment of switchgrass with 15% NH3 at 120°C was shown to be efficient in LCC solubilization whereas LCC were not solubilised by a 5% NaOH pretreatment at 85°C (Gupta & Lee, 2010).

4.3.3 Oxidative pretreatment

Oxidative pretreatment (H_2O_2, O_3, $FeCl_3$) can also be used to solubilize lignin and hemicelluloses and to increase the surface area of cellulose.

Hydrogen peroxide is usually used in association with alkali (pH=11.5) (Rabelo et al., 2008). For example, 50% lignin and most of the hemicelluloses were solubilized by 2% H_2O_2 at pH=11.5 and 30°C for 8h on sugarcane bagasse (Azzam, 1989). By applying 1% H_2O_2 at pH=11.5 and 65°C for 3h on corn stover, 66% delignification was observed compared to untreated corn stover (Selig et al., 2009). Gupta & Le (2010) assessed the impact of alkali pretreatment of switchgrass with 5% H_2O_2. Whereas 5% NaOH at 85°C and 15% NH3 at 120°C led to comparable levels of lignin and hemicellulose removal from solid fractions, the

use of ammonia led to significant degradation of xylan, galactan, arabinan and mannan originating from hemicellulose. A 5% H_2O_2, 5% NaOH, 85°C pretreatment was also shown to be effective in LCC solubilization.

Ozone can be applied to degrade lignin, though hemicellulose is slightly affected and cellulose not at all (Kumar et al., 2009). For example, a reduction in lignin content from 29% to 8% was observed after ozonolysis pretreatment of poplar sawdust (Vidal & Molinier, 1988). Ben-Ghedalia & Miron (1981) have shown 60% removal of lignin by applying ozone pretreatment to wheat straw. Although the process is carried out at room temperature and normal pressure, this pretreatment requires a large amount of ozone, making the process expensive.

Inorganic salts (NaCl, KCl, $FeCl_3$...) have also been tested as catalysts for the degradation of hemicelluloses in corn stover. $FeCl_3$ pretreatment was the most effective on corn stover, removing almost all the hemicelluloses; this pretreatment can disrupt almost all the ether linkages and some ester linkages between lignin and carbohydrates but had no effect on delignification. $FeCl_3$ significantly increased the degradation of hemicelluloses in aqueous solutions heated to between 140°C-200°C, with 90% xylose solubilization and only 10% cellulose removal (Liu et al., 2009a; Liu et al., 2009b).

4.3.4 Organosolv pretreatment

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts is used to break the internal lignin and hemicelluloses bonds. The solvents generally used are methanol, ethanol, acetone, ethylene glycol, triethylene glycol, tetrahydrofurfuryl alcohol; the acids used are HCl or H_2SO_4 (Kumar et al., 2009). For most organosolv processes at high temperatures (185-210°C), there is no need for the addition of acid because organic acids released from the biomass at this temperature act as catalysts for

the breakdown of the lignin-carbohydrate complex (Duff & Murray, 1996). Most hemicelluloses and lignin are solubilized, but the cellulose remains solid, making this process competitive for the bioethanol process (Zhao et al., 2009). During the organosolv process with poplar using aqueous ethanol; the recovery of lignin and hemicelluloses were 74% and 72%, respectively. (Pan et al., 2006). In the case of an ethylene glycol pretreatment of wheat straw, solubilization was observed at a level of 95 % for hemicelluloses and 64 % for delignification (Gharpuray et al., 1983). However, at the end of organosolv pretreatment, the solvents used in the process must be removed from the reactor because they may inhibit the growth of micro-organisms and they need to be recycled in order to reduce costs (Kumar et al., 2009).

4.3.5 Ionic liquids

Ionic liquids have also been investigated as an aid to dissolving lignocellulosic biomass (Dadi et al., 2007; Nguyen et al., 2010; Samayam & Schall, 2010). Ionic liquids offer several advantages: they have minimal environmental impact due to their low-volatility and can be reused after pretreatment (Dadi et al., 2007). Ionic liquid pretreatment of lignocellulosic biomass produces amorphous cellulose with little residual crystallinity (Samayam & Schall, 2010). Poplar and switchgrass were pretreated with (Emin)OAc (1-ethyl 3-methyl imidazolium acetate) for 30 min at 120°C. For poplar and switchgrass, the crystallinity dropped from 38% to 8 % (wt %) and from 21% to 6%, respectively (Samayam & Schall). Besides reducing crystallinity, ionic liquids can efficiently remove lignin. Wood flour was pretreated with (Emin)OAc for 90 min at temperatures ranging from 50 to 130°C. At 110°C, 44% of the lignin was removed and the crystallinity reduced from 63 % to 30% (Lee et al., 2009). At the present time, however, this process is too expensive for application as a lignocellulosic pretreatment due, mainly, to the high cost of ionic liquids (Nguyen et al., 2010).

4.4. Biological pretreatment

During biological pretreatment, industrial enzymes such as cellulase and xylanase or lignolytic enzymes (laccase, lignin and manganese peroxidase) are used to breakdown all components of lignocelluloses, including lignin, the polymer most refractory to microbial attack (Lopez et al., 2007). These enzymes can also be produced by micro-organisms such as brown-, white-, and soft-rot fungi that secrete extracellular enzymes (Galbe & Zacchi, 2007). White-rot fungi are the most effective in the biological pretreatment of lignocellulosic biomass (Fan et al., 1982). Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP) and laccase (Lee et al., 2007). To oxidize lignin, laccases, with low redox potential, need the presence of small compounds forming stable radicals that act as redox mediators. A mediating role is also played by the Mn^{3+} ion formed during the MnP and VP oxidation of Mn^{2+} (which acts as an oxidizer of phenolic structures and a starter of lipid peroxidation reactions), as well as by some aromatic radicals required for LiP oxidation of lignin, while VP can oxidise lignin directly (Martinez et al., 2009).

For example, white-rot fungi (*Ceriporiopsis subvermispora* and *Cyathus stercoreus*) were found effective in delignification of bermuda grass: after incubation with *Ceriporiopsis subvermispora* and *Cyathus stercoreus,* about 23 % and 41 % of total aromatics were removed respectively (Akin et al., 1995). Lopez *et al*. used *Coniochaet ligniaria* fungus to pretreat pepper plant residues. This microorganism produced cellulase, xylanase and two lignolytic enzymes (manganese peroxidase and lignin peroxidase). After 20 days of culture, reductions of about 75%, 50% and 40% were obtained for hemicelluloses, cellulose and lignin, respectively (Lopez et al., 2007).

The various types of biological pretreatment are considered environmentally friendly

and energy-saving as they are performed at low temperature and do not need any chemicals. However, biological pretreatment, because of its very low treatment rates, must be combined with other kinds of pretreatment (Sun & Cheng, 2002).

4.5. General remarks

Table 2 shows the results of the main types of pretreatment on the solubilization of cellulose, hemicellulose and lignin and on the crystallinity index, the surface area and digestibility of some selected biomass. Wheat straw is one of the most widely studied substrates. Measurements revealed high digestibility using (Emim)OAc ionic liquid (92%, Fu et al., 2010), organosolv process (85%, Sun & Chen, 2008) and SO_2 steam explosion (up to 70%, Piccolo et al., 2010). Where gramineae were involved, a high level of solubilization of hemicellulose was obtained by organosolv (Demirbas, 1998; Sun & Chen, 2008), wet oxidation (Bjerre et al., 1996), SO_2 steam explosion (Piccolo et al., 2010) and dilute acid (Kumar et al., 2009). A high level of solubilization of lignin was obtained with peracetic acid pretreatment , organosolv (Demirbas, 1998; Gharpuray et al., 1983; Sun & Chen, 2008) and ARP for corn stover (Kumar et al., 2009) . A significant decrease in the crystallinity index was observed after milling, peracetic acid pretreatment and pretreatment involving ammonia (Gharpuray et al., 1983; Kumar et al., 2009; Kumar & Wyman, 2009). However, several studies have shown an increase in the crystallinity index after ammonia treatment of wheat straw and after low-pH treatment of poplar (Kumar et al., 2009; Kumar & Wyman, 2009; Rémond et al., 2010). These results were explained by the greater break-down of amorphous cellulose in comparison to crystalline cellulose.

Where woody plants are concerned, the same trends are observed except for AFEX and ARP which are more effective on gramineae, notably in decreasing the crystallinity index (Kumar et al., 2009). Also noteworthy is that (Emim)OAc ionic liquid is highly effective in

reducing the crystallinity index and that a high level of solubilization of hemicellulose and lignin is obtained by pretreatment involving soda.

General trends for the effects of pretreatment on the lignocellulosic structure are summarised in Table 3. The majority of these studies were carried out with a view to bioethanol production. Thus the objective of pretreatment was to remove or dissolve hemicellulose and lignin from the cellulose which has to be retained in the solid fraction of the biomass. However, hemicellulose solubilization is a key feature in the bioethanol process though it is not indispensible to improve anaerobic digestion insofar as hemicellulose can be converted into biomethane. But solubilization of hemicellulose and cellulose are expected to improve anaerobic digestion rates. The shared aims of pretreatment for improving ethanol production and anaerobic digestion are the alteration of lignin structure (i.e. LCC cleavage and lignin solubilization) and an increase in the surface area, both of which should improve the extent and the rate of anaerobic digestion. Reduction of cellulose crystallinity, which is important for bioethanol production, may also impact on anaerobic digestion rates, though to a lesser extent.

When pretreatment conditions are too severe, especially in the case of thermal pretreatment at high temperature (LHW, steam explosion) and thermo-chemical pretreatment that requires high temperatures (acid or organosolv), byproducts such as furfural, hydroxymethylfurfural and soluble lignin compounds can be formed (Palmqvist & Hahn-Hägerdal, 2000). Such compounds become a problem during the fermentation stage of bioethanol production because they inhibit, or even stop, fermentation (Laser et al., 2002). In contrast to the bioethanol process, anaerobic digestion can convert these compounds. However, the methanogen microorganisms require a period of adaptation that decreases the hydrogen or methane production rate (Benjamin et al., 1984; Fox et al., 2003). Furthermore, the overly severe conditions of some forms of pretreatment such as oxidation may lead to the

partial degradation or mineralisation of sugars. Whereas in bioethanol production it is the degradation of glucose that must be avoided, in any pretreatment for anaerobic digestion degradation of sugars originating from either cellulose or hemicellulose should be avoided because this results in the partial loss of biohydrogen or biomethane potential.

Table 3: *General trends for the effects of pretreatment on lignocellulosic structure and characteristics and its impact on biohydrogen/biomethane and bioethanol processes*

5. Conversion of lignocellulosic materials to BioH_2 and BioCH_4 in one- and two-stage processes

Hydrogen (H_2) and methane (CH_4) are both valuable energy sources which can be used for the production of heat and electricity or as vehicle fuel. Hydrogen is regarded as an ideal type of renewable energy for the future because it can be converted either to electrical energy in fuel cells or burnt and converted to mechanical energy without producing CO_2 (Momirlan & Veziroglu, 2005). Over the past ten years, several studies have focused on the production of biohydrogen and biomethane using lignocellulosic residues which constitute a sustainable source thanks to their abundance and low cost (de Vrije et al., 2002; Koullas et al., 1992; Mosier et al., 2005; Panagiotopoulos et al., 2009). Methane can be produced from organic matter by a biological process known as anaerobic digestion while biohydrogen can be obtained by dark fermentation as a part of an anaerobic process. Indeed, the dark fermentation process is defined as an intermediate stage along the anaerobic digestion pathway when the last step (methanogenesis) does not occur or is inhibited (Figure 4). One of the major advantages of dark fermentation and anaerobic digestion processes is that all the

organic compounds can be transformed into biofuel except lignin, or only simple sugars for bioethanol and lipids for biodiesel (Frigon et al., 2008; Xiao et al., 2010). Consequently, hydrogen or methane production in a one-stage process or combined hydrogen and methane production in a two-stage process appears to be very promising (Pakarinen et al., 2009).

***Figure 4**: Principle of the conversion of lignocellulosic biomass to biohydrogen and biomethane.*

5.1 Dark fermentation

5.1.1 Principles

Biohydrogen can be biologically produced by bacterial fermentation (dark fermentation and photo-fermentation) or by a photosynthetic process carried out by microalgae (direct or indirect biophotolysis). One of the advantages in the use of the fermentation process rather photo-fermentation is that it performs concommitantly waste treatment and H_2 production (Saratale et al., 2008). In addition, dark fermention requires less space and is around 340 times cheaper than photosynthetic production because anaerobic fermentative bacteria produce hydrogen without photoenergy (Atif et al., 2005).

Dark H_2 fermentation is a simple process that requires low energy and can use various kinds of organic waste (Wang et al., 2008). Monosaccharides (*i.e* glucose, xylose, arabinose...) and also polymers such as starch, cellulose or hemicelluloses can be used as hydrogen feedstocks. There are two common pathways in the production of hydrogen by dark H_2 fermentation: one producing acetate and the second butyrate. These acidification processes are described by the following reactions (equations 1-4), using glucose and xylose as models.

The theoretical metabolic pathways of acetic acid (eq 1) and butyric acid (eq 2) from glucose are as follows (Antonopoulou et al., 2006):

$$C_6H_{12}O_6 + H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (eq1)$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (eq\ 2)$$

The theoretical metabolic pathways of acetic acid (eq 3) and butyric acid (eq 4) from xylose are as follows (Kongjan et al., 2009):

$$C_5H_{10}O_5 + 1.67H_2O \longrightarrow 1.67CH_3COOH + 1.67CO_2 + 3.33H_2 \quad (eq3)$$

$$C_5H_{10}O_5 \longrightarrow 0.83CH_3CH_2CH_2COOH + 1.67CO_2 + 1.67H_2 \quad (eq\ 4)$$

Theoretically, using pure cultures, 4 moles of hydrogen can be produced from glucose by the acetate pathway and 2 moles by the butyrate pathway; 3.33 moles of hydrogen can be produced from xylose by the acetate pathway and 1.67 moles by the butyrate pathway (Antonopoulou et al., 2006; Kongjan et al., 2009). Therefore, the butyrate / acetate ratio might be a quantitative indicator of substrate metabolism such that more hydrogen production is to be expected if more acetate and less butyrate are found in the system (Hawkes et al., 2007).

Of the heterotrophic bacteria that can be used for dark H2 fermentation, anaerobic (*Clostridium*) or facultative (*Enterobacter and Bacillus*) bacteria are the most efficient microorganisms. They can be found in pure, mixed or co-cultures. Pure cultures of selected hydrogen species that include strict anaerobes (*Clostridia, rumen bacteria, archaea*…) or facultative anaerobes (*E.Coli, Enterobacter, Citrobacter*...) are often used to produce hydrogen (Ntaikou et al., 2010). Among the hydrogen-producing bacteria, *Clostridium sp*. and *Enterobacter sp* are the most widely-studied bacterial species. Hydrogen production is about 2 mol H_2/ mol glucose by *Clostridium sp.* compared to 1 mol H_2/ mol glucose by *Enterobacter sp*. (Girbal et al., 1995; Yokoi et al., 1995). Hydrogen production using

Clostridium thermocellum on lignocellulosic substrates has been investigated by Levin et al. (2006). A hydrogen yield of 1.6 mol H_2 / mol glucose was observed from wood fibers (Levin et al., 2006). Thermophilic biohydrogen production from energy crops by *Caldicellulosiruptor saccharolyticus* was also studied by Ivanova et al. (2009). Wheat straw was found to be the best, with a H_2 production capacity of 2.09 mol H_2 / kg $_{DM}$ (Ivanova et al., 2009). Co-cultures can be a promising alternative (Yokoi et al., 2001). Wang et al, (2008) investigated biohydrogen production from microcrystalline cellulose using *Clostridium acetobutylicum* in a comparison with co-cultures (*Clostridium acetobutylicum* and *Ethanoigenens harbinense)*. An hydrogen yield of 3.6 mmol H_2 / g cellulose was observed using the pure culture and 8.1 mmol H_2 / g cellulose using the co-culture. *Ethanoigenens harbinense* rapidly removed the reduced sugars produced by *Clostridium acetobutylicum* through cellulose hydrolysis, resulting in improved cellulose hydrolysis and subsequent hydrogen production rates (Wang et al., 2008). However, pure cultures need to be isolated and require aseptic conditions, which significantly increases the overall cost of the process (Ntaikou et al., 2010).

Most studies have used mixed cultures originating from natural environments such as soils and anaerobic sludge to produce hydrogen (Ntaikou et al., 2010). Mixed cultures are easier to use because they are simpler to operate and a large range of feedstock can be transformed (Li & Chen, 2007). Moreover, unlike pure cultures they do not require aseptic conditions (Ntaikou et al., 2010). However, the use of mixed cultures has the disadvantage that non-hydrogen producing species such as methanogens, homoacetogens and lactic acid bacteria are present, leading to either the generation of byproducts like propionate, ethanol and lactate that involve the consumption of hydrogen; or to no hydrogen production (Guo et al., 2010; Ntaikou et al., 2010). Methanogens which are considered as the main hydrogen-consuming microorganisms can be inhibited by using pretreatment such as heat shock and pH

control. Heat-shock treatment methods utilise the capacity of some acidogenesic bacteria (*Bacillus and Clostridium*) to sporulate at high temperatures. In general, a heat-shock treatment of 110°C for 15 min to 2 hours is applied to eradicate non-spore-forming microorganisms (*e.g. methanogenic archaebacteria*) and to select spores of acidogenic bacteria that germinate when conditions become favourable again (Argun et al., 2008; Fang et al., 2006; Lay et al., 2003). Similarly, an acid/base pretreatment is an alternative to heat pretreatment. It consists in maintaining the seed microorganisms in acidic or basic conditions over a prolonged period to eradicate the methanogens that cannot survive in conditions of extreme pH (Ntaikou et al., 2010). Chemical inhibitiors such as bromoethanesulfonate, acetylene and chloroform can also be used (Guo et al., 2010).

5.1.2 Dark fermentation of lignocellulosic biomass

While carbohydrates and proteins are both basic components of organic materials, in terms of hydrogen yield during dark fermentation, carbohydrates are known to be a better substrate than proteins (Bai et al., 2004). The maximum hydrogen yields are about 6.25 mmole H_2 / g glucose and 3.43 mmoleH_2/g protein at initial neutral pH (Xiao et al., 2010). However, protein contents can improve the cell growth of hydrogen-producing bacteria and consequently increase hydrogen productivity (Brosseau et al., 1986). By way of example, substrates containing 60 % glucose and 40% peptone were tested and provided better conditions for cell growth and biohydrogen production than a substrate containing only glucose (Bai et al., 2004). Consequently, lignocellulosic materials that contain holocelluloses (hemicelluloses and celluloses) represent interesting substrates for hydrogen production, as has been shown extensive reviews of biohydrogen production involving a large range of substrates (Demirel et al., 2010, ; Guo et al., 2010).

Some studies using lignocellulosic feedstocks to produce biohydrogen are mentioned in Table 4. For lignocellulosic biomasses, Table 4 reports values ranging from 3.16 L H_2/kg $_{VS}$ (corn stalk) to 73 L H_2/kg $_{VS}$ (ryegrass). Better results for hydrogen potential were observed for starchy substrates such as fodder turnip and maize starch respectively, 188 L H_2/kg $_{DM}$ and 190L H_2/kg $_{DM}$.

***Table 4**: BioH_2 and Bi CH_4 production in one- and two-stage processes*

5.2. Anaerobic digestion

5.2.1 Principles

Anaerobic digestion of lignocellulosic residues consists of a complex series of metabolic interactions involving different anaerobic micro-organisms in an oxygen-free environment. Anaerobic digestion is made up of four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 4). Each stage requires the activity of its own specific group of micro-organisms. Hydrolysis is the conversion of carbohydrates into soluble sugar monomers (glucose, xylose, arabinose, mannose...). Acidogenesis is the transformation of soluble sugar monomers into volatile fatty acids (VFA). These two first steps correspond to dark H_2 fermentation during which the activity of hydrogen-consuming bacteria is not inhibited. During acetogenesis, VFA are transformed into acetate, CO_2 and H_2. Homoacetogenesis is of particular interest since it produces acetate from H_2 + CO_2. Finally, methanogenesis is the conversion of acetate, CO_2 and H_2 to methane by archae bacteria. The mixture CO_2/H_2 is transformed into methane by hydrogenophilic methanogens while acetate is transformed into methane by acetoclastic methanogens. Thus, the final product of anaerobic digestion is biogas which consists mainly of methane (55-75%) and CO_2 (25-45%). As

anaerobic digestion is a biological process, it is strongly influenced by the following environmental factors: temperature, pH, and toxic compounds. Anaerobic digestion is divided into psychrophilic (10-20°C), mesophilic (20-40°C) or thermophilic (50-60°C) digestion processes. The first stage in anaerobic digestion can occur at a wide range of pH whereas methanogenic micro-organisms are efficient only at neutral pH (6.5-7.5). At excessive concentrations, such compounds as VFA, hydrogen, ammonia or heavy metals may affect methanogenesis.

5.2.2 Anaerobic digestion of lignocellulosic biomass

The advantage to using anaerobic digestion is that not only simple organic compounds such as pentoses, hexoses, and volatile fatty acids are converted into methane but polymers, also (cellulose, starch, hemicelluloses...). Even inhibiting compounds from bioethanol fermentation (furfural, HMF and soluble lignin) can be transformed into methane if not highly concentrated. (Benjamin et al., 1984; Fox et al., 2003).

Based on the stoechiometric conversion of organic material into methane and carbon dioxide, the theoretical or maximum methane yield (100% conversion) can be calculated from the elemental composition of the substrate $C_aH_bO_cN_dS_e$ as follows (Frigon & Guiot, 2010; Lubken et al., 2010) :

$$Y_{CH_4}^{Theoretical}\ (L/g_{substrate}) = \frac{22.4\,(4a+b-2c-3d-2e)}{8\,(12a+b+16c+14d+16e)} \qquad \text{(eq 5)}$$

According to equation 5, the theoretical methane yields of carbohydrates ($C_{10}H_{18}O_9$) and lignocellulosic biomass ($C_5H_9O_{2.5}NS_{0.025}$) are 0.397 L CH_4/g $_{VS}$ and 0.475 L CH_4/g $_{VS}$, respectively (Frigon & Guiot, 2010). Based on measured elemental analysis, Lubken and al. calculated the theoretical methane yields of various form of lignocellulosic

biomass. Results ranged from 0.394 L CH_4/g $_{VS}$ for triticale straw to 0.490 L CH_4/g $_{VS}$ for rye grass (Lubken et al., 2010). However, actual methane yields from lignocellulosic biomass can be far lower than the theoretical amounts. According to Frigon and Guiot, they do not generally exceed 60% of theoretical values due to poorly- or non-biodegradable compounds (*eg.* lignin, peptidoglycan...) and polymers that are difficult to dissolve (cellulose, hemicelluloses and proteins) (Frigon & Guiot, 2010).

Several extensive reviews of methane production from biomass have been published (Frigon & Guiot, 2010; Gunaseelan, 1997; Ward et al., 2008). Some results are presented in Table 4 where values range from 25 L CH_4/kg $_{VS}$ (barley straw) to 455 L CH_4/kg $_{VS}$ (citrus peel).

It is generally accepted that the higher the crude fiber content, the lower the methane potential of the biomass. This has been confirmed on three types of paper (newsprint, paper tube residues and office paper) with different lignin content (30.3%, 23%, 3.6% respectively) which had a methane potential of 97 L CH_4 / kg VS $_{added,}$ 222 L CH_4 / kg VS $_{added}$, 364 L CH_4 / kg VS $_{added}$, respectively (Teghammar et al., 2009; Xiao et al., 2010). According to Kobayashi et al (2004)., a linear regression shows a strong negative correlation between the amount of methane produced and the amount of Klason lignin in bamboo. The lignin content plays a major role in methane production by limiting the access to holocelluloses. Holocelluloses, which are anaerobically-biodegradable compounds in their pure form, appear to be less biodegradable or even completely refractory when combined with lignin (Jimenez et al., 1990; Tong et al., 1990). Furthermore, Buffiere et al.(2006) showed a link between the methane potential of various lignocellulosic residues and the sum of their cellulose and lignin content : the higher the sum of cellulose and lignin, the lower the methane potential (Buffiere et al., 2006). As pure cellulose can be fully converted into biogas, previous results showed that the anaerobic digestion of lignocellulosic biomass is strongly conditioned by the

bioaccessibility of cellulose. However, Klimiuk et al. (2010) found that the methane yields of two varieties of micanthus having comparable lignin concentrations varied by a factor of 2: 0.10 and 0.19 m^3 CH_4/kg $_{VS}$ for *Miscanthus x giganteus* and *Miscanthus Sacchariflorous*, respectively (Klimiuk et al., 2010). Lignin concentration is thus a key parameter in anaerobic biodegradation, though not the only one.

Various studies have considered the anaerobic digestion of biomass sillage. Ensiling, the natural degradation of organic matter by aerobic lactic bacteria, favours nutrient conservation. Lehtomaki (2006) has shown that ensiling has a positive impact on methane production. He suggested that the structural polysaccharides contained in plant material, which are quite resistant to anaerobic degradation, can be partially degraded by aerobic lactic bacteria during storage (Lehtomaki, 2006). High methane potential was observed in sillage such as maize and grass, with methane production of 370 mL CH_4/g $_{VS}$ and 431 mL CH_4/g $_{VS}$ respectively (Bruni et al., 2010; Pakarinen et al., 2009). A 25 % increase in methane potential was observed for maize after four months ensiling. Ensiling can thus be considered as a natural pretreatment (Neureiter et al., 2005).

5.2.3 Comparison of energy production by anaerobic digestion with combustion and bioethanol production

In terms of energy potential (MJ / kg_{DM}), it is interesting to compare methane production from lignocellulosic waste with other energy sources such as combustion and bioethanol (Table 5). Calorific values that represent potential energy produced by combustion were directly taken from Voivontas et al. (2001) and McKendry (2002). Energy potential from bioethanol and methane production were calculated on the basis of yields of 23 200 kJ/L $_{bioethanol}$ and 39 790 kJ /Nm^3_{CH4}.

Because various energy potentials are considered from different studies, Table 5 does not provide a comparison of different routes for profitable use of the same substrate. Knowing that methane or ethanol potentials can vary significantly depending on several factors such as the plant species, maturity, values for energy potential are considered as rough estimates. Even so, methane production seems to lead to higher energy production than bioethanol, whatever the biomass. But energy production by combustion is far higher than by ethanol and methane conversion. These results can be explained by the high proportion of lignin that can be burnt during heating but not converted during bioethanol and biomethane production processes. Even so, apart from the amount of energy produced, the use of energy has to be considered: in fact biofuel and electricity are considered as more flexible energy sources than heat.

***Table 5**: Comparison of the different profitable uses of energy of lignocellulosic substrates*

5.3. Coupling BioH_2 and BioCH_4 two-stage processes

Only about 10-20% of the energy potential of an organic substrate is obtained through dark fermentation (Cooney et al., 2007). Dark fermentation residues contain VFA (mainly acetic and butyric acids) and other compounds which will not be degraded to H_2 due to thermodynamic restrictions (Hawkes et al., 2007). There are several routes for using such residues in a second stage; these include converting the by-products to H_2 using photosynthetic bacteria or converting VFA to CH_4 during an anaerobic process (Ren et al., 2009).

In the second stage, acetate and butyrate derived from soluble metabolites of the dark fermentation can be converted into hydrogen by photosynthetic bacteria, known for their dominant tendancy to convert organic acids to hydrogen in the presence of light, and by the action of the nitrogenase enzyme (Claassen et al., 2004). The combination of dark and photo

fermentation can achieve a theoretical maximum hydrogen yield of 12 mol H_2 / mol hexose. This kind of two-stage bioprocess has been investigated using lignocellulosic substrates such as potato steam peel and cassava starch (Claassen et al., 2004; Su et al., 2009). By a combination of dark and photo fermentation, the maximum hydrogen yield from cassava starch increased to 18 mmoles H_2 / g starch from the original 10.7 mmoles H_2 / g starch in dark fermentation only (Su et al., 2009). However, one of the major drawbacks of this process is its costs because photo-heterotrophic bacteria employ light as their primary energy source and organic compounds as the carbon source (Claassen et al., 2004).

Another promising route is the use of a two-stage H_2-CH_4 process which has shown greatly enhanced hydrolysis and higher energy yields compared to a one-stage methanogenic process (Hawkes et al., 2007). In the first stage, the operating conditions (acid pH and short retention time) are set to favour fermentation of the substrate to hydrogen by enhancing the growth of acidogenic bacteria. In the second stage, conditions are changed to suit methanogenesis (neutral pH and longer retention time). This kind of process presents several advantages because the first stage acts efficiently for solubilization and the combined hydrogen-methane mixture (20-30% H_2, 80-70% CH_4) has been shown to burn cleaner than methane alone (Bauer & Forest, 2001; Ueno et al., 2007).

Some studies have been carried out using a two-stage H_2 and CH_4 process (Table 4). Pakarinen *et al* (2009) compared mesophilic CH_4 production from grass sillage in a one-stage process to combined thermophilic H_2 and mesophilic CH_4 production in a two-stage process. As well as a hydrogen production of 5.6 ml H_2 / g $_{VS}$, an 8 % increase in CH_4 yields was obtained from grass sillage in the two-stage process compared to the one-stage process (467 mL CH_4/g $_{VS}$ and 431 mL CH_4/g $_{VS}$) (Pakarinen et al., 2009). In terms of energy, an increase of 7% in MJ / kg_{VS} was observed with the two-stage process, in which only 0.4 % came from hydrogen production. This higher methane yield in the two-stage process was attributed to the

fact that the thermophilic H_2 production stage enhanced hydrolysis of the solid substrates and resulted in increased solubilization and VFA production (Pakarinen et al., 2009).

A two-stage process was also applied to potatoes and maize: hydrogen production of 271 mL H_2 / g $_{VS}$ and 158 mL H_2 / g $_{DM}$ was observed respectively, the methane production being 130 mL CH_4/g $_{vs}$ and 426 mL CH_4/g $_{DM}$ respectively (Rechtenbach & Stegmann, 2009; Xie et al., 2007). Finally, this kind of process was also studied by Antonopoulou et al. (2006) using sweet sorghum (hydrolysate and solid fraction). The two-stage H_2-CH_4 process was applied to the hydrolysed part that was rich in readily-fermentable sugars whereas the one-stage CH_4 process was only applied to the solid fraction. Yields of 10.4 mL H_2 / g $_{DM}$ and 29 mL CH_4/g $_{DM}$ were achieved for the hydrolysate and 78 mLCH_4/g $_{DM}$ for the solid fraction. A two-stage process can increase methane production; however, the increase in the CH_4 yield has to be considered in the light of the investment required in the more complex two-stage process (Pakarinen et al., 2009).

6. Pretreatment for enhancing hydrogen and methane Production

Pretreatment of lignocellulosic substrates prior to biohydrogen or biomethane production favours the accessibility of holocelluloses to anaerobic microorganisms. Several kinds of pretreatment were used to enhance the production of bioH_2 and bioCH_4. The main results for bioH_2 and bioCH_4 potentials and the gain of energy (MJ/kg$_{VS}$) attributable to pretreatment are presented in Table 6.

***Table 6**: Effect of pretreatment on bioH_2 and bio CH_4 production*

6.1 Mechanical pretreatment

Mechanical pretreatment, in particular grinding, has been used to enhance anaerobic digestion. Grinding (0.5mm) led to a 53% enhancement of the energy yield from wheat straw (Sharma et al., 1988). The influence of particle size reduction on different types of organic solid waste was investigated. It was observed that such size reduction of a substrate led to an increase in methane production (≈ 20%) and a reduction in anaerobic digestion time (≈ 20%), particularly with substrates having a high fiber content such as hay and leaves (Palmowski & Muller, 2000). According to Palmowski & Muller (2000), comminution not only releases cell compounds usable more easily and rapidly but also supports hydrolysis of the solid compounds in the long term.

6.2 Thermal pretreatment

Of the kinds of thermal pretreatment used to enhance hydrogen and methane production, steam explosion has been one of the most widely investigated. This pretreatment removes lignin and improves the accessibility of holocelluloses (Kobayashi et al., 2004). When steam explosion at 180°C for 25 min was applied to wheat straw, methane production increased by 31% (Bauer et al., 2009). Teghammar *et al* (2009) combined chemical pretreatment with steam explosion. They observed that the combination of steam explosion with 2% NaOH and 2% H_2O_2 enhanced the methane yield of paper tube residues from 238 mL CH_4/g_{VS} to 493 mL CH_4/g_{VS} (Teghammar et al., 2009). Steam explosion has also been investigated for hydrogen production with corn straw, cornstalks and wheat bran, giving hydrogen production of 68 mL H_2/g_{DM}, 63.7 mL H_2/g_{DM} and 86 mL H_2/g_{VS}, respectively (Li & Chen, 2007; Lu et al., 2009; Pan et al., 2008).

6.3 Thermochemical pretreatment

Thermochemical pretreatment has also been studied with a view to enhancing hydrogen and methane production. Dilute acid pretreatment has been most widely investigated in relation to bioH_2 production. The main reason for using dilute acid treatment in hydrogen production is the effect of such pretreatment on the lignocellulosic structure: hydrolysis of hemicelluloses to sugars with high yields, a change in the structure of the lignin, an increase in the cellulosic surface area (Mosier et al., 2005; Wyman et al., 2005). This enhances hydrogen production which remains very slow or inhibited when hemicelluloses and cellulose are not transformed into monomer sugars. A great increase in hydrogen production, varying from 58% (wheat bran) to 5300% (cornstalk) was observed for lignocellulosic substrates subjected to a dilute acid (HCl) pretreatment (Pan et al., 2008; Zhang et al., 2006).

Types of alkaline pretreatment which delignify the lignocellulosic substrates and thus favour their degradation by anaerobic microorganisms have been widely studied for the enhancement of bioH_2 and above all bioCH_4 production. The application of a NaOH pretreatment to coconut fibers and corn stover led to big methane production increases of 50% and 89%, respectively (Kivaisi & Eliapenda, 1994; Zheng et al., 2009).

6.4 Biological pretreatment

Finally, biomethane production can be enhanced by using biological pretreatment involving micro-organisms (e.g. brown-, white- and soft-rot fungi) or enzymes (cellulases, xylanases, lignin peroxidase, manganese peroxidase...) (Ghosh & Bhattacharyya, 1999; Lehtomaki et al., 2004). Ghost et al (1999) studied the effect of white-rot fungi and brown-rot fungi on rice straw. Increases in methane of 32% and 46% were observed respectively for rice straw pretreated with brown- and white-rot fungi compared to untreated straw (Ghosh & Bhattacharyya, 1999). The use of enzymes to increase methane yield has also been studied

(Guiot et al., 2009; Lehtomaki et al., 2004). Lehtomaki et al (2004) applied enzymatic pretreatment to grass at 35°C for 24 h using two xylanases (GC 320 and Multifect) and two cellulases (IndiAge MAX L and Primafast 200). A slight increase, from 230 mL CH_4/g_{VS} to 271 mL CH_4/g_{VS}, was observed (Lehtomaki et al., 2004). Finally, Chairattanamanokorn et al (2009) investigated a combination of alkaline and enzymatic pretreatment. By applying 4% NaOH (w/v) at 100°C for 2h followed by the enzymatic pretreatment (Cellulase, 20U/g), a hydrogen yield of 300 mL H_2 / g_{VS} was observed versus 31.4 mL H_2 / g_{VS} for the enzymatic pretreatment alone (Chairattanamanokorn et al., 2009).

6.5 General remarks

Table 6 also shows the potential energy that can be recovered from pretreated and raw substrates by biohydrogen and/or biomethane production. If paper tube residues are excluded, as they do not actually belong to classic lignocellulosic biomass, the maximum amount of recovered energy was obtained from grass sillage, winter rye and maize sillage (ranging from 16 to 19 MJ/kg_{VS}) after their respective pretreatment by soda, wet oxidation and grinding (Bruni et al., 2010; Pakarinen et al., 2009; Petersson et al., 2007). With a rough approximation of 80% of volatile solids in dry matter, the range of energy potential is from 13 to 15 MJ/kg_{VS}. These values are still lower than the potential energy that can be recovered by combustion (Table 5) but they approach the calorific value of straw from different types of biomass (ranging from 17-18 MJ/kg_{VS}). Moreover, it is worth noting that when applied to sillage pretreatment does not lead to a very significant increase in the amount of energy recovered (+ 2.8% and 11% in the case of grass sillage and maize sillage, respectively). Infact, sillage can be considered as a biological pretreatment and it would be interesting to consider its impact on biomass features.

On the other hand, in biohydrogen production from substrates which initially had a very low hydrogen potential (corn stalks: 0.03 MJ /kg_{VS}, beer lees: 0.03 MJ/kg_{VS}, corncobs: 0.14 MJ/kg_{VS}, poplar leaves: 0.16 MJ/kg_{DM} and maize leaves : 0.18 MJ/kg_{VS}), massive gains were observed, from 200 to 5300 times the initial value (Cui et al., 2010; Cui et al., 2009; Ivanova et al., 2009; Pan et al., 2009; Zhang et al., 2006). Among these examples, the greatest enhancement was obtained after acid (Cui et al., 2009; Pan et al., 2009; Zhang et al., 2006) or alkali pretreatment (Zhang et al., 2006). Moreover, biological (*Bacillus amyloliquefaciens*) and enzymatic (viscozyme L) pretreatments were shown to be advantageous on maize and poplar leaves, with 333-fold and 206-fold increases, respectively (Cui et al., 2010; Ivanova et al., 2009).

A very few papers investigated pretreatment prior to two-stage H_2-CH_4 processes (Xie et al., 2007, Hawkes et al., 2008, Lu et al., 2009, Pakarinen et al., 2009). Among them, some studies concerned very low lignin-content substrates such as potatoes (Xie et al., 2007) or wheat flour industry co-products (Hawkes et al., 2008). Lu et al. (2009) studied steam explosion of cornstalks prior to two-stage process but they did not indiquate bioH_2 and bioCH_4 production from the untreated substrate. Only one study compared the two-stage bioH_2 and bioCH_4 production with and without pretreatment (4% NaOH, 20°C, 24 h, Pakarinen et al. (2009)). A 16% increase in bioH_2 production but no significant increase in bioCH_4 production was observed (Pakarinen et al., 2009). This poor result on bioCH_4 production may be explained by the methane yield increase by solubilization during the first H_2 stage, as mentioned previously. On the other hand, this study was carried out with grass sillage and, as mentioned above, sillage can be considered as a biological pretreatment and may have lowered the impact of the alkali pretreatment. If the impact of lignocellulosic biomass pretreatment on two-stage bioH_2 production can be deduced from the results on one-

stage bioH_2 process, the impact on bioCH_4 production is thus still not clear and needs further studies.

Kaparaju et al (2009) tested an original three-stage process on wheat straw, producing bioethanol, biohydrogen and biomethane. Initially, wheat straw was hydrothermally pretreated, giving a cellulose-rich solid fraction and an hemicellulose-rich liquid fraction (hydrolysate). After enzymatic hydrolysis of the solid fraction, an ethanol yield of 0.41g ethanol / g sugars was observed and a biohydrogen yield of 178mL H_2 / g sugars from the liquid fraction. The effluents from both the bioethanol and biohydrogen processes were further used to produce methane, with yields of 324 and 381 mL / g $_{VS,}$ respectively (Kaparaju et al., 2009).

As well as increasing biodegradability and, thus, biohydrogen and methane production, pretreatment is effective in increasing the hydrolysis rate of lignocellulosic biomass. For example, Fernandes *et al* (2009) have measured the first-order hydrolysis rate constant of anaerobic digestion of hay pretreated with 4 % ammonium (w/v). No significant increase in methane potential was observed but the hydrolysis rate increased from 0.088 to 0.409 d^{-1} (Fernandes et al., 2009).

To sum up, pretreatment can be effective in increasing biodegradability and, thus, the biohydrogen and biomethane potential. Pretreatment can be an advantage where it reduces the hydrolysis time. Solid retention time can be lowered in the digester, making for a higher organic load and a consequent increase in methane production in a given digestor. Acid pretreatment has been shown to be advantageous for hydrogen production whereas thermo-chemical (steam explosion, wet oxidation) and alkaline pretreatments are better for methane production. Over and above its performance-enhancing function, pretreatment must be considered for its environmental impact and its cost-effectiveness.

7. Conclusion

The profitable use of lignocellulosic biomass via anaerobic digestion or dark fermentation is of great interest for the production of renewable energy. The main advantage of these two processes compared to bioethanol or biodiesel production is that they use consortia of microorganisms which accept a large range of substrates (cellulose, hemicelluloses, lipids, proteins...). Moreover, biohydrogen and biomethane production can be combined in a two-stage process in which biohydrogen is produced in a first reactor by H_2 dark fermentation and methane is produced in a second reactor using the soluble metabolites of dark H_2 fermentation. The major advantage of using a two-stage process is an enhancement of solubilization during dark H_2 fermentation and, consequently, an increase in the methane yield as well as the production of hydrogen.

Due to the poor accessibility of biodegradable compounds, pretreatment can enhance the performance of the anaerobic digestion or dark fermentation of lignocellulosic materials. However, the complex structure and composition of biomass must be thoroughly understood in order to first decide on and then carry out effective pretreatment. All types of biomass pretreatment share common denominator: they must make more accessible biodegradable material as well as being cost-effective and environmentally friendly. However, pretreatment must also be adapted to subsequent biological processes. For examples, lignin and hemicelluloses have to be removed from cellulose for ethanol fermentation whereas delignification is the objective of anaerobic digestion or dark fermentation pretreatment. Further progress may come from a combination of a more highly refined characterisation of lignocellulosic materials and the optimisation of their pretreatment.

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CAPTIONS

Figure 1: Strategy of biohydrogen and biomethane production from lignocellulosic materials in integrated lignocellulosic biomass production.

Figure 2: Simplified scheme of the lignification, supramolecular organisation and composition of plant cell walls "lignocellulosic matrix". Monolignols oxidized by a peroxydase/H_2O_2 system form radicals. The coupling and oxidation of these radicals result in lignin polymer.

Figure 3: A) chemical structure of ferulic acid and p-coumaric acid and chemical structure of Lignin-Carbohydrates Complex (LCC) B) via glucuronic acid and arabinose (woody plant cell walls) and C) via phenolic acids and arabinose non-wood (grass cell walls).

Figure 4: Principle of the conversion of lignocellulosic biomass to biohydrogen and biomethane.

Table 1: Biochemical composition of different lignocellulosic biomass

Table 2 : Impact of pretreatment on the constituents of selected biomass

Table 3: General trends for the effects of pretreatment on lignocellulosic structure and characteristics and its impact on biohydrogen/biomethane and bioethanol processes

Table 4: BioH2 and Bio CH4 production in one- and two-stage processes

Table 5: Comparison of the different profitable uses of energy of lignocellulosic substrates

Table 6: Effect of pretreatment on bioH_2 and bio CH_4 production

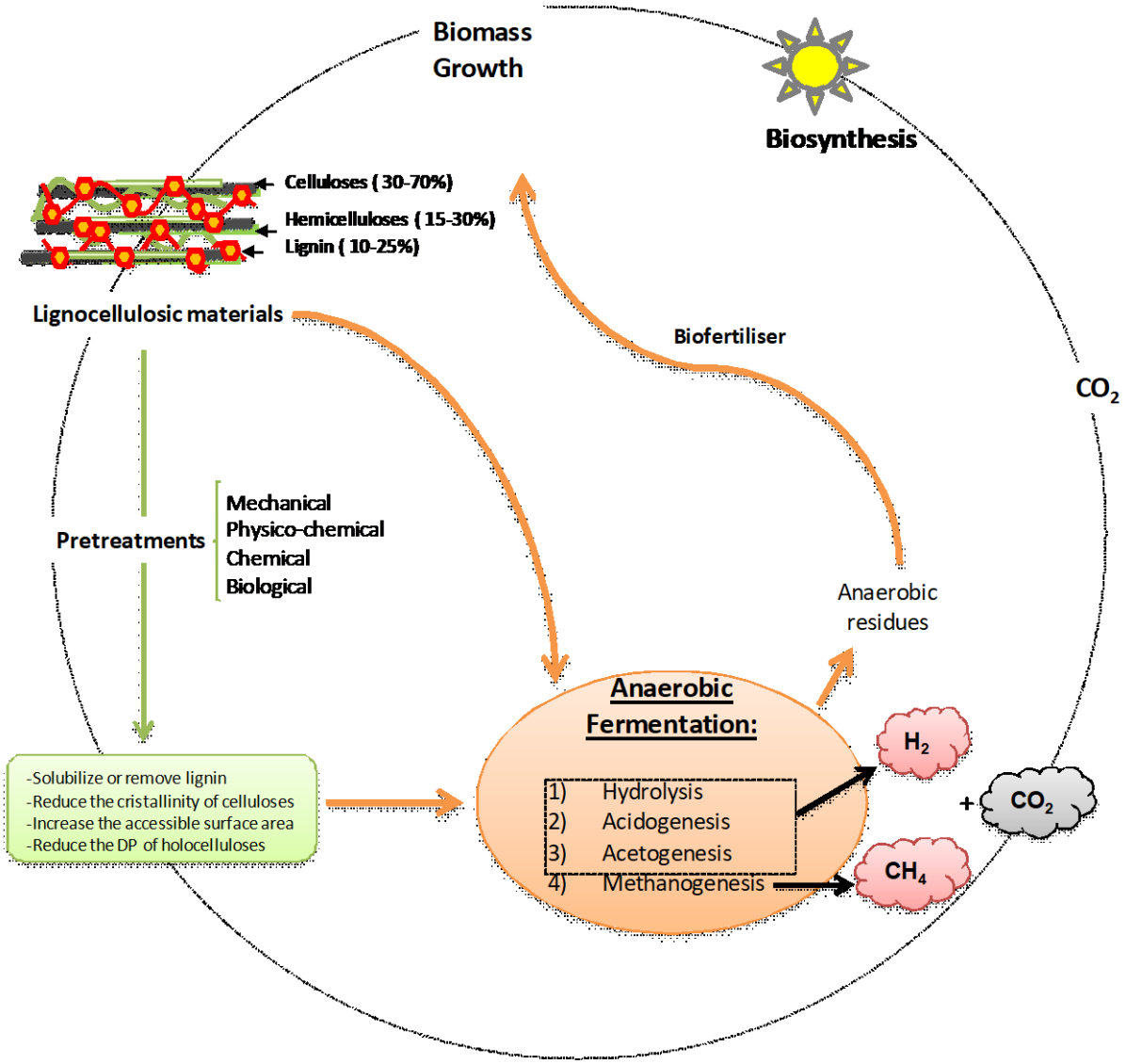

***Figure 1**: Strategy of biohydrogen and biomethane production from lignocellulosic materials in integrated lignocellulosic biomass production.*

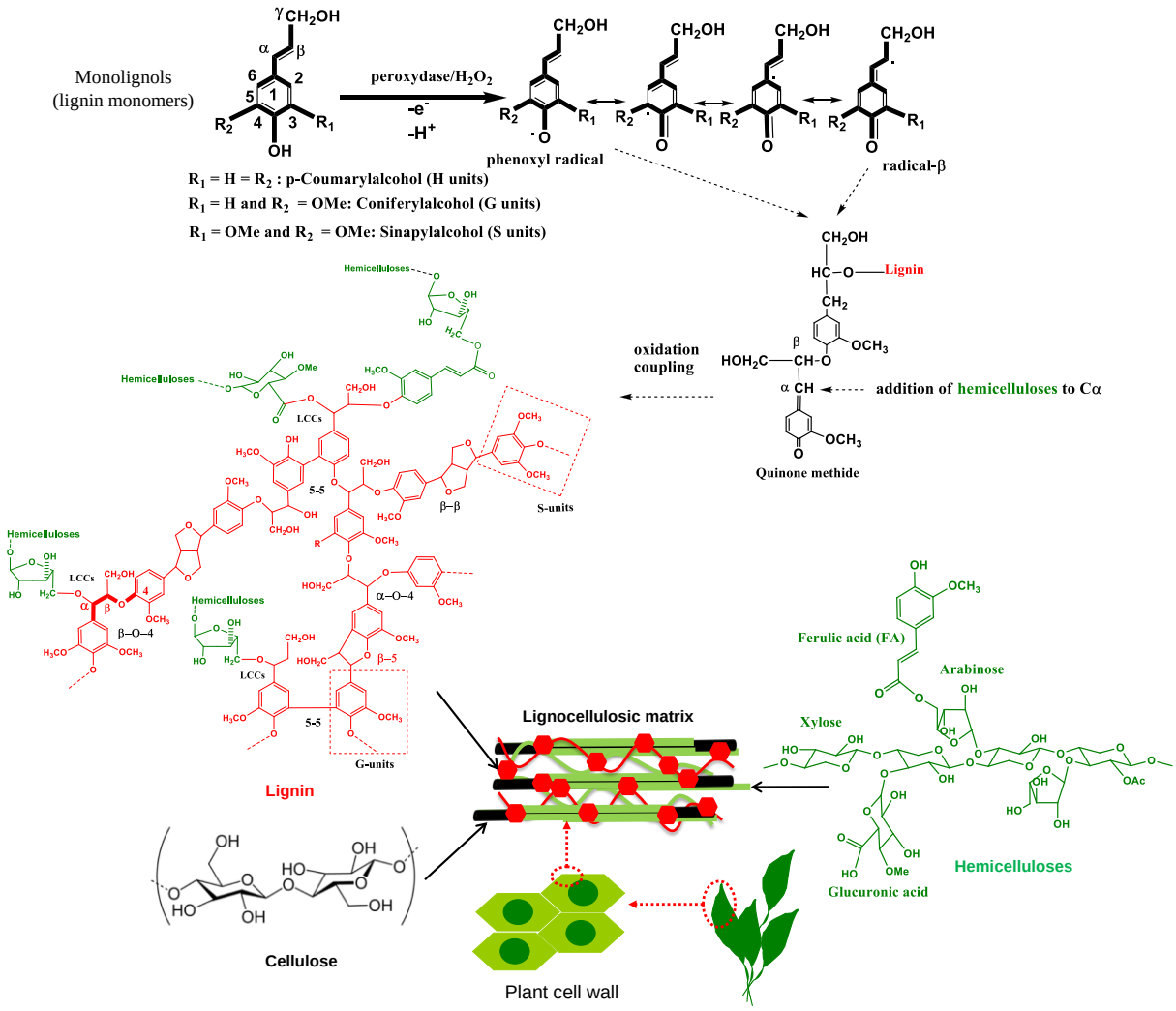

Figure 2 *Simplified scheme of the lignification, supramolecular organisation and composition of plant cell walls "lignocellulosic matrix". Monolignols oxidized by a peroxydase/H_2O_2 system form radicals. The coupling and oxidation of these radicals result in lignin polymer.*

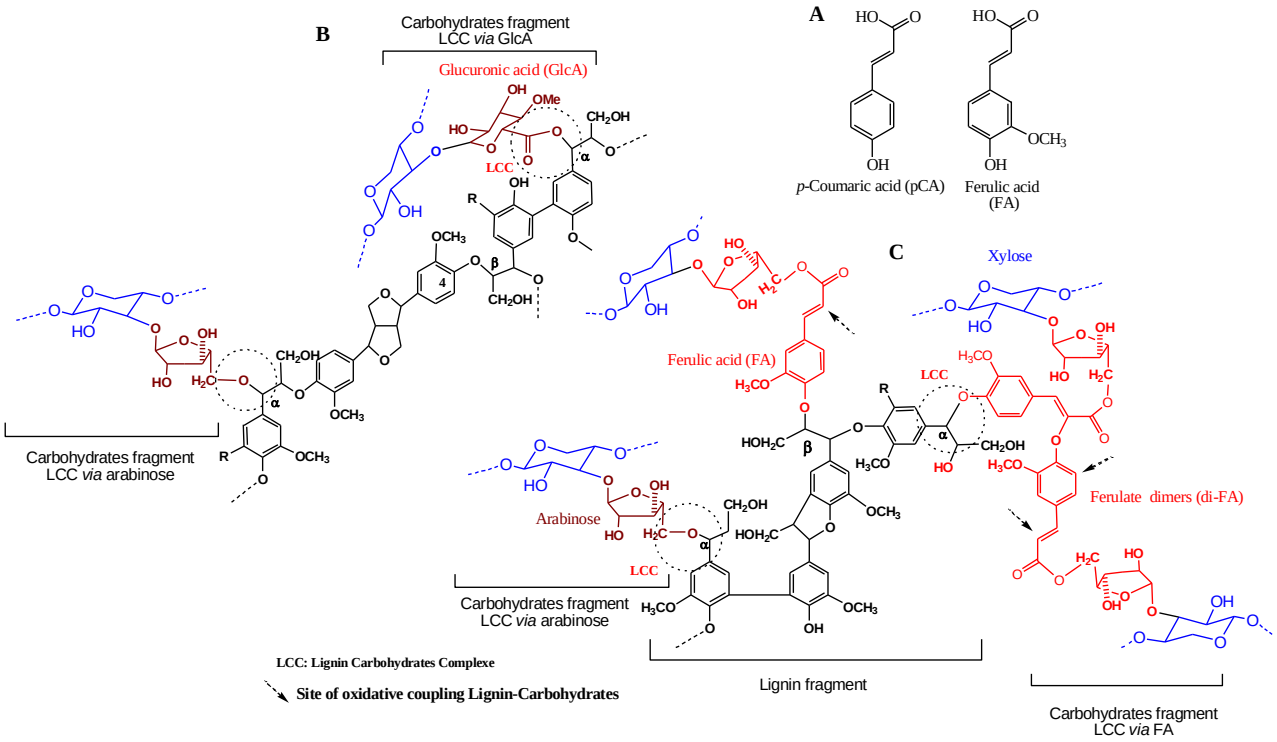

***Figure 3**: A) chemical structure of ferulic acid and p-coumaric acid and chemical structure of Lignin-Carbohydrates Complex (LCC) B) via glucuronic acid and arabinose (woody plant cell walls) and C) via phenolic acids and arabinose non-wood (grass cell walls).*

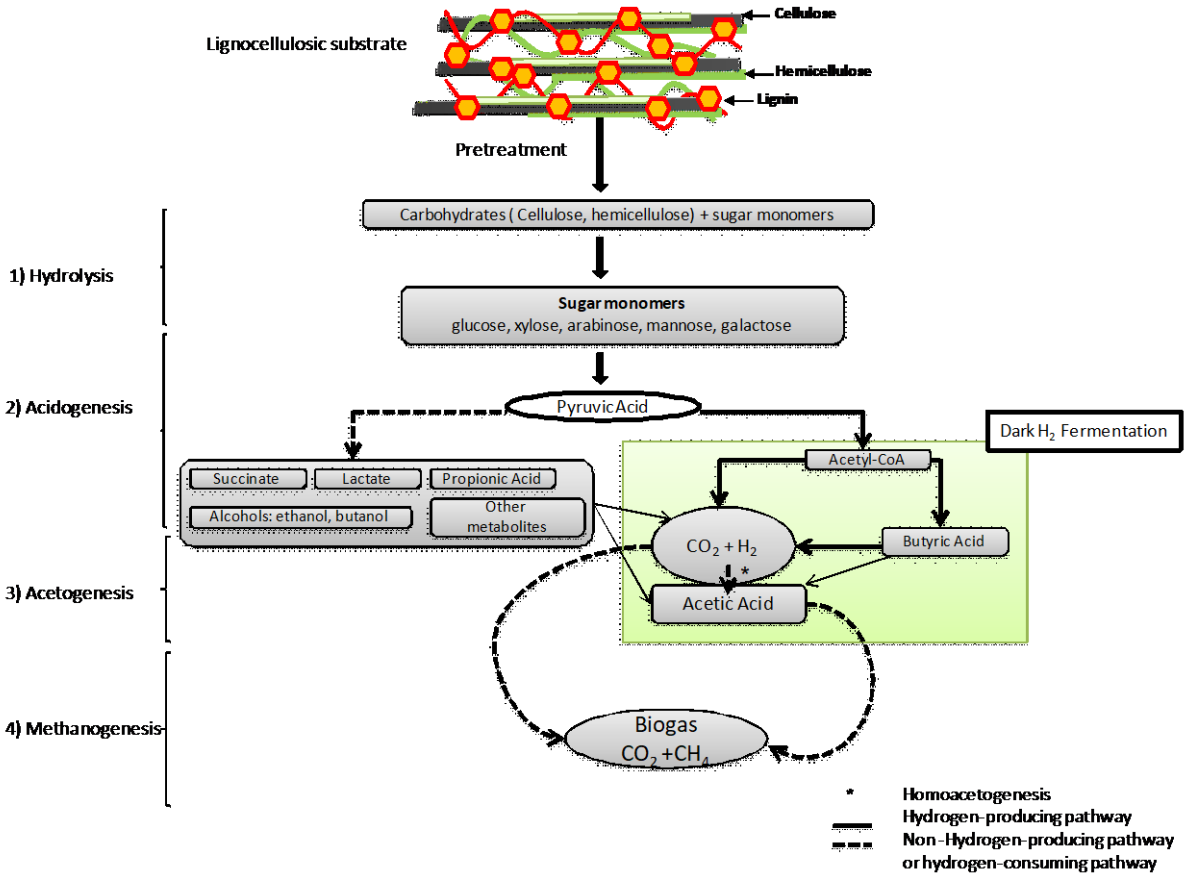

***Figure 4**: Principle of the conversion of lignocellulosic biomass to biohydrogen and biomethane.*

TABLES

***Table1**: Biochemical composition of different lignocellulosic biomass*

	Grasses or Gramineae								**Energetic plant**	**Hardwood**		**Softwood**	
Lignocellulosic compounds	Wheat straw	Wheat bran	Corn stover	Rice straw	Barley straw	Maize stover	Maize bran	Rye straw	Miscanthus	Poplar	Eucalyptus	Spruce	Pine
Celluloses (%)	**39.6**	**42.5**	**36.8**	**32.0**	**37.5**	**41.7**	**39.8**	**38.0**	**37.7**	**44.5**	**54.1**	**45.5**	**43.3**
Mw (g/mol)	250.720	-	-	272.130	337.820	382.210	-	241.830	-	171.950	-	-	-
DP	1547	-	7050	1680	2085	2360	-	1439	-	1091	-	-	-
CrI	**50.3**	-	**50.3**	**51.7**	-	-	-	-	-	**49.9**	**54.3**	**38.4**	-
Hemicelluloses (%)	**26.6**	**21.2**	**30.6**	**18.0**	**25.3**	**18.9**	**29.7**	**36.9**	**37.3**	**22.5**	**18.4**	**22.9**	**21.5**
Xylose (Xyl)	24.3	15.4	22.2	14.3	21.7	11.8	-	-	33.8	19.4	15.0	6.6	5.3
Arabinose (Ara)	2.1	3.1	5.5	2.3	2.5	1.5	-	-	2.8	0.5	0.5	1.2	1.6
Galactose	0.2	2.7	2.9	1.2	1.2	-	-	-	0.6	2.0	1.5	0.6	2.9
Mannose	-	-	-	0.2	-	-	-	-	0.1	0.6	1.5	13.5	10.7
Ara/Xyl	**0.09**	**0.20**	**0.25**	**0.16**	**0.12**	**0.13**	-	-	**0.08**	**0.025**	**0.03**	**0.18**	**0.3**
Lignin (%)	**21.0**	**3.4**	**23.1**	**11.2**	**16.0**	**26.1**	**2.6**	**17.6**	**25.1**	**19.5**	21.5	27.9	28.3
FA /*p*CA	1.2/0.64	0.44/0.01	-	1.22/0.61	1.27/1.28	0.82/0.32	-	1.42/0.45	-	-	-	-	-
G /S /H	**49/46/05**	-	**35/61/4**	**45/60/15**	-	-	-	**44/54/2**	-	**41/59/nd**	**38/62/nd**	**98/tr/2**	**82/tr/18**
β-O-4 (µmol/g of lignin)	**1040**	-	**610**	**630**	-	-	-	**1610**	-	**2390**	**2780**	**1230**	**1140**
Mw (g/mol)	2800	-	-	3600	3200	3600	-	3000	-	5500	≥5000	≥6000	≥8000
Others (%)	5.5	-	9.5	14.2	6.4	3.1	**7.3**	6.3	-	-	3.0	4.2	7.9
References	(Akpinar et al., 2009; Lapierre, 1993; Lequart et al., 1999; Sun et al., 2005; Sun et al., 2002)	(Lequart et al., 1999)	(Lapierre, 1993; Sun et al., 2005; Sun et al., 2001; Teramoto et al., 2009)	(Persson et al., 2009; Sun et al., 2005; Sun et al., 2002; Teramoto et al., 2009)	(Akpinar et al., 2009; Gullón et al., 2009; Sun et al., 2005)	(Kivaisi & Eliapenda, 1994)	(Lapierre, 1993; Sun et al., 2005)	(Aguilar et al., 2002)	(Brosse et al., 2009)	(Guerra et al., 2006; Lapierre, 1993; Popescu et al., 2009; Santiago & Neto, 2008; Sun et al., 2005)	(Galbe & Zacchi, 2007; Guerra et al., 2006; Lapierre, 1993; Santiago & Neto, 2008)	(Galbe & Zacchi, 2007; Lapierre, 1993; Palm & Zacchi, 2004; Popescu et al., 2009)	(Galbe & Zacchi, 2007; Lapierre, 1993; Tejado et al., 2007)

pCA :p-Coumaric Acid ; FA : Ferulic Acid ; G : Guaiacyl units in lignin ; S : Syringyl units ; H : p-Hydroxyphenyl; Mw: Molecular mass ; DP: Degree of polymerization; CrI: Crystallinity Index; nd: not detected; tr: traces.

***Table 2**: Impact of pretreatment on the constituents of selected biomass*

	Substrate	Pretreatment	Solubilisation (%)			CrI (%)	SA (m^2/g)	Digestibility(%)	Ref
			Cellulose	Hemicelluloses	Lignin				
ENERGY CROPS	Miscanthus	Dilute acid : 0.9 % H_2SO_4, 180°C, 2 min	1.6	57.8	3.6	-	-	40	(Brosse et al., 2009)
		Organosolv: 1.2% H_2SO_4 170°C, 80 min, Ethanol/water: 0.65	4.6	82.5	69.9	-	-	80	(Brosse et al., 2009)
		Milling 1 mm + 8% NaOH 25°C 24h	2	20	70	-	-	38	(de Vrije et al., 2002)
		Milling 17 µm + 8% NaOH 25°C 24h	24	36	67	-	-	58	(de Vrije et al., 2002)
		Extrusion + 8% NaOH, 25°C ,24h	9	3	75	-	-	48	(de Vrije et al., 2002)
	Switchgrass	Ionic liquid: 120°C, 20:1(Emim)OAc to biomass, 30 min	0	32	27	6/21[a]	-	71	(Samayam & Schall, 2010)
		AFEX: 120°C, 1.5:1 NH_3 to biomass, 24h	1.1	45.4	68	-	-	53.7/3.6[a]	(Gupta & Lee, 2010)
		120°C, 1.5:1 NH_3 to biomass + 5% H_2O_2, 24h	2.6	58	77	-	-	74.3/3.6[a]	(Gupta & Lee, 2010)
		5% NaOH, 85°C, 24h	1.4	60	76.2	-	-	70/3.6[a]	(Gupta & Lee, 2010)
GRAMINAE		Organosolv: 220°C, 3h glycerol/water :0.75	5	68	64	-	-	85	(Sun & Chen, 2008)
		Organosolv: 225°C, 8h, 1 % NaOH, glycerol/water :0.75	14.7	100	84.8	-	-	-	(Demirbas, 1998)

	Wheat straw	Ammoniac: 25°C, 1.8:1 NH_3 to biomass, 3 days	11	12	38	30/20[a]	-	40.1/18.2[a]	(Rémond et al., 2010)
		Ionic liquid: 90°C, 1:50 (Emim)OAc to biomass (0.5 mm) 24h	2.3	37	29.6	-	-	92.2/16.5[a]	(Fu et al.)
		Wet oxidation (130°C, 10 min, 10 bar O_2) + 10 g/L $NaCO_3$	0	68	35	-	-	75	(Bjerre et al., 1996)
		SO_2-Steam explosion 190°C, 2 min	6	46	-	-	1.9/1.1[b]	64.2/19.7[b]	(Piccolo et al., 2010)
		SO_2-Steam explosion 190°C, 5 min	6.5	65	-	-	2.1	70.9	(Piccolo et al., 2010)
		Milling, 4h	-	-	0.0	23.7/69.6[a]	2.3/0.64[a]	-	(Gharpuray et al., 1983)
		100°C, 10 % Peracetic acid, 30 min	-	-	87.5	28.4/69.6[a]	1.7/0.64[a]	-	(Gharpuray et al., 1983)
		129°C, 1:10 NaOH to biomass 2h	-	-	44.2	53.3/69.6[a]	1.7/0.64[a]		(Gharpuray et al., 1983)
	Corn stover	AFEX: 180°C, 2:1 NH_3 to biomass, 30 min	0.0	0.0	0.0	36.3/50.3[a]	-	-	(Kumar et al., 2009)
		ARP: 183°C, 3.66:1 NH_3 to biomass, 27.5 min	1.4	51.9	70.5	25.9/50.3[a]	-	-	(Kumar et al., 2009)
		Dilute acid: 0.6 % H_2SO_4, 190°C, 70s	6.6	72.8	18.0	52.5/50.3[a]	-	-	(Kumar et al., 2009)
		Lime: 0.5:1 Ca $(OH)_2$ to biomass 65°C, 4 weeks	2.9	-	-	56.2/50.3[a]	-	-	(Kumar et al., 2009)
		SO_2-Steam explosion 200°C, 5 min	3.1	-	-	-	-	-	(Kumar et al., 2009)
	Spruce	SO_2-Steam explosion 190°C, 2 min	2	85	-	-	2.0	39.6	(Piccolo et al., 2010)

WOOD PLANTS		SO_2-Steam explosion 190°C, 2 min and NaOH (1.5 mol/L, 70°C, 120 min)	10	100	14	-	-	-	(Li et al., 2009)
		Organosolv: 225°C, 8h, 1 % and NaOH, glycerol/water :0.75	6.8	100	76.8	-	-	-	(Demirbas, 1998)
	Beech	SO_2-Steam explosion 190°C, 2min and NaOH (1.5 mol/L, 70°C, 120 min)	10	100	86	-	-	-	(Li et al., 2009)
		Organosolv: 225°C, 8h, 1 % and NaOH, glycerol/water :0.75	9.6	100	89.8	-	-	-	(Demirbas, 1998)
	Poplar	Ionic liquid: 120°C, 1:20(Emim)OAc to biomass, 30 min	0	15	27	8/38[a]	-	76	(Samayam & Schall, 2010)
		AFEX: 180°C, 2:1 NH_3 to biomass, 30 min	0	0	0	47.9/49.9[a]			(Kumar et al., 2009)
		ARP: 183°C, 3.66:1 NH_3 to biomass, 27.5 min	6.8	31.8	39.2	49.5/49.9[a]	-	-	(Kumar et al., 2009)
		Dilute acid: 0.6 % H_2SO_4, 190°C, 70s	2.1	91.7	-	50.6/49.9[a]	-	-	(Kumar et al., 2009)
		Lime: 0.5:1 Ca $(OH)_2$ to biomass 65°C, 4 weeks	1.9	3.8	49.9	54.5/49.9[a]			(Kumar et al., 2009)
		SO_2-Steam explosion 200°C, 5 min	3.1	90.7	-	56.5/49.9[a]	-	-	(Kumar et al., 2009)

[a:] without pretreatment

[b:] surface area (SA) and digestibility of cellulose Avicel treated in the same conditions.

Table 3: General trends for the effects of pretreatment on lignocellulosic structure and characteristics and its impact on biohydrogen/biomethane and bioethanol processes

	Impact on CH4/H2 production	Impact on Bioethanol production	Achieved by	Results if conditions too severe
Increase of surface area	+	+	Mechanical treatment Irradiation Liquid hot water Steam explosion CO_2 explosion Acid Alkali AFEX Oxidation Organosolv Lignolytic enzymes	
Reduction of cellulose cristallinity	+	+	Ionic liquids Mechanical treatment Afex Oxidation	
Alteration of lignin structure (Cleavage of lignin-carbohydrate complex)	+	+	Alkali AFEX Oxidation CO_2 explosion Lignolytic enzymes Rot fungi Acid	
Solubilisation of hemicellulose	+	+	Acids Liquid hot water Steam explosion CO_2 explosion Oxidation $FeCl_3$ Organosolv Xylanase	oxidation O_3
Solubilisation of cellulose	+	- *	Acids/thermal Ionic liquid	Mechanical treatment Acids/thermal
Solubilisation of lignin	+	+	AFEX alkali oxidation O_3	

			Organosolv Ionic liquid Lignolytic enzymes Rot fungi	
Formation of furfural/HMF	No/low impact	-	Acids/thermal Organosolv Liquid hot water	
Mineralisation of hexoses	-	-		oxidation
Mineralisation of pentoses	-	No impact		oxidation

nd : not determined

+ : positive impact, - : negative impact

**: Effect of pretreatment before enzymatic hydrolysis of cellulose*

***Table 4**: BioH2 and Bio CH4 production in one- and two-stage processes*

BioEnergy	Substrate	Hydrogen yield ($L\ H_2 / kg\ VS_{added}$)	Methane yield ($L\ CH_4 / kg\ VS_{added}$)	Energy yield ($MJ / kg\ VS_{added}$)[a]	References
Bio H_2	Corn stalk	3.16		0.03	(Zhang et al., 2006)
	Corn-cob	13.1		0.14	(Pan et al., 2009)
	Poplar leaves	15		0.16	(Cui et al., 2010)
	Maize leaves	17		0.14	(Ivanova et al., 2009)
	Sweet sorghum	30.5		0.33	(Ivanova et al., 2009)
	Wheat straw	46		0.50	(Ivanova et al., 2009)
	Wheat bran	51		0.55	(Pan et al., 2008)
	Sweet sorghum stalk	52.1		0.56	(Shi et al., 2010)
	Fodder maize	61[c]		0.66	(Kyazze et al., 2008)
	Ryegrass	73[c]		0.79	(Kyazze et al., 2008)
	Beer residues	3.11[b]		0.034[b]	(Cui et al., 2009)
	Sweet sorghum	59[b]		0.63[b]	(Ntaikou et al., 2007)
	Wheat flour	60[b]		0.65[b]	(Hawkes et al., 2008)
	Fodder turnip	188[b]		2.02[b]	(Rechtenbach & Stegmann, 2009)
	Maize starch	190[b]		2.05[b]	(Rechtenbach & Stegmann, 2009)
Bio CH_4	Coconut fibers		66	2.63	(Kivaisi & Eliapenda, 1994)
	Newsprint		97	3.86	(Xiao & Clarkson, 1997)
	Corn stover		114	4.54	(Zheng et al.)
	Switchgrass		125	4.97	(Guiot et al., 2009)
	Wheat grass		160	6.37	(Romano et al., 2009)
	Sisal fibre		180	7.16	(Mshandete et al., 2006)
	Rice straw		190	7.56	(Zhang & Zhang, 1999)
	Corn silage		194	7.72	(Frigon et al., 2008)
	Willow		200	7.96	(Uellendahl et al., 2008)
	Miscanthus		200	7.96	(Uellendahl et al., 2008)
	Paper tube residues		222	8.83	(Teghammar et al., 2009)

	Rice straw		224	8.91	(Ghosh & Bhattacharyya, 1999)
	Barley straw		229	9.11	(Dinuccio et al., 2010)
	Grass hay		230	9.15	(Lehtomaki et al., 2004)
	Bagasse		237	9.43	(Kivaisi & Eliapenda, 1994)
	Wheat straw		276	10.98	(Bauer et al., 2009)
	Wheat straw		297	11.82	(Kaparaju et al., 2009)
	Sugar beet tops		310	12.33	(Lehtomaki et al., 2004)
	Red banana peel		322	12.81	(Gunaseelan, 2003)
	Potatoes		328	13.05	(Frigon et al., 2008)
	Potato pulp		332	13.21	(Kryvoruchko et al., 2008)
	Winter rye		336	13.37	(Petersson et al., 2007)
	Office paper		364	14.48	(Xiao & Clarkson, 1997)
	Maize sillage		370	14.72	(Bruni et al., 2010)
	Corn kernels		397[b]	15.8[b]	(Frigon et al., 2008)
	Summer switchgrass		403	16.04	(Frigon et al., 2008)
	Grass sillage		431	17.15	(Pakarinen et al., 2009)
	Citrus peels		455	18.10	(Gunaseelan, 2003)
Bio H_2 + Bio CH_4	Potatoes	200.4	130	7.33	(Xie et al., 2007)
	Grass sillage	5.6	467	18.36	(Pakarinen et al., 2009)
	Sweet sorghum	10.4[b,c]	107[b,c]	4.36[b,c]	(Antonopoulou et al., 2006)
	Maize	108[b]	426[b]	18.11[b]	(Rechtenbach & Stegmann, 2009)

[a] Energy yield 1Nm^3 CH4 = 39790 kJ; 1Nm^3 H2 = 10780 kJ.
[b] mL CH4 / kg DM added or mL H2 / kg DM added or MJ / kg DM added.
[c] adapted from the literature

***Table 5**: Comparison of the different profitable uses of energy of lignocellulosic substrates*

Lignocellulosic substrates	**Energy potential from bioethanol (MJ / kg $_{DM}$)**	**Energy potential from methane (MJ / kg $_{DM}$)**	**Calorific value combustion (MJ / kg $_{DM}$)**
Wheat straw	**6.7** (Kim & Dale, 2004)	**10.2** (Bauer et al., 2009)	**17.9** (Voivontas et al., 2001)
Barley straw	**7.2** (Kim & Dale, 2004)	**8.6** (Dinuccio et al., 2010)	**17.5** (Voivontas et al., 2001)
Rice straw	**6.5** (Kim & Dale, 2004)	**7.9** (Ghosh & Bhattacharyya, 1999)	**16.8** (Voivontas et al., 2001)
Rye straw	-	**12.3** (Petersson et al., 2007)	**16.8** (Voivontas et al., 2001)
Sugarcane bagasse	**6.5** (Kim & Dale, 2004)	**8.8** (Kivaisi & Eliapenda, 1994)	**19.4** (McKendry, 2002)
Miscanthus	-	**7.64** (Uellendahl et al., 2008)	**18.5** (McKendry, 2002)
Switchgrass	**6.5** (Guiot et al., 2009)	**5-15** (Frigon et al., 2008)	**17.4** (McKendry, 2002)
Willow	-	**7.64** (Uellendahl et al., 2008)	**20** (McKendry, 2002)
Poplar	-	**11.93** [a] (Chynoweth et al., 1993)	**18.5** (McKendry, 2002)

[a] MJ / kg $_{vs}$

***Table 6**: Effect of pretreatment on bioH_2 and bio CH_4 production*

Pretreatment method	**Processes**	**Lignocellulosic material**	**Pretreatment conditions**	**BioH_2 production (L H_2 / kg VS_{added})**	**BioCH_4 production (L CH_4 / kg VS_{added})**	**Energy from pretreated biomass (MJ / kg VS_{added})**	**Energy from raw biomass (MJ / kg VS_{added})**	**Energy gain (%)**	**References**
Physical	Grinding	Wheat straw	0.4mm		248	9.87	6.45	53	(Sharma et al., 1988)
		Bermuda grass	0.4mm		228	9.07	5.45	66	(Sharma et al., 1988)
		Grass	5mm		320	12.73	-	-	(Kaparaju et al., 2009)
		Sisal fibre	2mm		220	8.75	7.16	22	(Mshandete et al., 2006)
		Maize sillage	2-8mm		410	16.31	14.72	11	(Bruni et al., 2010)
Thermo-chemical	Steam explosion	Corn straw	1.5 MPa,10 min, + cellulase (25 FPU / g)	68[b]		0.73[b]	-	-	(Li & Chen, 2007)
		Wheat bran	0.27 MPa, 60 min, 0.01M HCl	86		0.93	0.55	69	(Pan et al., 2008)
		Bamboo	5min / 243°C		215	8.55	-	-	(Kobayashi et al., 2004)
		Potato pulp	15min / 107°C		373	14.84	13.21	12	(Kryvoruchko et al., 2008)
		Wheat straw	10 min / 170°C		361	14.36	10.98	31	(Bauer et al., 2009)
		Paper tube residues	10min / 220°C / 4% H_2O_2 (w/w) + 4% NaOH (w/w)		493	19.62	8.83	122	(Teghammar et al., 2009)
		Cornstalks	5 min / 1.6 MPa	63.7[b]	114.6[b]	5.25[b]	-	-	(Lu et al., 2009)
	Wet oxidation	Miscanthus	-		360	14.32	7.96	80	(Uellendahl et al., 2008)
		Willow	-		360	14.32	7.96	80	(Uellendahl et al., 2008)
		Winter rye	2g/L Na_2CO_3 / 195°C / 15 min / 12 bar O_2		447	17.79	13.37	33	(Petersson et al., 2007)
Chemical	Oxidative	Wheat flour	2 % H_2O_2 (w/v), 4h, 60°C	31[b]	264[b]	10.84[b]	-	-	(Hawkes et al., 2008)
		Paper tube residues	4% H_2O_2 (w/w) / 190°C / 30min		233	9.27	8.83	5	(Teghammar et al., 2009)

	Alkaline	Cornstalks	0.5 % NaOH	57		0.62	0.03	1966	(Zhang et al., 2006)
		Bagasse	4% NaOH (w/v), 100°C, 2h + Cellulase, 20 FPU/g	300		3.23	-	-	(Chairattanamanokorn et al., 2009)
		Miscanthus hydrolysate	12 % NaOH (w/w), 70°C, 4h	29.5[a]		0.32[a]	-	-	(de Vrije et al., 2002)
		Sweet sorghum stalk	0.4% NaOH, 20°C, 24h	127		1.37	0.56	144	(Shi et al.2010)
		Paper tube residues	4% NaOH (w/w) / 190°C / 30min		269	10.70	8.83	21	(Teghammar et al., 2009)
		Bagasse	1M NaOH / 25°C / 30days		-			44	(Kivaisi & Eliapenda, 1994)
		Coconut fibers	1M NaOH / 25°C / 30days		-			73	(Kivaisi & Eliapenda, 1994)
		Grass hay	4% NaOH (w/w) / 25°C / 24h		270	10.74	9.15	17	(Lehtomaki et al., 2004)
		Sugar beet tops	2% NaOH (w/w) / 20°C / 24h		350	13.93	12.33	13	(Lehtomaki et al., 2004)
		Corn stover	2% NaOH (w/w) / 20°C / 3 days		215	8.55	4.54	89	(Zheng et al., 2009)
		Bagasse	1M NH_4OH / 25°C / 30days		-			22	(Kivaisi & Eliapenda, 1994)
		Coconut fibers	1M NH_4OH / 25°C / 30days		-			46	(Kivaisi & Eliapenda, 1994)
		Grass hay	3% $Ca(OH)_2$ (w/w) + 4% Na_2CO_3 (w/w) / 25°C / 72h		270	10.74	9.15	17	(Lehtomaki et al., 2004)
		Rice straw	2% NH3 / 90°C / 10mm		245	9.75	7.56	29	(Zhang & Zhang, 1999)
		Grass sillage	4% NaOH (w/w) / 20°C / 24h	6.5	473	18.89	18.36	2.8	(Pakarinen et al., 2009)
	Acid	Cornstalk	0.2 % HCl , boiled 30 min	150		1.62	0.03	5300	(Zhang et al., 2006)
		Beer lees	2 % (w/v) HCl	53[b]		0.57[b]	0.03[b]	1800	(Cui et al., 2009)
		Poplar leaves	4 % (w/v) HCl	33.5[b]		0.36[b]	0.16[b]	125	(Cui et al., 2010)
		Wheat straw	2 % HCl + 4 min microwave	68		0.73	0.005	136	(Fan et al., 2005)
		Wheat bran	0.01M HCl, boiled 30 min	81		0.87	0.55	58	(Pan et al., 2008)
		Wheat bran	0.01M HCl + 9 min microwave (800 W)	93		1.00	0.55	81	(Pan et al., 2008)
		Corncorb	1% HCl / 100°C / 30 min	108		1.16	0.14	728	(Pan et al., 2009)

		Newsprint	30% acetic acid / 2% HNO3		271	10.78	3.86	179	(Xiao & Clarkson, 1997)
		Bagasse	1M HCl / 25°C / 30 days		-			32	(Kivaisi & Eliapenda, 1994)
		Coconut fibers	1M HCl / 25°C / 30 days		-			76	(Kivaisi & Eliapenda, 1994)
Biological	Micro-organisms	Rice straw	White rot-fungus *Phanerochaete chrysosporium*		328	13.05	8.91	46	(Ghosh & Bhattacharyya, 1999)
		Rice straw	Brown rot-fungus *Polyporus ostreiformis*		295	11.74	8.91	32	(Ghosh & Bhattacharyya, 1999)
		Grass hay	White rot-fungus *Pleurotus ostreatus*		240	9.55	9.15	4	(Lehtomaki et al., 2004)
		Maize leaves	Aerobic bacterium *Bacillus amyloliquefaciens*	73.13		0.78	0.18	333	(Ivanova et al., 2009)
	Enzyme	Bagasse	2mm / 100°C, 2h + cellulase (20FPU/g)	31.36		0.34	-	-	(Chairattanamanokorn et al., 2009) 9
		Poplar leaves	2 % (v/v) viscozyme L	45[b]		0.49[b]	0.16[b]	206	(Cui et al., 2010)
		Switchgrass	Lignin peroxidase		202	8.04	4.97	62	(Guiot et al., 2009)
		Switchgrass	Manganese peroxidase		223	8.87	4.97	78	(Guiot et al., 2009)
		Grass hay	2 Xylanases + 2 cellulases, 0.1 % (w/w)		280	11.14	9.15	22	(Lehtomaki et al., 2004)
		Potatoes	0.1 % (w/w) α amylase + 0.2% (w/w) glucoamylase	271	145	8.69	7.33	18.5	(Xie et al., 2007)

[a] adapted from the literature

[b] mL CH4 / kg DM added or mL H2 / kg DM added or MJ / kg DM added.