1. Introduction

The first generation of biofuels, made out of starch (ethanol) or triacylglycerol (biodiesel) uses expensive homogeneous feedstocks (sugar cane, corn, wheat and edible oils) coupled with relatively inexpensive technologies known and practiced for years at an industrial level. First generation biofuels have had a bad press: high water and energy consumption (very significant is the energy used in the production of the fertilizers needed by agriculture) and the fuel versus food controversy. Increased use of biofuels requires alternative sources of biomass that lower water and energy consumption and do not compete with food supplies. Lignocellulosic biomass, either from forestry or agriculture offers such potential. Cellulose, the most abundant carbohydrate on the planet, is a fraction of the complex lignocellulosic matrix along with other macromolecules, lignin, hemicelluloses, and extractives. The cellulose macromolecule is composed of glucose units linked together via β-1,4-glycosidic bonds (or acetal bonds) creating long chains that combine together to form fibrils and eventually fibres. The polar hydroxyl groups are oriented one toward the other so that interaction with a polar medium (as a solvent) is fairly difficult making cellulose water resistant. The natural macromolecule is usually present in nature in two forms: crystalline and amorphous. A typical fibril will have zones that are crystalline separated by zones that are amorphous. Whilst the crystalline form is difficult to disassemble with hydrolyzing agents, the amorphous phase has a certain level of disorder that makes relatively easy the penetration and action of hydrolysing agents, either enzymes or ionic species. The other macromolecules found in the lignocellulosic matrix are also of interest. Lignin is a macromolecule composed of phenylpropane units bond together via, predominantly, ether bonds although C-C between moieties are also significant. Lignin, has low oxygen content and thus a high energetic value. Hemicelluloses are, as cellulose, macromolecules composed of carbohydrates. Upon hydrolysis, the C6 fraction of these carbohydrates can effectively be converted to ethanol via fermentation using classical yeast strands (Gírio, 2009). Studies have shown that fermentation of all the glucidic part of the hemicelluloses, both C6 and C5 sugars, was feasible using nontraditional microorganisms (Agbogbo & Coward-Kelly, 2008; Casey et al., 2009; Chu & Lee, 2007). It is also well known
that hydrolysis of hemicelluloses produces / liberates organic acids that could inhibit fermentation of the carbohydrate.

Cellulosics being an alternative for ethanol production, there is still an important aspect that has to be considered: the cost of the feedstock. Lignocellulosics for sugar production and subsequent fermentation can be considered to belong to three categories which interlink biomass cost, quality and transformability: homogeneous, quasi-homogeneous and non-homogeneous. The first category comprises structural and furniture wood and chips for pulp production which requires a single species (or a mixtures of comparable species). Such homogeneous biomass has also a rather homogenous chemical composition and it is used for high end products with well established markets. Homogeneous biomass is expensive, above $US 100 / tonne (anhydrous basis equivalent) in the NorthEast of America (2011 basis, prices range is a courtesy of CRB Innovations). Besides cost, such biomass also has a large market, in structural wood and in the pulp and paper industry. Quasi homogeneous biomass is usually composed of a mixture of species and to a certain extent, of tissues. This category embraces the residual lignocellulosic biomass produced during forest or agricultural operations. Cost range for this biomass will vary, FOB conversion plant in 2011, NorthEast of America, from $US 60 to 80 per tonne, dry basis, mostly related to transportation costs in a radius of a maximum of 100 km from harvesting operations (prices are a courtesy of CRB Innovations). Contrary to the homogeneous feedstock which has a wide and diversified market (yet very competitive), the quasi-homogeneous feedstock, since such biomass is of lesser homogeneity and often includes a higher quantity of ashes, is less coveted. Therefore, this biomass could be the main feedstock of the upcoming cellulosic ethanol market since the competition is actually low, the feedstock does not compete with food supply and the biomass is readily available close to major cities in the world. The last category of biomass is non-homogeneous. It is of lesser quality than the previous categories and usually costs close to 0 USD ( it may even come with a tipping fee in some cases). The low cost is of course attractive but the conversion process will have to use such biomass as a whole complex mixture converting it to a more homogeneous intermediary. Although we acknowledge the availability and the potential of each type of biomass, this chapter will be focused on the residual lignocellulosic material generated from established forest and agricultural industries (quasi-homogeneous biomass) as well as on plantation biomass.

Plantation biomass can be identified as “energy crops” which are grown, ideally on marginal lands, with two objectives; sequestering carbon and bioconverting it into carbon-based structured macromolecules that could be used for the production of bioenergy. In North America, some cultures that have gained attention during the last 10 years, amongst many: willows, poplars, miscanthus, switchgrass, panic, reed canary grass, etc. Depending on the targeted market, these energy crops could be oriented towards high yields of cellulose (if the ethanol market is the main target) or high yield of lignin and less ashes (if the combustion market is targeted).

Biomass cost and composition are the main concerns of a cellulosic ethanol plant. A technological issue is how to convert the carbohydrates to low cost monomeric sugars in high yields. Cellulose has been separated from plants for decades by the pulp and paper industry, the latter having developed industrial scale facilities that converted large quantity of lignocellulosic biomass to pulp and paper. However, the established processes to isolate the cellulose use large quantities of water putting a stress on water supplies. Furthermore, the pulp and paper does not actually use the hemicellulose and lignin other than for CHP production. Research around the world have been focusing in the past decades toward processes that recover and use most of the carbon present in biomass to create true biomass
refineries from which multiproducts would be obtained. This requires a careful consideration of which biomass to use to achieve valuable multiproducts and which biomass to use to provide heat and power.

The key technological challenge for the production of cellulosic ethanol is depolymerizing the cellulose to obtain high yields of glucose. As mentioned earlier, cellulose is a compact macromolecule, particularly its crystalline fractions, and it requires specific enzymes or chemicals to allow hydrolysis of the β-1,4-glycosidic bonds. Accessibility of enzymes and chemical hydrolytic agents is a function of the three-dimensional ultrastructure of cellulose. Therefore, before going forward with production of cellulosic ethanol, the composition of the original feedstock and the ultrastructure of its isolated cellulosic fraction has to be known in order to adapt the hydrolytic processes to such ultrastructure.

This chapter focuses on three aspects that should be closely related to the production of second generation ethanol. In a first section, the composition of different substrate will be review as for their cellulosic, hemicellulosic and lignin contents. These data are essential for adaptation of the downstream process of a biorefinery. The second section of this chapter will be aimed at reviewing the steam treatments from our experience with the Feedstock Impregnation Rapid and Sequential Steam Treatment (FIRSST) process developed through two and a half decades of effort within our extended team (fundamentals at the academic level; engineering and technology via the spin-off company, CRB). Finally, the third section of this chapter will be an overview of the chemical treatments for cellulose hydrolysis compared with the CRB decrystallization and depolymerization process whose fundamental basis was developed by our team at the Université de Sherbrooke.

2. Biomass

Lignocellulosic biomass is a readily available feedstock that can be purchased yearly from forest and agricultural operations. Forest residues comprise unusable trunk sections, limbs and tops. Typical composition of these residues is similar to that of common wood chips shown in Table 1. We define, as agricultural residues, the non-edible part of the plant which is let on the field after the harvest and the latter are usually composed of straw and stalk. It also comprises the parts of the cultivated plants that are thrown out after industrial processes. A specific example of such biomass includes but is not limited to corn cobs. Agricultural residues are the most probable feedstock that will be the original source for the production of ethanol from lignocellulosic materials due to their availability, their quantity and their proximity to the existing grain to ethanol platform. Non conventional plantation crops (i.e. ‘energy crops’ ) are also to be considered as feed for biorefineries. Most of these crops have not reach industrial scale production (in North America) but an increasing amount of information has been published during the last few years about their chemical composition. Pricing for this biomass has been evaluated to 100-120$ per (dry basis; prices courtesy of CRB Innovations) metric ton but it tends to decrease because of a reduced use of fertilizers and the utilisation of marginal lands instead of high value agricultural land. The characteristics which make energy crops, especially perennial grasses, attractive for ethanol production, are the high amount of cellulose and hemicellulose as well as, under certain restriction, the favorable environmental impact.

Lignocellulosic biomass is composed of cellulose, hemicelluloses, lignin, extractives and ashes. The quantities of each fraction are detailed below for a large range of lignocellulosic materials including agricultural residues, energy crops and forest residues which are
divided into leafy hardwoods and coniferous families (Table 1). Cellulose is the principal constituent of lignocellulosic plants representing 30-50 wt% of its composition. It is a polymer composed of D-glucose. Contrarily to cellulose, hemicelluloses are a heterogeneous polymer principally composed of pentoses (β-D-xylene, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic, α-D-galacturonic acids). Among them, xylans and glucomannans are the most common compounds. Hemicelluloses represent 15-35 wt% of the plant. We can define lignin as a relatively hydrophobic amorphous polymer which consists of phenylpropane units. This macromolecule occurs primarily between the fibre cells, acting as a cementing material and giving the wood its rigidity and its impact resistance. It is always associated with hemicelluloses through carbon-carbon and ether linkages (Xu et al., 2008) and can be classified following 2 major classes (Gibbs and Thimann, 1958): (1) the guaiacyl which includes most of the lignins of softwoods (gymnosperms), (2) the guaiacyl–syringyl which comprises the lignins of hardwoods (angiosperms) and the lignins of grasses (non woody or herbaceous crops) (angiosperms). Extractives are composed of resins, fats and fatty acids, phenolics, phytosterols, terpenes, salts and minerals. This fraction is not used for the production of ethanol, and, for obvious reasons, neither are the ashes. The latter is defined as the residue remaining after total combustion. It is composed of elements such as silicon, aluminum, calcium, magnesium, potassium, and sodium. Typically, the amount of each fraction can also differ within a single biological species (following the environment: soil composition, water supply and weather patterns) and also during the growth of the plant making the quantification of sugar present in the holocellulose (sum of hemicelluloses and cellulose) difficult to specify.

The valorization of the lignocellulosic materials and more particularly of the carbohydrates (composing the holocellulose) into ethanol is made possible through their fermentation. Each plant has different composition (Table 1) but, as detailed before, contains the same major compounds. All the biomasses show comparable characteristics (Table 2) with the following order by quantity: Glucan>Xylan>Mannan-Galactan-Arabinan, except for the coniferous forest residues which show a high amount of mannan, which leads to a shift between the glucan and the xylan. Table 2 also shows an average of the 6 carbons sugars (C6) which can be fermented by most common yeast (including but not limited to S.cerevisiae) to give ethanol. Agricultural residues, energy crops and leafy forest residues present high averages of 41.5, 46.6 ans; 48.2 wt% respectively. Furthermore, coniferous forest residues could potentially produce more ethanol as they possess a very high amount of C_6 (56 %) sugars which can be explained by a high amount of mannose (about 10 % more than the other species).

North America produced 46% of the world biofuels in 2008 (IEA, 2009) and the R &D efforts on second generation biofuels have been widely orientated toward the production of ethanol. From the results in Table 2, it is possible to estimate the ethanol production directly from C_6 sugars using S. cerevisiae. Production of ethanol from C_5 sugar was not taken into account in our study as these sugars require the use of special microorganisms. C_5 sugars, although hard to ferment to ethanol could be converted into other value added products as ethyl levulinate (considered as part of the extended P-fuel pool) by successive dehydration, reduction and ethanolysis. Table 3 shows a comparison between the actual possible production of ethanol (not operational) using the forest residues, the agricultural residues and the unexploited forest biomass available in Quebec, Canada and North America versus the operational ethanol production from energy crop (first generation of biofuel) and the consumption of gasoline. Only 25 % of the forest and agricultural residues have been taken
into account for ethanol production estimate as the rest of the biomass is already dedicated for others purposes. In the case of the unexploited forest, our study presents a result based on the forest zone which can be used without causing damage on biodiversity (Ministère des Resources Naturelles et de la Faune MNRF, Quebec, 2009).

| Agricol residues               | Cellulose (wt%) | Hemicellulose (wt%) | Lignin (wt%) | Extractives (wt%) | Ashes (wt%) |
|--------------------------------|-----------------|---------------------|--------------|-------------------|-------------|
| Rice straw<sup>a</sup>         | 41.2            | 19.5                | 21.9         | -                 | -           |
| Wheat straw<sup>a</sup>        | 39.7            | 36.5                | 17.3         | -                 | -           |
| Rye straw<sup>b</sup>          | 37.9            | 36.9                | 17.6         | -                 | -           |
| Tritical straw<sup>v</sup>     | 34              | 31.7                | 17           | 12.4              | -           |
| Flax straw<sup>c</sup>         | 53.8            | 17.1                | 23.3         | -                 | 3.6         |
| Sunflower stalk<sup>a</sup>     | 37.6            | 29.3                | 10.3         | -                 | -           |
| Sorghum stalk<sup>a</sup>      | 41.5            | 24.4                | 15.6         | -                 | -           |
| Cotton stalk<sup>a</sup>       | 58.5            | 14.4                | 21.5         | -                 | -           |
| Corn stover<sup>d</sup>        | 38              | 26                  | 19           | -                 | 6           |
| Barley straw<sup>e</sup>       | 42              | 28                  | -            | -                 | 11          |
| Vine shoots<sup>a</sup>        | 41.1            | 26                  | 20.4         | -                 | -           |
| Olive pruning<sup>s</sup>      | 35.7            | 25.8                | 19.7         | -                 | -           |

| Energy crops                   |                 |                     |              |                   |             |
|--------------------------------|-----------------|---------------------|--------------|-------------------|-------------|
| Switchgrass                    | 37              | 28                  | 16.4         | 15                | 3.7         |
| Miscanthus                      | 40              | 18                  | 25           | -                 | -           |
| Big bluestern<sup>f,g,h</sup>  | 37              | 28                  | 18           | -                 | 6           |
| Little bluestern<sup>h</sup>   | 35              | 31                  | -            | -                 | 7           |
| Prairie cordgrass<sup>i</sup>  | 41              | 33                  | -            | -                 | 6           |
| Indian grass<sup>j,h</sup>     | 39              | 29                  | -            | -                 | 8           |
| Intermediate wheatgrass<sup>s</sup> | 35 | 29 | - | - | 6 |
| Reed canarygrass<sup>i,k</sup> | 24              | 36                  | -            | -                 | 8           |
| Smooth bromegrass<sup>j,k</sup>| 32              | 36                  | -            | -                 | 6           |
| Tymothyb<sup>l,m</sup>         | 28              | 30                  | -            | -                 | 6           |
| Tall fescue<sup>n</sup>        | 25              | 25                  | 14           | -                 | 11          |
| Sundan grass<sup>j,k</sup>     | 33              | 27                  | -            | -                 | 8           |
| Jatropha stem<sup>b</sup>      | 37.1            | 30.6                | 22.3         | -                 | -           |
| Jatropha seed cake<sup>e</sup> | 13.5            | 26.8                | 12.4         | -                 | -           |
| Cannabis sativa<sup>v</sup>    | 43              | 26                  | 14.5         | 16                | 3.2         |
| Salix viminalis<sup>v</sup>    | 30.2            | 33.5                | 29.2         | 8.8               | -           |

| Forest residues                |                 |                     |              |                   |             |
|--------------------------------|-----------------|---------------------|--------------|-------------------|-------------|
| Leafy                          |                 |                     |              |                   |             |
| Soft maple                     | 41              | 35                  | 24           | -                 | -           |
| Red oak<sup>r</sup>            | 35.5            | 18.8                | 29           | -                 | -           |
| European oak<sup>q</sup>       | 38              | 29                  | 25           | 4.4               | 0.3         |
| White oak<sup>r</sup>          | 44              | 24                  | 24           | 5.4               | 1           |
| Chesnut oak<sup>r</sup>        | 41              | 30                  | 22           | 6.6               | 0.4         |
| Post oak<sup>r</sup>           | 38              | 30                  | 26           | 5.8               | 0.5         |
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|                | Cellulose (wt%) | Hemicellulose (wt%) | Lignin (wt%) | Extractives (wt%) | Ashes (wt%) |
|----------------|-----------------|---------------------|-------------|-------------------|------------|
| White birch $^r$ | 45              | 33                  | 18          | 5                 | 0.3        |
| Yellow birch   | 40              | 39                  | 21          | -                 | -          |
| Quaking aspen $^r$ | 49           | 29                  | 19          | 6                 | 0.4        |
| White elm      | 49              | 27                  | 24          | -                 | -          |
| Beech          | 42              | 36                  | 22          | -                 | -          |
| Basswood       | -               | -                   | -           | -                 | -          |
| Poplarwood     | 41.4            | 23.7                | 24.5        | -                 | -          |
| Eucalyptus $^a$ | 52.8            | 27.7                | 20          | -                 | -          |

Coniferous

- Pinus pinaster $^a$: 35.9 13.7 26.2 - -
- Pinus radiate chips $^c$: 53 15.8 23.7 - -
- Cedar $^a$: 43.5 20.3 - - -
- Eastern Hemlock: 42 26 33 - -
- Eastern white cedar: 44 25 31 - -
- White spruce: 44 29 27 - -
- Jack pine: 41 30 29 - -
- Tamarack: 43 28 29 - -
- Spruce $^t$: 54.1 21.4 24.4 - -
- Loblolly pine $^u$: 43.6 21.2 26.8 3.2 0.4
- Balsam-fir: 44 27 29 - -

Table 1. Chemical composition of various lignocellulosic materials

In the case of forest residues, we can assume that for 1 m$^3$ of roundwood exploited, 0.6 m$^3$ of residual biomass is left behind (Smeets & Faaïj, 2007). In the province of Quebec, forest residues have been estimated to 6.9 millions of tons per year (Goyette & Boucher, 2009). Thus, the production of ethanol from glucose fermentation can be determined assuming that the average of this sugar in such materials is about 52.4% (calculated from the Table 2) and that the maximum yield is equal to 0.51g of ethanol per g of glucose. Thus, 584 millions liters of ethanol could be produced in Quebec. To put such a value in perspective, consumption of refined petroleum in Quebec reached 9 billion liters in 2007 (Ministères de Ressources Naturelles et de la Faune, MNRF, Quebec, 2009). The production of ethanol from forest residues is sufficient to reach the objective fixed by the government (5 vol% in gasoline in 2012) since it represents 6.5 vol%. The North American consumption of gasoline for transport was estimated in 2008 at 518 739 millions liters with respectively 479 243 millions liters for the United States of America and 39 496 millions liters for Canada (IEA energy statistic, 2010). More ethanol can be produced by using agricultural residues, energy crops and unexploited forest zone. In Quebec, the latter represents 14,100,000 m$^3$ or 5.6 millions tons assuming an average density of 400kg/m$^3$. Thus, 1 638 millions liters of ethanol can be produced per year. Quebec will be able to replace 24.7 vol% of its gasoline by ethanol just by using exploited forest zone and forest residues, thus using only residual...
biomass. The comparison between the gasoline consumption and the possible production of ethanol from residues shows undeniably the importance of such source of raw material. Furthermore, the production of ethanol coming from the latter could rise above the production of ethanol coming from the first generation of biofuel. As an example, in North America, the nameplate production of ethanol from grain represents 53,949 millions of liters per year while the exploitation of residues could give more than 60,000 millions of liters per year.

|                 | Glucan (wt%) | Xylan (wt%) | Mannan (wt%) | Galactan (wt%) | Arabinan (wt%) | Lignin (wt%) |
|-----------------|--------------|-------------|--------------|----------------|----------------|--------------|
| Agricol residues|              |             |              |                |                |              |
| Corn stover     | 39           | 14.8        | 0.3          | 0.8            | 3.2            | 13.1         |
| Rice hulls      | 41           | 14.8        | 1.8          | 0.4            | 4.5            | 9.9          |
| Rice straw      | 36.1         | 14          | 3            | 0.1            | 2.6            | 19.4         |
| Wheat straw     | 36.6         | 19.2        | 0.8          | 2.4            | 2.4            | 14.5         |
| Triticale straw | 43.5         | 17.7        | -            | -              | 2.3            | 17           |
| Sugar cane      | 41.3         | 21.8        | 0.3          | 0.5            | 1.8            | -            |
| C6 Average      | 39.5         | 1.2         | 0.8          |                |                |              |
| Energy crops    |              |             |              |                |                |              |
| switchgrass     | 35.2         | 21.7        | 0.2          | 0.9            | 2.8            | 27.4         |
| Miscanthus      | 44           | 21          | -            | -              | -              | -            |
| Hemp            | 51           | 14.3        | 1.5          | 0.7            | 1.3            | 14.5         |
| Sweet sorghum   | 44.6         | 25.3        | -            | -              | -              | 18           |
| Bagasse fiber   | 38.1         | 13          | 8            | -              | 2              | 20           |
| C6 Average      | 42.6         | 3.2         | 0.8          |                |                |              |
| Forest residues |              |             |              |                |                |              |
| Leafy           |              |             |              |                |                |              |
| Populus tristis | 40           | 13          | 8            | -              | 2              | 20           |
| Oak             | 45.2         | 20.3        | 4.2          | -              | -              | -            |
| Red Maple       | 46.0         | 19.0        | 2.4          | 0.6            | 0.5            | 24           |
| Aspen           | 45.9         | 16.7        | 1.2          | 0              | 0              | 23           |
| Salix           | 41.4         | 15.0        | 3.2          | 2.3            | 1.2            | 26.4         |
| Yellow poplar   | 42.1         | 15.1        | 2.4          | 1              | 0.5            | 23.3         |
| Eucalyptus      | 48.1         | 10.4        | 1.3          | 0.7            | 0.3            | 26.9         |
| C6 Average      | 44.1         | 3.2         | 0.9          |                |                |              |
| Coniferous      |              |             |              |                |                |              |
| Spruces         | 43.2         | 5.7         | 11.5         | 2.7            | 1.4            | 28.3         |
| Lodgepole pine  | 42.5         | 5.5         | 11.6         | 2.1            | 1.6            | 27.9         |
| Ponderosa pine  | 41.7         | 6.3         | 10.8         | 4.7            | 1.8            | 26.9         |
| Douglas-fir     | 44           | 2.8         | 11.0         | 4.7            | 2.7            | 32           |
| Loblolly pine   | 45           | 6.8         | 11.0         | 2.3            | 1.7            | 28           |
| Red pine        | 42           | 9.3         | 7.4          | 1.8            | 2.4            | 29           |
| C6 Average      | 43.1         | 10.6        | 3            |                |                |              |

a(Lee, 1997); b(Sørensen et al., 2008); c(Ballesteros et al., 2004); d(Wang et al. 2008); e(Sassner et al., 2008); f(Zhu & Pan, 2010); g(Zhu et al., 2009); h(Youngblood et al., 2009); i(Pettersen, 1984); jMesured in our laboratory

Table 2. Details of carbohydrates and lignin amounts present in various lignocellulosic materials
### Table 3. Comparison between the actual possible production of ethanol from C<sub>6</sub> sugars contained in the lignocellulosic biomass, the operational ethanol production from energy crop and the gasoline consumption

|                | Possible Production of ethanol (Millions of liters per year) | Production of ethanol operational from energy crop (Millions of liters per year) | Gasoline consumption (Millions of liters per year) |
|----------------|-------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------|
|                | Agricultural residues (25%) | Forest residues (25%) | Unexploited Forest | Quebec | Canada | North America |
|                | - | 584 | 1638 | 155<sup>(a)</sup> | 9000<sup>(d)</sup> |
|                | 5097<sup>(e)</sup> | 3353 | - | 1821<sup>(e)</sup> | 39496<sup>(f)</sup> |
|                | 41483<sup>(e)</sup> | 20322<sup>(e)</sup> | - | 53949<sup>(b)</sup> | 518739<sup>(f)</sup> |

(Canadian Renewable Fuels Association, 2010), (b) (Renewable Fuels Association, 2010) (c) Estimated from the production of roundwood; (d) (Natural Resources Canada, 2007) (e) Estimated from FAOSTAT (FAOSTAT, 2010) thanks to the coefficient of residues proposed by D. Bellerini (Bellerini, 2006); (f) IEA energy statistic (IEA, 2011)

In this estimation of the possible production of ethanol, marginal lands have not been taken into account. The potential of surplus land for the cultivation of energy crops like willows, poplars, miscanthus, switchgrass, panic, reed canary grass (second generation of biofuel) considerably depends on the regions. Numerous constraints exist for the implementation of new energy crops, making the estimation of biofuel production very approximative. The first one is food competition. In fact, in major countries, populations are growing, consequently increasing the food demand therefore reducing surplus land, which overall limits the additional production of energy crops. Among the others constraints we can mention, the water shortages, the implementation of indigenous species (could be a problem for biodiversity), the type of plant, improvement of agricultural system (allowing the cultivation of other land), etc. Depending on the scenario, disparate results are obtained; bioenergy could reach between 39EJ to 204 EJ in 2050, furthermore, Smeets et al. 2007 show that the biggest energy apport will be done by dedicated energy crops with 20-174 EJ of biomass against 6-11 EJ of agricultural residues and 6 EJ of forestry residues. More than replacing gasoline coming from fossil resources, the employment of biofuels in well defined conditions can contribute to reduce the Greenhouse Gas (GHG) emissions. The GHG balance varies significantly following the choice of biomass, the technology employed throughout the full « fuel cycle » from biomass production to final fuel consumption, the characteristic of the land and climate, the crop management, etc. Thus, the choice of biomass is essential. As for ethanol production, the potential of lignocellulosic biomass to reduce GHG is comprised between 60 and 120 % and it is comparable to the high diminution of GHG observed with sugar cane (90%). In comparison, production of ethanol from wheat grain brings a lesser gain of 20 to 50% (IEA, 2004). The reduction, especially for lignocellulosic biomass, is due to the composition of plant itself, to fertilizer loading and to the efficiency of vehicles. The high reduction of carbon dioxide emissions in the case of lignocellulosic biomass (cellulose to ethanol) essentially comes from the use of the other part of the plant (mainly lignin) as a source of energy for the process. However the previous
estimations do not take into account the modifications affecting the lands. In fact, the GHG balance of the second-generation biofuels are closely related to the land use change (LUC) and the indirect use change (ILUC) which could in certain case conduct to a negative GHG balance. When a prior land-use like forest is replaced by culture for biofuel production, a direct land-use change occurs which can change the carbon stock of that land. This aspect has been widely studied and factors of changing balance can be found in the literature. However, the changes on GHG are induced by ILUC (takes place when land use change implies the displacement of the previous activity on another land). Thus, the replacement of sparsely vegetated and certain grassland by energy crops could generate a positive effect on GHG and in the mean time participate to the stockage of carbon in soil. Contrarily, the estimation made by Farrel and O’hare (2008) on the ILUC GHG emission shows that if the actual crops of soybean are used for the production of ethanol, the result could lead to the expansion of soybean for food into forests and will conduct to more emission than the use of fossil resources (6 times more). These cultivations can also have several positive or negative impacts on soil, water and biodiversity. ILUC are normally less important if residues are used as feedstock since there is no need for additional land to be cultivated.

The second-biofuel generation, and in particular in the case of lignocellulosic ethanol production, should start with a sustainable development of agricultural and forestry residues which are at that time very interesting in terms of productivity (as it was shown on the Table 3) and environment. Even if some species contains much more C$_6$ sugars like coniferous and notably loblolly Pine, the use of a wide range of biomass genotypes is advised and needed. All the species presented show an interest for ethanol production. In fact, the use of just the best species would be catastrophic for biodiversity. In general, lignocellulosic biomass is a promising source of fuel as it is shown on the figure 1. As an example, in about ten years the production of biofuel via the lignocellulosic biomass in the US could almost reach the same production as from the other sources of biofuel.

![Fig. 1. Biofuel Mandate from lignocellulosic materials in the United States Renewable Fuels Standard](www.intechopen.com)
3. Fractionation

3.1 Basics informations about steam treatments

The first technological challenge restraining the commercialisation of the cellulosic biomass industry is the fractionation of the lignocellulosic biomass to isolate the cellulose macromolecule. Similar processes have been applied by the pulp and paper industry for decades but there is a new objective now, the complete utilisation of the carbon-based structures of the biomass as well as a reduction of the water consumption, hence, the concept of biorefinery. Although the traditional pulping processes are being remoulded to fit with the biorefinery approach, other processes are also investigated among which are the organosolv process and steam treatments. There is a significant variety of different steam treatments which all rely on the same concept: biomass is first saturated with a solvent (usually water) with or without the utilisation of a catalyst (acid or basic depending on the targeted macromolecule). The mixture is then “cooked” by addition of steam in a pressure-resistant vessel for a certain period after which a valve is open, and the vapor phase exits the vessel through a nozzle entraining the solids. The exiting vapor reaches very high velocities as a function of the geometry of the nozzle thus reaching a sonic velocity. A “explosion” takes place while induced by the sonic field. The water saturating the biomass in its pores rapidly expands to vapour causing cell changes which vary from simple fibrilar disaggregation to fragmentation. During cooking, water, at high pressure and temperature, has a high dissociation constant leading to the occurence of a larger quantity of hydronium ions directly formed in the saturated pores of the biomass. Hemicelluloses, which are highly ramified and relatively easy to hydrolyse (in comparison to cellulose), will be affected by the increasing concentration of ions in the solution. The reaction of water and biomass is of course a major concern when considering steam treatments and it was found that in the absence of a catalyst, the two most important factors that were related to fractionation of the biomass and the hydrolysis of hemicelluloses were temperature and cooking period. The relationship between both parameters has been related to a mathematical equation called the “severity factor”. This equation, reported first by Overend and Chornet (1987) has been from this point forward a significant contribution for the homogeneization of the steam processes.

\[
R_o = \int_0^{(\text{min})} \exp \left( \frac{T[^\circ C] - 100}{14.75} \right)^{\cdot} dt
\]

\[
S_o = \log R_o
\]

Where \(S_o\) is the severity factor, \(T\) is the temperature (expressed in degree celcius) and the overall equation relates on the integral of the temperature curve between the start and the end of the cooking period, including the preliminary heating leading to operating temperature. Severity factors were also related to the relative hydrolysis of the hemicelluloses macromolecule and (Overend and Chornet, 1987) showed that at a severity factor of 4, hemicelluloses were completely hydrolysed and there was starting to be an impact on the cellulosic fiber. This concept, can serve as a guide for other substrates although it was shown to vary from one feedstock to another, mostly because of the varying nature and amounts of hemicelluloses found in the biomass (Lavoie et al., 2010a, 2010b, 2010c). Impact of the calculated severity factor has also been reported for other feedstocks as
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residual cotton and recycled paper (Shen et al., 2008), aspen wood (Li et al., 2005), douglas fir (Wu et al., 1999), from rice husk and straws (Gerardi et al., 1999), from yellow poplar, from peanut hulls and from sugar cane (Glasser et al., 1998). In most of the cases reported previously, the ideal severity factor for the isolation of cellulose and hydrolysis of hemicelluloses was found to be between a severity factor of 3 and 4. A non-catalytic steam process of biomass usually leads to a brown lignocellulosic fibre as depicted in Figure 2 below:

![Softwood chips, Hardwood chips, Straws](image)

Fig. 2. Lignocellulosic matrix obtained after a non-catalytic steam treatment of different biomasses with a severity factor between 3 and 4

Although hydrolysis of hemicelluloses can be performed only using the natural dissociative potential of water, many researches have investigated the effects of including a catalyst on the overall outcome of the process. Both acid and basic catalyst has been considered and each will have the tendency to target one type of macromolecule more than the other. Whilst acids will have a more pronounced effect on cellulose, bases will have a more significant effect on lignin. Among the acids that were used for catalysis of steam explosion reaction, sulphuric acid is one of the most common but also one of the less expensive at an industrial level. Utilisation of the latter has been reported repetitively in literature (Lawford et al., 2003; Emmel et al., 2003; Ballesteros et al., 2001). Directly comparable to sulphuric acid, sulphur dioxide was also widely used as a catalyst for steam explosion. The latter will interact with biomass and react with water to produce in turn sulphuric acid. The main difference might be that utilisation of SO$_2$ would allow a more homogeneous distribution of the acid catalyst in the biomass since the diffusion of the gas should be higher than the sulphuric acid molecule. Such a treatment has been effectively applied on lodgepole pine (Ewanick et al., 2007), poplar (Lu et al., 2009), aspen (De Bari et al., 2007) and eucalyptus (Ramos et al., 1999). The acid catalyst will have a direct effect on the hydrolytic potential of the mixture increasing the natural hydrolytic potential of water considerably. As for the previously mentioned severity factor, researchers have tried to translate this phenomenon into an equation. Abatzoglou et al. (1992) were able to introduce the concentration of acid into the calculation of the severity factor as depicted below:

$$R_{\text{ref}} = \exp \left( \frac{X - X_{\text{ref}}}{\lambda X_{\text{ref}}} \right) \exp \left( \frac{T - T_{\text{ref}}}{\omega} \right) t_r$$

(3)
Where $X$ and $X_{\text{ref}}$ is the acid loading (g of acid/g of dry biomass) and reference (acid loading, g of acid/g of dry biomass) respectively, $\lambda$ is a parameter expressing the acid catalyst role in conversion of the system, $\omega'$ is parameter expressing the temperature role in conversion of the catalysed reaction system and $t_R$ is the reaction time. A couple of years later, Montane and co-workers (Montane et al., 1998) developed a new version of the equation which included slight modifications over the equation proposed by Abatzoglou et al. The equation is depicted below:

$$R_i = \exp\left(\frac{1}{\omega_0} \left(1 - \frac{T_{\text{ref}}}{T}\right)^\frac{\omega}{\gamma}\right)$$

(4)

In this equation the $\omega_0$ parameter express the energetic of the process respect to a reference reaction temperature, $T$ and $t$ remains the temperature and time whilst $\gamma$ defines the shape of the distribution of activation energies. The research also showed that it was possible, for a specific species, to estimate the whole conversion of the process using a single equation:

$$(1 - f) = \exp\left(-1.06 \times 10^{10} \exp\left(-\frac{9733}{T}\right) C^{0.674} \frac{t^{0.608}}{0.608}\right)$$

(5)

Where $f$ is the conversion parameter and $C$ is the catalyst concentration. Equation 5 has been developed by Montane et al. using birch as substrate for the steam explosion process. Utilisation of an acid catalyst, for similar temperatures and times, should allow a more complete hydrolysis of the hemicelluloses but should also attack the cellulose molecule which is overall sensitive to the occurence of protons. It has been mentioned and it is still widely studied that the interactions between the hydronium and the cellose macromolecule may lead to a more efficient hydrolysis to glucose when used as a pretreatment for an enzymatic treatment (Dererie et al., 2011; Khunrong et al., 2011; Zhang et al., 2009). In most of the previously mentioned situations, utilisation of the catalyst leaded to increased value for conversion following the enzymatic hydrolysis, although in some specific cases, even if the conversion to glucose was increased, the fermentation was strongly inhibited by the production of furfural-derived compounds. Dehydration of xylose to furfural is depicted in Figure 3.

Dehydration of carbohydrates is strongly induced by acid catalysts at temperature higher than 150 °C with a classical inorganic catalyst although lower operating conditions were reported for the utilisation of ionic liquids (Tao et al., 2011). Five-carbon carbohydrates will dehydrate to furfural whilst dehydration of C₆ sugars will lead to 5-hydroxymethylfurfural (5-HMF) (Zhang et al., 2010). The latter will usually be less concentrated in a steam explosion process since it will require an isomerisation of the aldohexose sugars to a ketohexose form. Furthermore, under acid catalyst, 5-HMF has been reported to undergo spontaneous hydrolysis to levulinic acid and formic acid which are both fermentation inhibitors. The minimal concentration at which furfural starts to inhibit fermentation has been reported to be at 2-3 g/L (Palmqvist et al., 1999) whilst as for 5-HMF, it has been reported that the concentration that causes 50% inhibition of fermentation was of 8 g/L. Base-catalysed steam explosion may also be a potential pathway since the occurence of hydroxide ions, as in the case of kraft pulping, would lead to the hydrolysis of the hemicelluloses as well as the lignin whilst allowing the isolation of cellulose. Utilisation of NaOH as a catalyst for steam explosion has been reported in literature (Zhuang et al., 1997;
Li et al., 2005), although less frequently in comparison to the acid-catalysed reaction. Another process called ammonium fiber explosion is also slightly comparable to a base-catalysed steam explosion since it will allow defibration of the feedstock in a first time, then the interaction of ammonia with water can be directly related to an hydroxide ion catalyst although part of the hydrolysis process could be related to the ammonia itself although it is highly soluble in water and it interacts in an classical acido-basic reaction to produce ammonium hydroxide. This concept was efficiently tested on rice straws (Vlasenko et al., 1997) and the process itself has been patented by Dale et al. (2008).

**Fig. 3. Example of dehydration of a carbohydrate to furfural under acid catalyst (in this case xylose)**

### 3.2 The Feedstock Impregnation Rapid and Sequential Steam Treatment (FIRST)

Since a non-catalytic and an acid steam treatment allowed targeting the carbohydrate-based macromolecules from the biomass and the based-catalysed reaction allowed partial depolymerisation and solubilisation of lignin, our group has developed the two step FIRST (Feedstock Impregnation Rapid and Sequential Steam Treatment) process. The biomass is first reduced in size to a range 3 to 6 cm long. It then follows the process flow diagram depicted below (Figure 4).

Biomass is first extracted with water, solvent and/or a mixture of both to extract the secondary metabolites. Two reasons justify the preliminary extraction, first some of the compounds could have a bioactive potential thus leading to applications in cosmetics and pharmaceuticals. Secondly, the extractives could act as inhibitors for fermentation and depending what is the targeted application for the broth obtained after the first steamexplosion, it might be beneficial to remove such compounds. After extraction, biomass is rinsed with a minimal amount of water to remove traces of the residual solvent or to ensure maximal removal of extracts. Typically, at the bench scale level, a 5/1 massic ratio of water/biomass is used at this point. Biomass is then impregnated with water to ensure
Fig. 4. Process flow diagram for the two step FIRSST process allowing isolation of the cellulose fibre as well as lignin, hemicelluloses and extractives with minimal purification required.
maximal penetration of the aqueous medium in the biomass’ pores. Impregnation could be performed with or without pressure, either positive or negative. Saturation with water is one of the key elements for performing an efficient steam process and has to be monitored carefully. Impregnation could be performed by letting the biomass soak in water for a time period (typically up to 24h) allowing the water molecules to fill the small pores via capillarity. Whilst both positive and negative pressure might be used, utilisation of a positive pressure to ensure water penetration is by far the most efficiently scalable approach. After impregnation, excess water has to be removed, a pressure of 100 psi is sufficient both for the pressurized impregnation process as well as the following excess water removal. Once excess water is removed, the biomass is transferred in the FIRSST reactor where it is cooked for 2-4 minutes whilst monitoring the severity factor of the whole process. In a two step FIRSST process, one must ensure that the severity factor of the first process is not excessively high or the following delignification process, although efficient, will lead to excessive conversion and a lignin-carbohydrates broth after the second process.

Once the first FIRSST process is completed, the biomass is once more impregnated but this time with an aqueous diluted NaOH solution (typically 1-10%). Impregnation as well as removal of excessive solution was performed at 100 psi and room temperature. The residual lignocellulosic matrix is then cooked at temperature comparable to the first process although typically in a 10 ºC inferior temperature range. Once the second cooking period is completed, biomass is rinsed with a 10/1 ratio water/fibre to ensure removal of the remaining sodium ions. Such process has been tested on different feedstock including energy crops (Lavoie et al., 2010a), residual forest biomass (Lavoie et al., 2010b) and different agricultural residues (Lavoie et al., 2011). When using based-catalysed steam process, the catalyst itself, as in the case of acid hydrolysis, becomes an important factor of the reaction. Increasing the concentration of the basic catalyst showed to have a direct impact on lignin removal and using the same conditions (time and temperature), it was shown that an increasing alkali concentration in the mixture allowed the production of a whiter fibre. The texture of the fibres are strongly affected by the severity of the steam processes, example of the different textures of fibres produced according to the two-step FIRSST process used on triticale straws are depicted below (Figure 5).

At this point, the two-step FIRSST process has allowed the isolation, in high yields, of all the fractions of lignocellulosic biomass whilst producing pulp with good mechanical properties. Example of mechanical properties obtained from FIRSST pulps are depicted in the Table 4 for *Salix viminalis* (Lavoie et al., 2010a), for a mixture of softwood (Lavoie et al., 2010b) and for *Cannabis sativa* (Lavoie et al. unpublished results). Opportunity to produce quality pulp is yet another advantage of this technique which would, from the same feedstock, allow the production of many derived products including ethanol and cellulose.

For the first step of the steam explosion and as mentioned earlier, no catalyst is used since the hemicelluloses and/or protein found in the lignocellulosic matrix were shown to be directly affected by water at the operating conditions. The first step of the FIRSST process is usually performed between temperatures of 180-230 ºC depending on the nature of the biomass used as a feedstock. As an example, residual forest biomass was shown to require more severe conditions in comparison to residual agricultural biomass as triticale or hemp. Cooking period is usually ranging from 2-4 minutes, but it most of the cases investigated by our team, a 2 minute cooking period was shown sufficient. The uncatalyzed steam explosion process can be related to the severity factor that is calculated from the cooking temperature and time, needless to underline the fact that similar severity could be obtained by increasing the cooking period whilst decreasing the temperature. The 2 minutes cooking time usually
excludes the heating period where biomass is heated to the operating conditions, nevertheless, this heating period is taken into consideration when calculating the severity factor. The heating period for the FIRSST process varies from 10-30 seconds and the temperature of biomass is monitored with thermocouples strategically located in the steam explosion reactor and recorded on an acquisition system allowing control and downstream calculations with regards to the conditions of operation.

Fig. 5. Lignocellulosic and cellulosic fibres obtaineds with the two step FIRSST process applied to triticale straws

Another important factor that has to be taken into consideration when applying the 2-steps FIRSST process concerns the production of high quality pulp which may be tricky because of the severity of the reaction. Therefore, even if the second steam process is often based catalyzed, the severity of the treatment will lead to an indirect attack on cellulose which will reduce the fibre length if not suitably controlled. If ethanol production is inteded, then the quality of the fibres produced after the FIRSST process is of lesser interest and both FIRSST treatments can be relatively severe. When dealing with quality cellulose fibres production, one must think about reducing the strength of the first FIRSST treatment in order to cope with the severity of the second treatment. So far the approach that has been developed in order to cope with such problem was to evaluate the composition of the lignocellulosic matrix at different severities for a first uncatalyzed FIRSST process. Optimal severity for the first process would lead to a drastic decrease of the hemicelluloses content whilst altering minimally the long cellulose fibres (which are determined by quantification of the hemi,
holo and α-cellulose). During the first FIRSST process, the holocellulose concentration will progressively decrease whilst α-cellulose will remain constant. We identify the maximal severity at the point where α-cellulose starts to significantly decrease. An example of such is depicted in Table 5 below:

| Standard test | Willow | Softwood | Hemp |
|---------------|--------|----------|------|
| ATPPC C.1     | 454    | 664      | 407  |
| ATPPC C.12    | 0.16   | 5.58     | n.d. |
| ATPPC C.5U    |        |          |      |
| + 14          |        |          |      |
| + 28          |        |          |      |
| + 48          |        |          |      |
| + 100         |        |          |      |
| + 200         |        |          |      |
| - 200         |        |          |      |
| ATPPC B.4P    | 0.41   | 2.08     | n.d. |
| ATPPC D.3     | 60.1   | 59.6     | 59.2 |
| ATPPC E.1     | 33.3   | 24.8     | 29.4 |
| ATPPC E.5     |        |          |      |
| L*            | 71.45 ± 0.01 | 67.26 | 68.90 |
| a*            | 2.18 ± 0.01 | 4.41 | 2.74 |
| b*            | 12.42 ± 0.01 | 18.31 | 13.79 |
| ATPPC E.2     | 99.5   | 98.5     | 99.0 |
| ATPPC D.4     | 1.79   | 2.24     | 2.90 |
| ATPPC D.9     | 3.14   | 8.04     | 11.8 |
| ATPPC D.8     | 1.56   | 3.05     | <0.34 |
| ATPPC D.34    | 4.24   | 5.03     | 2.29 |
| Length of rupture |        |          |      |
| ATPPC D.34    | 19.6   | 37.8     | 8.8  |

Table 4. Mechanical properties of FIRSST for different types of feedstock.

| Conditions | Severity Eqn [1,2] | Conversion %wt | Lignin % wt ± SD | Holocellulose % wt ± SD | α-cellulose % wt ± SD |
|------------|-------------------|----------------|-----------------|-------------------------|-----------------------|
| 210 °C, 2 min. | 3.64 | 20.03 | 28 ± 2 | 51 ± 2 | 36.2 ± 0.7 |
| 220 °C, 2 min. | 3.95 | 22.00 | 29.8 ± 0.5 | 47 ± 5 | 37 ± 1 |
| 220 °C, 4 min. | 4.20 | 23.02 | 32 ± 3 | 44 ± 6 | 34.6 ± 0.4 |
| 230 °C, 2 min. | 4.27 | 24.94 | 31 ± 8 | 43 ± 3 | 33 ± 1 |

Percentages are expressed in terms of bone dry biomass. Deviations from 100% closure are due to ash content.
* Comprised in the holocellulose

Table 5. Composition of the lignocellulosic fibers following the first steam treatment under various conditions for softwood (From Lavoie et al. 2010b)
For the specific case depicted in Table 5, we can assume the severity factor varying from 3.64 to 3.95 only had a minor impact on the alpha cellulose and therefore we can assume that the treatment targeted the hemicelluloses principally. The quantity of removed hemicelluloses can be assimilated to the conversion also presented in the same table which shows an increasing mass content in the lignin broth produced from the FIRSST process. In a situation where quality pulp would be intended, although the best conditions for removing all the hemicelluloses would be at a severity factor of approximately 4.00, it would be more strategic to use a lower severity first process (210°C, 2 minutes as an example) to ensure that the second steam treatment will not affect to much the pulp quality downstream.

The second process is also tricky since ideally and economically, lignin should be recuperated at >80%. Increasing the severity of the treatment will lead to very low lignin content but will also affect the cellulose fibres. To a certain extent, concentration of lignin inferior to 1% can be obtained using steam treatment. The downside will be that the higher severity will also affect the cellulose fibres and overall, the conversion will be higher and the quantity of pulp recovered downstream will also be lower with regards to the original quantity of biomass used for the process. An example of this is depicted in Table 6 below:

| Conditions     | Yield* | Lignin    | Holocellulose |
|----------------|--------|-----------|---------------|
|                | %wt    | %wt ± SD  | %wt ± SD      |
| 190 °C, 2 min. | 48.52  | 24.8 ± 0.2| 75.3 ± 0.2    |
| 200 °C, 2 min. | 41.35  | 14.5 ± 0.1| 85.6 ± 0.1    |
| 210 °C, 2 min. | 37.60  | 6.9 ± 0.1 | 93.2 ± 0.1    |
| 220 °C, 2 min. | 37.23  | 3.2 ± 0.4 | 96.9 ± 0.4    |

*Yield is expressed in terms of %wt of dry biomass and can therefore be considered as the rate of pulping for the overall process.

In light of the results depicted in Table 5, it is clear that increasing severity will also affect the overall conversion which will lead, at higher severity to a lower production of pulp. Other less severe technique could be used for removing the residual lignin and it should be considered to accentuate the pulping yields and to reduce to chemical alterations that the process may have induce to the cellulose fibres. On the other hand, if hydrolysis of glucose is intended, it was previously reported that severe steam explosion process could have a beneficial effect on enzyme hydrolysis and therefore, reaching higher severity may be beneficial to the whole process even if part of the cellulose is solubilized in the lignin broth.

Typical energy consumption for each of the steam process is approximatively 7% of the net biomass calorific value which represents approximatively 1.4 GJ per tonne of biomass process. A two step process could easily lead to a 15% which now justifies investigations towards a one step process. Using triticale, our group compared the two- and one- step steam process. Since lignin has to be partially hydrolysed and removed, the one step steam process will require utilisation of a base-catalyst.
Although the process was shown efficient for the isolation of cellulose from triticale straws (unpublished results), the downside of such process is that it will generate a broth containing both hemicelluloses and lignin. Although lignin can be precipitated by reducing significantly the pH and mildly heating, it will nevertheless produce an ion-rich solution that will be overall hard to ferment without prior purification. Depending on the targeted downstream processes for hemicelluloses and lignin, the one-step or the two-step process may be more suitable and the choice of one or another should be influenced by the economic of the added-vapue products that will be generated from this biorefinery.

4. Hydrolysis

The cellulose macromolecule is composed of glucose units that are linked via a β 1-4 acetal bond. Despite the fact that the glucose units composing it are highly polar and completely soluble in water, cellulose itself is rather hydrophobic. The reason explaining such a phenomenon is because the polar functional groups of the cellulose macromolecules in the fibrils are oriented facing one another in such a fashion that render penetration by a polar solvent as water fairly hard to achieve. Furthermore, cellulose is composed of highly structured crystalline form as well as a less structured and fairly more ramified amorphous phase. The amorphous phase is more accessible to hydrolysing agents as acids or enzymes and usually bares the sites where original hydrolysis is going to take place. Although it is fairly uncommon for solvent to slide between the cellulose macromolecules, some solvent have been shown to be able to accomplish such task. The latter are usually highly polar and often have an ionic part.

The key to chemical hydrolysis of cellulose is to break the hydrogen bonding between the cellulose macromolecules in the fibrils. To do so, a compound or a mixture of compound that can efficiently rupture the hydrogen bonding is used. Furthermore, the compound has to be made in a specific shape capable of penetration in the tight hydrogen bonded ultrastructure. The compounds that have shown such potential all have in common an “arrow head” structure the ionic part of the molecule being the tip of the arrow. Once the ion is inserted between the cellulosic fibril layers, much effort has to be made in order to avoid reformation of the previous hydrogen bonding. Therefore, at this point, the utilisation of a larger quantity of solvent is of the essence. A classical example of this theory is the ASTM D1106-56 method which allows the quantification of lignin in a lignocellulosic matrix. The process relies on the acidification of the fibers with a strong sulphuric acid mixture. The mixture is kept at low temperature, allowing the ionic sulphuric acid to progress through the cellulosic matrix after which water is added to the mixture prior to hydrolysis. In this specific case, once the cellulose molecules have been spaced one from the other, water is added in large quantity to avoid reformation of hydrogen bonding between the cellulose macromolecules, thus isolating them one from the other. The solution is still acidic enough to allow hydrolysis and since the process requires energy to activate the bond, heating the solution close to ebullition of water allows the hydrolysis of cellulose to glucose. This process is highly effective and allows the conversion of cellulose up to a 100% into glucose. The downside of this process is directly related to the low cellulose concentration in the solution and the high residual ionic composition which makes this classic ASTM method fairly hard to scale up into an industrial fully functional process.

Sulphuric acid, although probably the more inexpensive of the potential reactants for cellulose hydrolysis is not the only one that has been reported so far. Formic acid, the smallest organic acid, has also been reported to be efficient for the swelling and overall
hydrolysis of water. A few researchers have reported the utilisation of formic acid in relation with cotton (Sun et al. 2008). Cotton is a good example of a cellulose rich medium and it was reported that formic acid was shown effective for the hydrolysis of these fibres. Nevertheless, formic acid was never efficient at a 100% concentration and for a suitable hydrolysis, it had to be diluted somewhat with water. This organic acid, as sulphuric acid, absolutely requires water as a medium for propagation of the protons. In a pure formic acid medium, the auto-hydrolysis process will generate far less free hydronium ions which will overall reduce the catalytic potential on the mixture. On the other hand, adding too much water will lead to dilution of the acid which will not be able to performed efficiently as a swelling agent in cellulose, since, as mentioned earlier, water is not strong enough to break the actual bonds between the cellulose macromolecules. Other reports have mentioned that they could increase efficiency of a formic acid hydrolysis by adding a specific amount of sulphuric acid in the mixture. In this specific case, sulphuric acid is not used as a swelling agent (small concentration has been reported to be efficient) but only as a proton provider, accentuating the actual contribution made by formic acid. And since sulphuric acid is significantly more acidic than formic acid, there will be available protons in the mixture, allowing hydrolysis of cellulose without however justifying the addition of water to the mixture. Another catalyst that has been reported often for the hydrolysis of cellulose using formic acid as a solvent is chlorhydric acid (Sun et al., 2010). Although it was shown effective for cellulose hydrolysis, such a mixture also has major drawbacks, first of which is the high concentration of formic acid which is a fermentation inhibitor. Production of glucose according to this process would therefore require a very extensive purification process to ensure minimal occurrence of formic acid in the mixture. Another problem that may arise from a formic-acid process would be the removal of sulphuric acid. Althought it may not be too much of a problem at low concentration, removing formic acid would concentrate the sulphuric acid in the mixture leading to a strong acidic catalyst at the end of the distillation process. Even in small amount, the inorganic acid would more likely interact with glucose at the boiling point of formic acid and induce dehydration of the glucose molecule leading yet to another major problem via the occurrence of another major inhibitor, 5-hydroxymethylfurfural (5-HMF).

Another compound that was shown to be effective to separate the cellulose macromolecules is cupryethylene diamine, an organometallic compound currently used to evaluate the polymerisation degree of the cellulose fibres. This compound also demonstrates the “arrow head” structure and the major interaction between this molecule and the cellulose fibrils is made via the amine groups (see Figure 6). The target of cupryethylene diamine is to solubilise cellulose in order to evaluate the polymerisation level of the fibres, therefore, it does not allow hydrolysis of cellulose. Nonetheless, insertion of an acid catalyst may also lead to an efficient hydrolysis although the acid would most probably interact with the strongly basic amine compounds.

In recent literature, other compounds have also been reported to interact favourably with cellulose and most of these compounds are related to the ionic liquids group. Such liquids have gained attention around the 90’s and are currently involved in many scientific studies and specially in the field of high efficiency electrochemical cells. There also have been some reports that ionic liquids could have an impact on cellulose, allowing dissolution of the latter. A recent patent by Braun et al. (2010) reported that they were able to produce cellulose beads by dissolving a cellulose medium in a solution containing an imidazolium compound (see Figure 7 below).
Our group has developed a process “the CRB-UdeS process” by which cellulose is hydrolysed to glucose (Lavoie et al., 2010a, 2010b; Chornet et al., 2008) being scaled to a demonstration level by CRB. The production of glucose out of lignocellulosics is overall a tricky process and one should keep in mind that before attacking the acetal bonding of the cellulose, it is primordial that the targeted bonds are exposed to the catalyst. The process has been divided in three steps: swelling, hydrolysis and purification. Swelling of cellulose implies the insertion of a suitable molecule, that breaks the strong hydrogen bonding among cellulose macromolecules in the fibrils. A stable gel is formed. Our group has investigated the different parameters which favour an efficient swelling and the most important factors to be taken into account are temperature and time of swelling. The concentration of the swelling agent is of crucial importance if cellulose macromolecules in the fibrils are to be
separated one from the others. Figure 8 below depicts the influence of the concentration of sulphuric acid as well as the acid/cellulose ratio in relationship with total hydrolysis of cellulose.

![Figure 8](image-url)

**Fig. 8.** Acid quantity and concentration during swelling vs tritical cellulose hydrolysis yield (for 10 min hydrolysis at 121°C in autoclave with H₂SO₄ concentration of 208-267 g/L and with H⁺/OH⁻ molar ratio of 2.3 during NaOH 18-23 % addition)

Results shows that the there is an optimal concentration of acid leading to an optimized swelling which facilitates the hydrolysis of cellulose with a yield close to 100% at around 72-74 wt% acid in solution. A 72 %wt of sulphuric acid correspond to a 2.0 molar ratio of water/sulphuric acid which is stoichiometrically sufficient to completely ionise sulphuric acid which leads back to the arrowhead structure. The swelling period is also fairly important for such process, getting optimal after half an hour. Results (depicted in Figure 9 below) shows that increasing the time of swelling does not lead to significant increases in terms of rate of hydrolysis downstream. The temperature at which the cellulose is swelled can be varied from 20-40 °C after which degradation of the cellulose is observed (Belanger, 2005).

![Figure 9](image-url)

**Fig. 9.** Time of swelling vs tritical cellulose hydrolysis yield (for 72% H₂SO₄ during swelling at 30°C, 10 min hydrolysis at 121°C in autoclave, H₂SO₄/Cellulose massic ratio of 36, H⁺/OH⁻ molar ratio of 2.5 during NaOH 20 % addition)
Once the hydrogen bonding between the cellulose macromolecules is replaced by hydrogen bonding to the aqueous solvent, the next step leading to the production of glucose is hydrolysis. The latter is performed under a decreased free acid concentration. The effect of the pH on the hydrolysis process is depicted in Figure 10 below.

![Figure 10](image1.png)

**Fig. 10.** H+/OH- molar ratio vs tritical cellulose hydrolysis yield (for 72% H2SO4 during the 2h at 30°C swelling, with 10 min hydrolysis at 121°C in autoclave, with NaOH 40% addition)

The previously mentioned results show that a minimal amount of hydronium ions are required in order to perform hydrolysis to an optimal value. The concentration of available protons is of course an important factor but temperature and time are also parameters that have to be assessed in order to control optimally the production of cellulosic glucose. Hydrolysis of the acetal bonding requires activation. Figure 11 below shows the effect of temperature on the hydrolysis process.

![Figure 11](image2.png)

**Fig. 11.** Hydrolysis time and temperature vs tritical cellulose hydrolysis yield (for 72% H2SO4 during the 2h at 30°C swelling, with H2SO4/Cellulose massic ratio of 12, with H+/OH- molar ratio of 2.0 and partial neutralization. All the results at 121°C include 20 min more above 97°C due to the preheat and the cool down of temperature in the autoclave.)
Figure 11 shows that for both temperatures, a maximum hydrolysis yield is reached but the required time for achieve such hydrolysis is different which is perfectly in accordance to the kinetics of the hydrolysis reaction. As for steam explosion, hydrolysis of cellulose could as well be depicted in terms of a severity factor but no mention of such mathematical relationship has been reported so far. Finally, time of hydrolysis is of course an important factor when considering hydrolysis of cellulose. Once cellulose has undergone a sufficient swelling, it is completely exposed to acid attacks on the acetal bonds of the glucose units. Results depicted in Figure 12 below clearly shows that the hydrolysis is fast and efficient at 121°C.

![Figure 12. Hydrolysis time and H+/OH- molar ratio vs critical cellulose hydrolysis yield](image)

5. Conclusions

The production of cellulosic ethanol via the fractionation process requires an understanding of the availability of the cellulose in different lignocellulosic substrates. The cellulose content of a biomass can lead to the estimation of ethanol productivity via glucose fermentation. In order to convert biomass to glucose, two important steps have to be controlled and optimized, fractionation of the biomass to yield cellulose and hydrolysis of the latter. Aqueous/Steam treatments are an efficient alternative to isolate cellulose. They rely on the utilisation of water with or without catalyst to saturate the biomass and induce, via steam addition, the increase in temperature that results in, the hydrolysis of bonds between constitutive fractions liberating hemicelluloses and lignin. The impact of steam processes on biomass can be correlated by a severity factor which allows estimating the quantity of hemicelluloses and to certain extent cellulose that is hydrolyzed during the process. Utilization of a base catalyst can allow hydrolysis of lignin and production of a relatively clean cellulosic fibre. The two step FIRSST process first hydrolyzes the hemicelluloses whilst lignin is separated from the cellulose in a second based-catalyzed treatment. This process thus allows the fractionation of biomass while producing a high quality pulp and a variety of smaller fibers called fines which can be hydrolyzed via penetration of strong ion solution, (concentrated sulphuric acid) which swells the crystalline cellulose. The “swelling” was shown to be more efficient when using ionic compounds that show an “arrowhead”
configuration. Such a pattern is representative of a few compounds including sulphuric acid. The step for hydrolyzing cellulose is the acidic attack on the acetal bonds. The latter can be performed by an hydronium ion and accessibility to the acetal bonding of cellulose is directly related to the efficiency of the previous swelling. It was shown that once the targeted bonds are exposed, the swelling is of the order of minutes (and can be performed at different temperatures near the boiling point of water. It is an alternative to enzyme hydrolysis.

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This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

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