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Mechanochemistry: a new approach to depolymerize cellulose via solid-solid reaction

Tesi di laurea sperimentale

CANDIDATO

Massimiliano Caiti

RELATORE

Prof. Stefania Albonetti

CORRELATORE

Dr. Ceri Hammond

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Abbreviation

AGU	Anhydrous glucose unit
DP	Degree of polymerization
HMF	5-hydroximethyl furfural
FDCA	2,5-furandicarboxylic acid
THF	Tetrahydrofuran
TGA	Thermogravimetric analysis
p-TSA	para-toluene sulfonic acid
HPLC	High performance liquid chromatografy
ELSD	Evaporative light-scattering detector
UV/VIS	Ultraviolet / Visible
MS	Mass-Spectroscopy
ESI	Electrospray ionization
RI	Rifractometry index
EI	Electron ionization
ICP	Inductively coupled plasma
NMR	Nuclear magnetic resonance
TOF	time-of-flight
LC	Liquid chromatography
WPS	Water soluble products - Cellulose conversion
Glc	Glucose monomer unit
Lg	Levoglucosan unit
Glc_{3-5}	Oligosaccharides composed by 3-5 AGUs

CONTENT

AIM AND MOTIVATIONS OF THIS THESIS

Since fossil oil isn't a renewable neither sustainable source of energy and matter, alternative more green friendly founts are researched especially for the production of chemicals due that for the energy purpose different solutions are already found, such as wind, sun and the heat present under the earth's crust exploited by the geothermal technology, though they must be deeply developed and improved.

Biomass is the largest natural carbon feedstock present on earth with renewable features due that it is able to regenerate itself in a reasonable period of time. For the same reason its carbon dioxide emission could be considered almost null when it is exploited also as combustible. Moreover nowadays are present enormous amounts of biomass both from industrial and municipal wastes. Thus the biomass exploitation as a carbon feedstock enhance the green features of the industrial processes that use it as a starting material due that they transform matter previously considered as a refusal in valuable products. Indeed if biomass is unpacked in its mainly constituent: cellulose, hemicellulose and lignin could be further converted in platform molecules from which could be originated plenty of derivates and useful compounds. For example cellulose can be depolymerized in its building block glucose that could be transformed by both fermentation or chemical reactions in a proper wide tree of important chemical derivates. In this way biomass gains value and could be considered a new renewable replace of fossil oil, today considered the main not renewable carbon atoms source for the production of chemicals. Nowadays are already know hydrolysis reactions to convert cellulose in glucose, such as enzymatic process or trough inorganic acids, but both of them show drawbacks in contrast with the green chemistry approach and furthermore don't exploit the whole biomass potential because don't lead in full yields of desired products. Recently the scientific community has enhance its attention toward a new chemical approach called mechanochemistry due that with the mechanical forces exerted on the reactants it is a able to perform solid-solid reactions, thus preventing or highly restricting the use of hazardous and toxic solvents that are today commonly used by the chemical industry. Hence the aim of this work is explore the possibility of depolymerize cellulose trough solid-solid reactions catalyzed by solid acid species and assisted by mechanical energies provided by special devices.

Since it is important to obtain, at the end of the solid depolymerization reaction, products in a suitable form that permit their qualitative and quantitative determinations an experimental methodology of the overall process had to be developed. This involves the selection of the best cellulosic substrate to be treated, the setting of a catalytic benchmark on which compare the solid acids performances, the tuning of the milling parameters and the establishment of a method to transforming the solid products in a physical state available for its analytical determinations, without affecting its conversions and yields arisen from the milling process. Moreover, since the classical analytical techniques resulted not suitable to obtain reliable conversion values, an analytical method was developed to determine the total amount of water soluble products at the end of the milling process, that it was considered in this way as the conversion value of the studied reaction. Thanks to these preliminaries studies, it was possible to compare the catalytic performances of the solid acids under investigation, confronting water soluble products conversion and sugar yields resulted from the grinding in the planetary ball mill. Furthermore analyzing the results obtained could be deduced important information regarding the dependence of the depolymerization rate on some catalytic features such as the number of the acid sites or the distribution effect. Moreover it was possible to understand trough which mechanism the cellulose depolymerize toward shorter oligosaccharides in reactions assisted by mechanical forces. At end of this work the same topics were addressed in the mixer ball mill to study its performances and compare them with the ones of the planetary ball mill.

CHAPTER 1: INTRODUCTION

1.1 NOWADAYS FOSSIL FUEL ECONOMY

Today the world's primary source of energy for the transport sector and production of chemicals is oil. World oil demand is approximately 84 million barrels per day and it is projected to increase to about 116 million barrels a day by 2030 due to the world economical progress and the continuously demographic increment[1]. In particular the transport sector accounts for some 60% of this fossil fuel demand increasing. Moreover in the emerging economies of India and China is predicted to grow again by at least 3% per year[2]. Concerning chemicals, their dependence on fossil resources is even stronger. The majority of the chemical products are produced from oil refinery and almost 4% of oil is worldwide used for chemicals and plastics production[3]. Crude oil, known also as petroleum, is formed when large quantities of dead organisms, usually zooplankton and algae, are buried underneath sedimentary rocks and subjected to both intense heat and pressure in anoxic conditions for millions of years. Hence it is a natural resource of energy and matter but, because the time required for its formation cannot be compared with the time of its consumption, it is considered not renewable. Indeed it is predicted that the crude oil resource will run out in the next few centuries. Moreover as already well known, there are clear scientific evidences that emissions of greenhouse gases, such as carbon dioxide, methane and nitrous oxide, arising from fossil fuel combustion and land-use change as a result of human activities, are perturbing the earth's climate[4]. Furthermore the increasing exploitation of the fossil fuels connected with their diminishing causes political concern besides the increasing prizes of this energy source. For these reasons scientific society began to recognize the opportunities offered by a future sustainable economy based on renewable sources and has been starting to finance R&D activities for its implementation. For the energy and heat supply several alternative renewable sources were already found based on the exploitation of wind, sun, water or geothermal heat, whereas for the production of chemicals and transportation fuels the only sustainable source of carbon was be identified in biomass.

1.2 BIOMASS AS A RENWABLE SOURCE OF CARBON ATOMS

Commonly with the term biomass the scientific community refers to non-fossilized and biodegradable organic material originating from plants, animals, and microorganisms derived from biological sources. The vegetable biomass, or lignocellulose, is grown up by the plant kingdom organisms as a support structure of their cell wall, developed through the carbon dioxide reduction, a process known as chlorophylline photosynthesis. Hence biomass could be considered a massive storehouse of carbon source that could be exploited as a chemical feedstock. Biomass includes products, byproducts, residues and wastes from agriculture, forestry and related industries, as well as the non-fossilized and biodegradable organic fractions of industrial and municipal solid wastes[5]. Also algae are an important biomass feedstock in the world due that can grow practically in every place where there is enough sunshine, and some of their species also in saline water. Moreover must be considered that microalgae are the fastest growing photosynthesizing organisms due that they can complete an entire growing cycle every few days[6]. Thus since biomass is the major natural carbon feedstock can be considered as renewable, potentially sustainable and environmentally benign source of energy. Plenty of studies are been conducted in the last century to essay to exploit its huge carbon composition as replacement of the fossil sources which our modern economy is based on. Its green features as starting material for the industrial processes are represented by its renewability as it can regenerate in a reasonable period of time and its recycling due that nowadays it represent a massive quantity of wastes, especially from the agricultural sector. Nowadays the worldwide biomass production is estimated to be 170 billion of tons per years that is divided in 75% of carbohydrates, 20% of lignin and 5% of oil, fats and proteins[7]. Thus this huge amount of carbon source is considered to be sufficient to cover the today overall production of chemicals. To have an idea of the potential of this replacement must be considered that the annual corn production of United States contains the carbon equivalent of 500 million barrels of crude oil. Nonetheless one of the main ethic problem discussed by the scientific and political communities is represented by the competition between the biomass exploitation for the energetic or chemical purpose and the human feeding, due that nowadays it is destined to this latter purpose the 62% of the vegetable origin fraction of the entire biomass supply[9]. Hence it is clear why, for energetic and chemicals production purpose, it is preferred to exploit the biomass fraction that constitute wastes or residues. As a confirm of its increasing consideration as renewable resource, in the last decades was developed and coined the biorefinery concept

as new setup of complex industrial system for the production of fuels, heat and energy that is based on biomass as the main starting material.

1.2.1 Biomass structure

The effectively fraction of the complex biomass matter that can be used as a source of carbon feedstock is represented by the natural hetero-matrix polymer called lignocellulose. As Figure 1.1 shows it is situated in plant cell walls which are constructed by a multitude of macrofibrils where each of those is the result of a microfibril ensemble, that in turn it represents the properly strand where cellulose, hemicellulose and lignin are strongly linked together. Indeed this three natural compounds are the main polymers that compose lignocellulose. It also contains smaller amounts of pectins, inorganic compounds, proteins and extractives, such as waxes and lipids, which also have potential value. Depending on the origins and the species where the plants come from, the composition of this constituents vary in terms of quantities and how they are link together conducing in a greatly diversified biomaterials. For instance hardwood have greater amount of cellulose, whereas wheat straws and leaves contain more hemicelluloses[9].

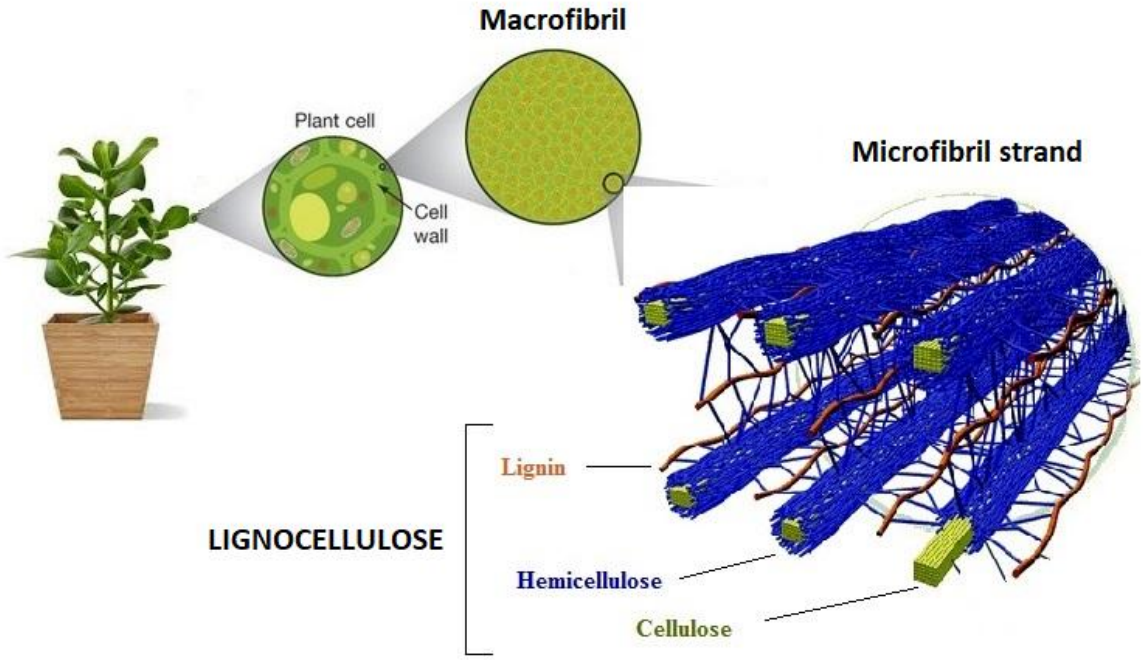


Figure 1.1 Lignocellulose physical structure and where could be found in the plant organism.

- Cellulose

Cellulose, with its 35% to 50% of presence, is the largest compound that the lignocellulosic mycrofibrils are made of. As can be seen in Figure 1.2 it could be described as an syndiotactic polymer of glucose where the anhydroglucose units (AGUs) are linked together by 1,4-β-glycosidic bonds[10].

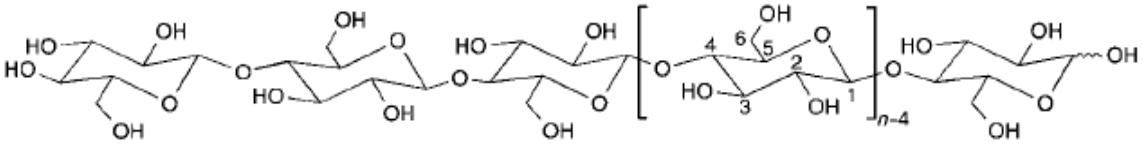


Figure 1.2 Cellulose is a polymer constituted by anhydrous glucose units.

Its syndiotactic structure is due on the glucose units that in the polymeric chain are rotated by 180° against each others. Despite this can be also considered as a macromolecule constructed by cellobiose monomers appearing in this way in a isotactic conformation. Both definition are accepted by the scientific community and satisfy the regularity requirement to exhibit a crystalline morphology. Anyhow the degree of polymerization (DP) of cellulose is defined by the number of repeating AGUs present in the polymeric chain. The macromulecules's length varies with the origin and the treatments that the raw materials undergo, make cellulose a high polydisperse polymer with its wide range of molecular weight distribution. For example cotton and other fibers show DP values between 800 and 10000 units, for wood and pulps the values are typically 300 and 1700. Through a mild hydrolysis process of these natural substrates a partial chain degradation occurs, yielding in the microcrystalline cellulose powdery form. Thanks to this pretreatment this latter kind of cellulose shows an average DP value of 225 and a composition of 100% of glucose units differing to α-cellulose that is constructed by 76% of glucans and 16% of xylans, thus appearing as a heterogeneous solid in its granular and fibrous shape[11]. The linear conformation of the shaccaride polymer enables the packing of numerous cellulose strands into crystalline fibrils[12]. In this native structure the glycosidic chains arrange themselves in layers linked together by a strong inter and intramolecular hydrogen bonds, resulting in the formation of flat sheets represented in Figure 1.3, that moreover, are held one on another by Van der Waals forces [13]. The hydrogen bonds network create a supermolecular structure in which the cellulosic strands are really well packed, this make cellulose one of the most recalcitrant substrate in nature as can be demonstrated by its insolubility in most of the

known solvents, including, first among all, water. Hence it can be understood the difficulties that arise when chemical treatments or analysis are carried out to try to exploit this kind of natural starting material.

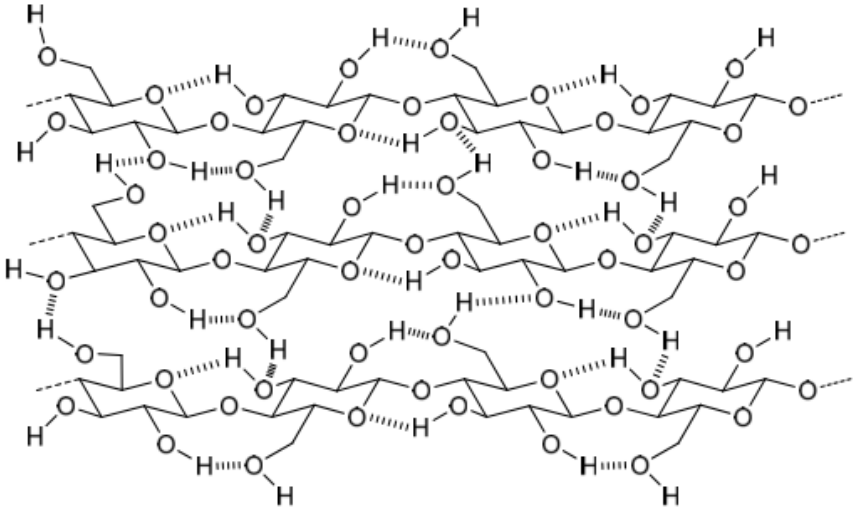


Figure 1.3 Cellulose sheets linked by intermolecular hydrogen bonds.

- Hemicellulose

Hemicellulose differing from cellulose, is a copolymer made of both hexose and pentose monosaccharide such as glucose, mannose, xylose, and arabinose. Due to its heterogeneous composition shows an amorphous morphology and a lower polymerization degree. Thus it represents the softer components of the lignocellulosic polymers. Generally it acts as a matrix material that hold the stiff cellulose fibrils in place, enveloping closer the divers strands[14]. As it is illustrated in Figure 1.4 hemicellulose can be branched and differentiated with divers functionalities. Moreover if one of hydrophilic group of the several sugar units is substitute with a hydrophobic group such as methyl or acetyl, the affinity with lignin results enhanced, acting in this way as a compatibilizer and aiding the cohesion between the three major lignocellulosic polymers. It constitutes about the 25% of the biomass material and its composition varies on the considered kind of substrate [15]. For instance in hardwood and grasses the major sugar compound in hemicellulose is xylose, whereas in softwood the most common monomer is mannose. Hence, as cellulose, it could be exploited as a bio source of sugar starting material for further added value chemical products [10].

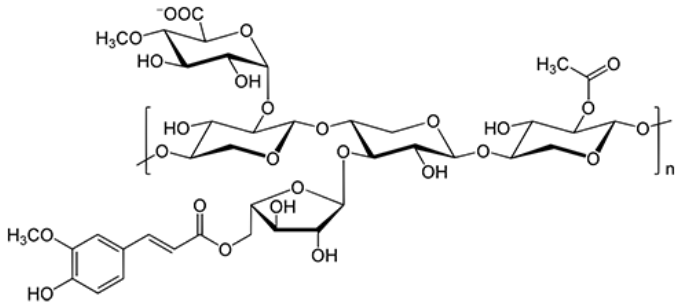


Figure 1.4 Hemicellulose polymer chain. It is clear the more heterogeneous composition than cellulose.

- Lignin

Lignin is an aromatic polymer biosynthesized when the plant growth has already ceased. The three major monomers are represented by coniferyl, sinapyl and p-coumaryl alcohols units as it is depicted in Figure 1.5. It is link to hemicelluloses both by physical entanglement and through covalent cross-links. For instance some molecules of ferulic acid are initially bonded to hemicellulose via ester bonds and during the following lignification process, their aromatic ring can be incorporated into the lignin network by participating in the radical polymerization reaction[17]. The cross-linking degree is strictly correlated with the cell walls rigidity and with the enzymatic digestion resistance.

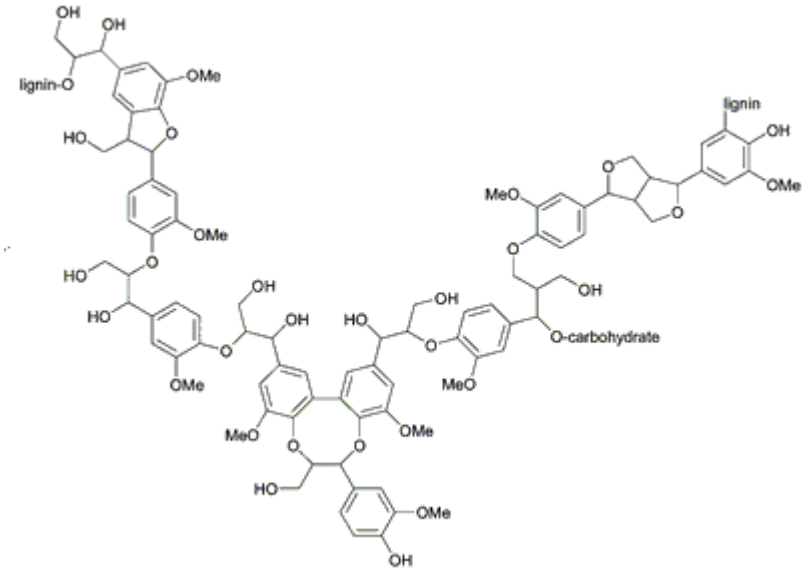


Figure 1.5 Example of the complex lignin chemical composition. The major constituents of lignin are aromatic alcohols.

It represents the hardest polymer in the lignocellulose macrofibril and supporting both carbohydrate chains, cellulose and hemicelluloses, it works as structural reinforcement for the whole plant organism. Thanks to its resilience it provides several shields against the natural attacks such as the bacteria enzymatic hydrolysis or the toughest weather conditions that has allowed the vegetable specie survival during the entire earth life. On the other hand if the cellulose core wanted to be reached as a feedstock supply this rigid network must deconstructed as illustrated in Figure 1.6 trough physical, chemical or even biological pretreatments, often performed at elevated temperatures and pressures. One of the most known pretreatment in the paper production area is the Kraft process. This consist in the heating at 130°C – 180°C of the lignocellulosic biomass for several hours in aqueous mixture of alkali substances. The obtained product is a carbon concentrated liquor where the lignin and hemicellulose are dissolute. This doesn't perfectly match the optimum substrate for the biorefinery industries which require high purity of saccharides with the lowest possible amount of by-products. Furthermore the basic liquor obtained must be neutralized with mineral acids, overall resulting in a huge utilization of toxic and dangerous substances that at the end of the process must be disposal, not matching at all the green chemistry approach. Alternatively, lignocellulose can be treated with mixtures of organic solvents, for example ethanol and water, in the so called organosolv process, to separate lignin from the cellulose fraction[18]. Another well know pretreatment is the steam explosion where lignocellulosic material is exposed to a high-pressure saturated steam at a temperature of 160–260 °C for few minutes. The pressure is gradually released, and the steam expands within the lignocellulosic matrix, causing the separation of individual fibers and the deconstruction of the cell wall structure. Acid can be added as a catalyst during the steam explosion, though their addition is not mandatory, considering that lead in variation of the chemical composition of the final products [19].

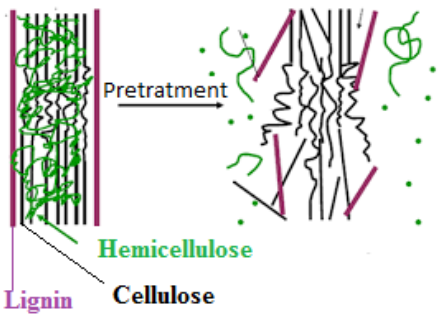


Figure1.6 Deconstruction pretreatment of lignin to make cellulose available for further transformation.

1.2.2 Biorefinery system

The biorefinery system embraces a wide range of technologies able to separate biomass resources into their building blocks such as, carbohydrates, proteins and triglycerides, which can be further converted to value added products, biofuels and chemicals. A biorefinery is considered a network of facilities where biomass conversion processes and equipments are smartly integrated to produce transportation biofuels, power, and chemicals. This concept is analogous to today's petroleum refinery, which produces multiple fuels and chemical products from petroleum. An important stage in the biorefinery network is the provision of a constant and regular supply of feedstock. Indeed one of the main drawbacks of the use of the biomass as a renewable resource is its collection from all the disconnected places where it is stored, like agricultural fields and industries or forest and its following transportation to the biorefinery centre. Moreover its really poor energy density involves high costs and thus economic losses for its transportation. To enhance its energy density should be better dried soon after its harvesting in order to avoid the transportation of useless masses of water that only increase the shipping costs and the energy waste. Thus it is clear that to optimize the biorefinery profits and the environmental benefits a logistic study on the feedstock collection and transportation is mandatory to be developed, whereas with the fossil refinery it is not necessary due that oil is extracted directly in liquid form and thus could be sent trough pipe lines. Furthermore biomass experiences seasonal changes since its collection is not possible throughout the entire year. Thus in order to ensure the continuous request of the chemical market, seasonal switch to fossil source are necessary. Moreover the biomass composition is not consistent because varies greatly from species to species and season to season thus biorefinery operation parameters require to be flexible in order to accept and work with every kind of substrate. Hence some drawbacks are present in the use of biomass instead of fossil oil, but its renewable features that promise a possible endless source of energy and matter push the research toward more studies to overcome these obstacles. For example numerous crops have been proposed or are being tested for commercial energy farming in order to have a constant composition and a controlled amount of biomass feedstock during the whole year. Potential energy crops include woody crops and herbaceous plants like grasses, starch and sugar crops and oilseeds. Some common characteristics are researched for an ideal energy crops such as

high yield of dry matter per hectare, low cost, low energy input to be grown and composition with the least amount of contaminants.

In the biorefinery system biomass feedstock is converted into valuable products by several technologies applied jointly such as thermochemical, biochemical, mechanical, and chemical processes.

- Thermochemical processes convert biomass into energy and chemical products. Basically there are two main processes, the gasification and pyrolysis. The first consists in keeping biomass at temperature major than 700°C with poor oxygen environment to produce syngas, a mixture of H_2, CO, CO_2 and CH_4. Syngas can be used directly as a biofuel or as chemical intermediate for the production of fuels and commodities. The latter thermochemical technique, pyrolysis, uses lower temperatures than gasification, about 500°C in total absence of oxygen to convert the feedstock into liquid pyrolytic oil, solid charcoal and light gases. The most desirable of these three compound phases is the pyrolytic oil which its yield could be maximized through the flash treatment in which biomass is processed with high heating rates that use contact times in the order of few seconds. This technique is also recently studied as in situ method for the conversion of biomass into liquid products, directly performed where the feedstock is collected, such as in a agricultural field. In this way the energy density of the biomass is enhanced and could be easer transported because in form of liquid rather than solid, thus reducing the shipping cost. Obviously in biorefinery could be also performed the third thermochemical technique in which the biomass is burned in rich oxygen atmosphere to provide heat generation. However this latter treatment is not preferred due that doesn't produce any added value products and also produce negative environmental impacts. For instance lot of dangerous compounds are emitted during the biomass combustion such as polycyclic aromatic hydrocarbons including polycyclic aromatic hydrocarbons, dioxins, furans, volatile organic compounds, and heavy metals, especially when combusted in traditional stoves.

- Unlike thermochemical processes, biochemical processes are conducted at lower temperatures and have lower reaction rates. The most common types of biochemical processes are fermentation and anaerobic digestion. Fermentation uses microorganism and enzymes to convert the substrate in value added products. The main one produced is ethanol but also the production of hydrogen, methanol and succinic acid are deeply investigated. A mixture of methane and CO_2 called biogas can be formed by anaerobic digestion which involves the breakdown of organic material by bacterial in absence of oxygen at temperature about 50°C. Biogas can be upgraded up to 97% of methane and used as a replace of natural gas.

- Usually mechanical treatments are performed on the biomass not to change its state or composition but to reduce its size in order to making it more available for the following processes or to separate the feedstock components.

- The most common chemical processes in biomass conversion are hydrolysis and transesterification. Hydrolysis uses acids, alkalis or enzymes to depolymerize polysaccharides and proteins into their components or chemical derivates. Transesterification is the chemical process by which vegetable oils can be converted to biodiesel, a mixture of methyl and ethyl esters of fatty acids. In biorefinering could also be exploited Fisher–Tropsch synthesis, methanization reaction and steam reforming[20].

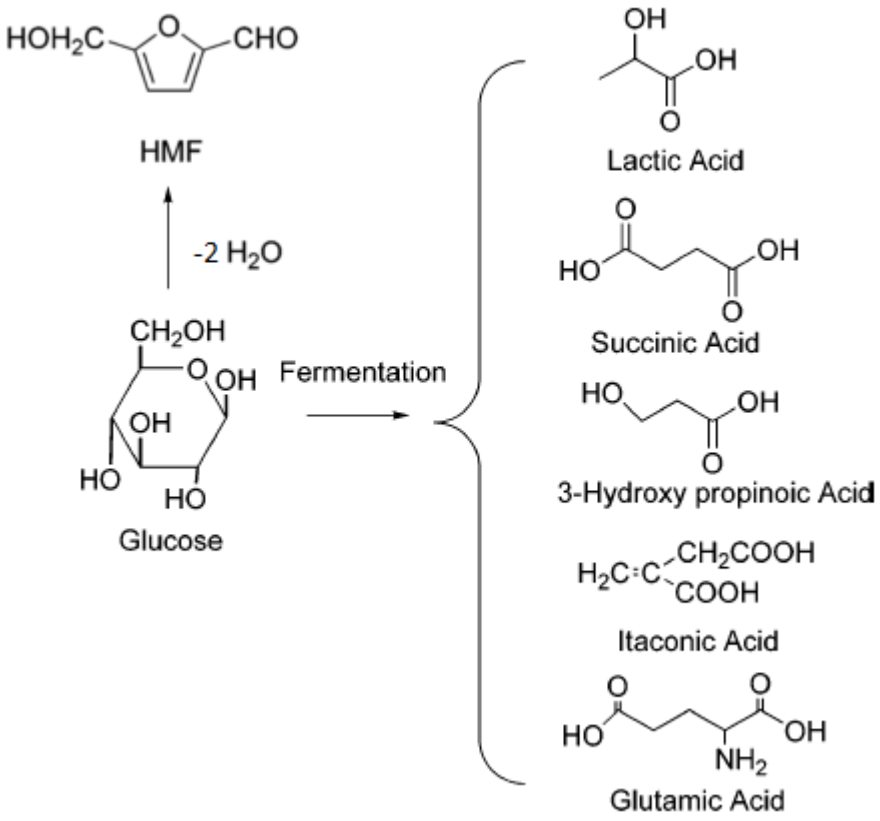
1.2.3 Glucose

The cellulose consistent composition, made of only by glucosan units, makes it the favorite constituent of biomass exploitable in biorefinery. Indeed the more homogeneous chemical composition of the biorefinery feed, rather than when hemicelluloses or lignin are utilized, represent an advantage in term of more constant operational parameters that it is translated in more easier process conduction and in a greater industrial productivity. Moreover also the chemical composition of the converted products is more homogeneous and it is a benefit for the faster separation processes that follow the conversion step and the easier exploitation in further chemical transformations. Furthermore cellulose could

be ideally totally converted in its building block glucose which is a really interesting platform molecule due that it can undergo in a variety of chemical transformations thus providing a huge world of derivates, usable as a commercial products.

1.2.3.1 Glucose chemistry tree

To better understand the economic potential of glucose as a platform molecule it is following described the main chemistry and the market products that could be originated from this compound, trough both fermentation and chemical processes. The most important chemical derivates are represented in Scheme 1.1 , thus originating the first step of the glucose chemistry tree.



Scheme 1.1 The main chemical derivates obtainable from glucose.

- **5-hydroximethyl furfural (HMF)**, it's only one, and probably the first for industrial interest, of the several platform molecules that could be obtained from chemical transformations of glucose. It is the product of glucose dehydration reaction supported by acid catalyst. Nowadays plenty of studies are conducted on the catalytic systems including organic and inorganic acids or salts, lewis acids, ion-exchange resins and zeolites. Moreover this reaction could be conducted both in aqueous and non aqueous media, or also in mixed solvents, thus lot of studies are today focus to find and improve the best reaction environment[21]. Unfortunately the chemistry of the HMF formation is very complex: besides dehydration, it includes a series of side reactions such as isomerization, fragmentation, and condensation which highly influence the yield of the process[22]. Despite these drawbacks HMF could be transformed in a plenty of derivates. Indeed it is called a "sleeping giant"[23] because posses a high potential industrial demand but lot of efforts must be still carry out to improve the whole yield of its production. The main chemical intermediates that can be obtained from HMF are six carbon molecule, such as 5-hydroxymethylfuranoic acid, 2,5-furandicarboxylic acid, 2,5-bis(hydroxymethyl) furan, and 2,5-furandicarboxaldehyde that could replace other petrochemical-based compounds[24]. 2,5-furancarboxaldehyde is a starting material for the preparation of Schiff bases, and 2,5-bis(aminomethyl)furan is able to replace hexamethylenediamine in the preparation of polyamides.

  2,5- bis(hydroxymethyl)furan is used in the manufacture of polyurethane foams, and the fully saturated 2,5-bis-(hydroxymethyl)tetrahydrofuran can be used like alkanediol in the preparation of polyesters[25]. Moreover, HMF has been used for the production of special phenolic resins, and numerous other polymerizable furanic monomers with promising properties have been prepared[26].

  Again HMF could be transformed in 2,5-furandicarboxylic acid (FDCA) by oxidation using conventional oxidants such as nitric acid or better trough elettrochemical oxidation that conduce in greater selectivities. FDCA is a compound with high potential applications in the polymers field because it can replace terephthalic, isophthalic, and adipic acids in the manufacture of polyamides, polyesters, and polyurethanes[27]. Moreover hydration reaction of HMF in acid condition lead toward the formation of levulinic acid. It is a low-molecular weight carboxylic acid having a ketone carbonyl group. Levulinic acid

is useful as a solvent, food flavoring agent, and starting material for the preparation of a variety of industrial and pharmaceutical compounds. Its potential uses as a resin, plasticizer, textile, animal feed, coating material, and antifreeze have been reported [28].

- **Lactic acid** is commercially produced mainly through the fermentation of glucose, though the process require many improvements to overcome several drawbacks of its productivity and selectivity. Nonetheless it is produced around 350000 ton/year and the worldwide growth is believe to be 12-15% per year due that is used by many industries such food, chemical, pharmaceutical and cosmetic[29]. An interesting application which is currently receiving great attention is its use as a monomer for the synthesis of biodegradable polymers. Indeed polylactic acid polymers could be an environment friendly alternative to plastics derived from petrochemical materials. Moreover lactic acid could be chemically esterified to produce linear or cyclic compounds that could be useful as monomer for the production of copolymers[30]. Lactate esters are also used as plasticizers in cellulose and vinyl resins and enhance the detergent properties of ionic surfactants. These compounds are even useful in the preparation of herbicidal formulations. A further product coming from the transformation of lactic acid is propylene glycol obtained by hydrogenation reaction. It could be exploited as commodity by several industries for its solvent features. Nowadays propylene glycol is produced by hydration of propylene oxide that its production involve the use of chloroydrin and hypoclorous acid, thus once again the route from glucose could be considered a greener path to the petroleum-based process. Acrylic acid, with its annual production of 14.2 million metric tons, is considered an important monomer for the production of acrlylate polymers because in turn found numerous application in surface coatings, texitiles, adhesives, paper treatments and detergents[31]. Currently acrylic acid is produced out 100% of fossil oil mostly via direct oxidation of propene. Therefore if it is produced by dehydration of lactic acid is an attractive target for new bio-based compound, hence lot of studies are focus on this reaction. Lactic acid could be transformed also in 2,3-pentanedione via condensation reaction. This diketone has application as a flavor agent, photoinitiator or biodegradable solvent[32].

- **Succinic acid** is another platform molecule that could be obtained from the glucose fermentation and could undergo in several chemical reactions thanks to which can found plenty purposes. The market potential for products based on succinic acid is estimated to be 270 000 ton/year, including 1,4-butanediol, tetrahydrofuran (THF), *ç*-butyrolactone, *N*-methylpyrrolidone, and linear aliphatic esters. Currently succinic acid is mainly produced from butane or maleic anhydride[33]. Direct hydrogenation of succinic acid, succinic anhydride, and succinates leads to the formation of the product family consisting of 1,4-butanediol, THF, and *ç*-butyrolactone. 1,4-Butanediol is a compound of great interest as a starting material for the production of important polymers such as polyesters, polyurethanes, and polyethers. THF is a solvent for polyvinyl chloride and it is used as a monomer in the manufacture of polytetramethylene glycol, which is used as an intermediate for Spandex fibers and polyurethanes[30].

- **Hydroxypropionic acid** is a structural isomer of lactic acid, also produced by the glucose fermentation. At the moment there isn't a commercially viable production route from fossil fuel feedstocks. Like lactic acid, it has bifunctionality that allows for multiple chemical transformations. The acid group can be reduced to alcohol or can be converted to a variety of esters, amides, and derivatives. For example the reduction of that acid conduce to the formation of 1,3-propanediol that is a starting material for the production of polyester or by dehydration could conduct to acrylic acid and its derivates. Furthermore it could be converted in the precursor of vitamins B1 and B6 like malonic acid trough oxidation pathway. Polymerization of hydroxypropionic acid conducts in a bio-derived plastic similar to the polylactic acid with attractive mechanical properties such as ductility, rigidity, and exceptional tensile strength in draw films. Additionally, the polymer could be degraded trough enzymatic or hydrolytic process, thus making it more environmentally attractive[34].

- **Itaconic acid** can be considered an exceptionally helpful compound for the presence in its structure of the methylene group along with two carboxylic functions. The double bond makes it available for the polymerization and thus a potential substitute for acrylic or methacrylic acid. The polymerized methyl,

ethyl, or vinyl esters of itaconic acid are used as plastics, adhesives, elastomers, and coatings. This compound is also used as a co-monomer in polyacrylonitrile and styrene-butadiene copolymers[35]. Nowadays the total marked size is regarded around 10000-15000 ton/year. The higher part of this market is for polymers, with another small part for additives, detergents, and biologically active, derivatives, particularly in the pharmaceutical industry and in agriculture. The basic chemistry of itaconic acid is similar to that for maleic and succinic acids and their derivatives[36].

- **Glutamic acid** is used in food, drugs, dietary supplements, cosmetic, personal care product and fertilizers. The polymer derived from glutamic acid, poly *ç*-glutamicacid, is a polypeptide that contains both enantiomers of glutamic acid units connected by amide linkages between amino and *ç*-carboxylic acid groups. Therefore, it is a potentially optically active polymer having a chiral center in every glutamate unit. It is water soluble, biodegradable, edible, independent of oil resources, and nontoxic. The numerous potential applications in medicine, food, cosmetic, plastics, and water treatment have recently attracted particular attention. Moreover it could be converted in useful precursor of chemical intermediates such 1,5-pentanediol and glucaric acid[37].

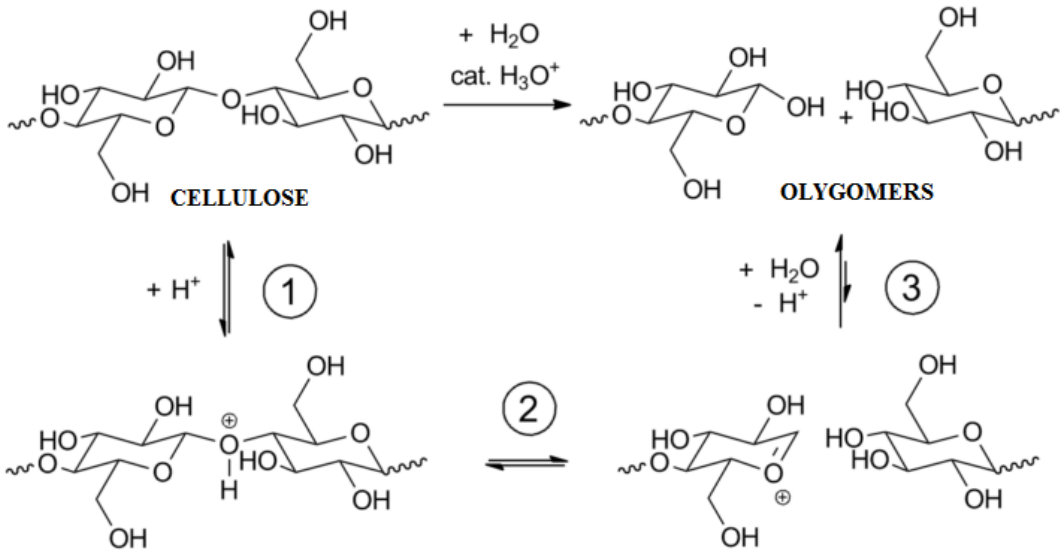
1.2.3.2 Nowadays glucose production

Nowadays glucose is produced on industrial scale from starch by enzymatic or inorganic acid hydrolysis making corn the main source of glucose. For instance one ton of corn is able to produce about 590Kg of glucose. Many crops can be used as the source of starch like maize, rice, wheat, cassava and corn husk, depending on the parts of the world where they are cultivated. In the enzymatic process the enzyme α-amilase breakdown the starch structure in shorter sugar oligosaccharides that could be further fermented into ethanol and this represents the worldwide major enzymatic process for the production of glucose trough cleavage of bigger carbohydrates. Despite the high selectivity that provide the enzymatic via some drawbacks restrict the potential glucose productivity. Indeed enzymes can operate only in narrow windows of operational parameters like pH, temperature, and concentration otherwise their protein structure undergoes in denaturation reactions that prevent their ability to convert the starting material into the

desired products. Thus the strictly conditions in which the enzymatic hydrolysis must be conducted doesn't allow to reach high conversions that most of the times are also limited by the crystalline morphology of cellulose that prevent the enzymes approach to the glycosidic bonds. Moreover the high cost of the α-amilase, which its recycling is still a challenge, in addition to the long dead times of the batch operations where the enzymatic hydrolysis is conducted and the following concentration process that is required to have a market competitive product, make the enzymatic conversion an expensive technique. On the other hand inorganic acid such as H_2SO_4, HCl or HF permit to reach higher conversions but lower selectivities are achieved. In this processes, after the starch conversion into glucose, the sugar solution must be neutralized with basic chemicals yielding in high undesired production of salts that must be recovered and subsequently disposed. Thus the use of toxic and dangerous substances, the large waste production and the not selectivity features that imply following product purifications contrast the Green Chemistry objectives and neither satisfied the optimum economic purpose. Furthermore the concentrate or dilute acidic conditions mandatorily require stainless steel equipments that enormously increase the invested capital for the plant construction. The most known worldwide sugar production process, the hot water extraction from sugar beets, is not sufficient to overcome these drawbacks due that yields in sucrose, a disaccharide composed by glucose and fructose, thus an hydrolysis reaction followed by a separation processes is still required to obtain pure glucose as platform molecule. Moreover the utilization of sugars beets for chemical purposes is in competition with the human feeding supply. Hence it is clear that large profit margins are present, also in term of environmental benefits, for the glucose exploitation from cellulosic substrates. Thus lot of studies are recently concentrated in this field to find alternative methods that can overcome the major drawbacks previously described, in order to make glucose a competitive alternative platform molecule to the ones coming from the fossil sources.

1.2.4 Reaction mechanism

If useful platform molecules want to be obtained from cellulose, the 1,4-β-glycosidic bond should undergo on a hydrolysis reaction yielding in shorter sugar oligosaccharides or even in glucose units. Obviously if microcrystalline cellulose would be stirred in water even for infinite time no one product would be obtained. Indeed for the hydrolysis reaction is essential a little quantity of an acid or basic compound, as well as the presence of water. Due that this farther reactant doesn't take place in the end products, but remains totally in the reaction environment, it could be defined as a catalyst. An acid catalysis is preferred rather than a basic catalysis because in alkaline conditions the hydrolysis exhibit poor selectivity in sugar compound, yields mainly in C_1 to C_6 carboxylic acids. As a confirm, the alkaline hydrolysis was commercially explored by the chemical industries for the oxalic acid production.



Scheme 1.2 Hydrolysis mechanism of the β-1,4-glycosidic bond.

The main importance of an acid catalyst, if an acid hydrolysis is considered, is due to the fact that an elettrofilic compound must approach the oxygen atom among two consecutive glucose monomers to purchase a fraction of its electronic density, thus allowing an impairment of the glycosidic linkage (step 1). This permits the cyclic carbocation formation upon cleaving the glycosidic bond (step 2). Finally a water molecule connection on the resulting elettrofilic carbon atom releases a shorter oligosaccharide and regenerates the elettrofilic specie (step 3)[38]. The whole hydrolysis reaction mechanism of the β-1,4-glycosid bond is summarized in Scheme 1.2.

Over the hard morphology exhibited by the crystalline structure, a farther impediment of this selective chemical decomposition is represented by the other oxygen atoms present in the glucose monomer units that show a more basic behavior than the glycosidic oxygen. The estimated pKa values for the hydroxyl oxygens is calculated as -2.4, while the pKa values for the protonated acetalic oxygens wander around -4 [39] as clarified in Figure 1.7.

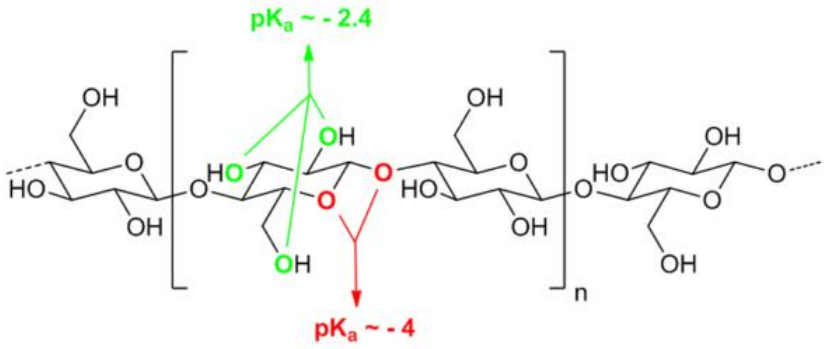


Figure 1.7 Estimated pKa of the hydroxyl oxygens and protonated acetalic oxygens in the cellulose chain polymer.

In this way the reactive site of the reaction is hide by the substrate molecular conformation and this provides a chemical protection mechanism of the cellulose against the acid hydrolysis. As confirm, from DFT calculations emerge that the protonation step require almost the 90% of the total activation energy needed for the glycosidic bond hydrolysis. In that conformation it is clear that the substrate is unable to react and so is called 'latent sate' [40]. Here we have understood that the cellulose morphology, for more than one reason, represent one of the harder obstacles to exceed if smaller and uncomplicated building block molecules want to be obtained and this cannot be totally achieved only with the use of the hydrolysis processes previously described. As a confirm, with the enzymatic process, neither with the inorganic acid hydrolysis a total conversion of cellulose into glucose is achieved. Hence mechanochemistry could play a key role because introduce mechanical forces in the reaction system that could be able to induce conformational changes that transform the 'latent state' in an 'activated state' as better illustrated in Figure 1.8. Indeed when the grinding balls impact the sample particles could hypothetically cause two important effects: weaken the intermolecular hydrogen bonds of the recalcitrant substrates leading in slightly delamination of the

cellulose sheets and expose the hide glycosidic oxygen atoms in a more open structure where they result farter from the neighbor hydroxyl oxygens. This conformational changes could enhance the basicity of the glycosidic oxygen sites, thus the acid protons or the solid acid particles can easier approach it, resulting in a decrease of the energy barrier of the difficult protonation step, facilitating in this way the overall hydrolysis reaction. In this way, thanks to the mechanical energies, the hardest obstacles could be overtook and thus the reagents can easily proceed toward the desired products.

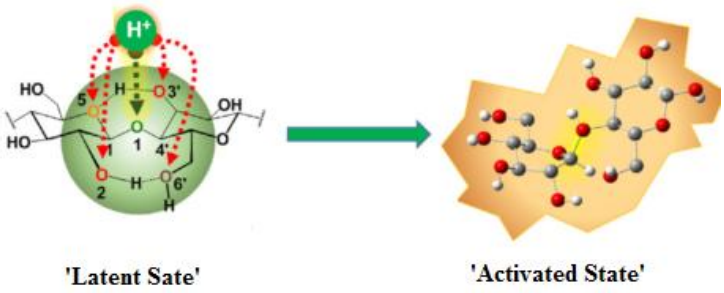


Figure 1.8 Transition from the cellulose 'latent state' to the 'activated state' assisted by mechanical forces.

1.3 MECHANOCHEMISTRY

In literature could be found plenty of different definitions of mechanochemistry depending on the years when they were formulated and on the kind of phenomena that were studied with this subject. Today mechanochemistry is considered a research field intersecting chemistry and mechanical engineering in which mechanical forces are exerted on the reaction materials thanks to special devices in order to provide physical and chemical transformation. The energy purchased by the matter can be converted in temperature and pressure increases, formation of hot spots and generation of radicals or new surfaces that overall contribute to change intrinsically the reactants nature. Moreover friction, wear and fracture phenomenon must be considered when substrates are processed with this treatments because together can lead to particles sizes reduction or in inter and intramolecular bonds breaking[41]. Despite its long history, mechanochemistry has been introduced as a proper branch of chemistry only in the recently times. During the new millennium the scientific community has increased its interest in this part of research field because totally match the Green Chemistry point of view. Indeed the use of mechanical forces instead of the batch or plug flow systems permits the occurring of solid-solid reactions. This avoids the use of solvents, that since today are essential when also a heterogeneous solid catalyst reaction was performed. Thus huge advantages arise in terms of starting materials saving, waste disposal and more safety conditions for the technical personnel[42]. Typically the major research works developed in this scientific discipline are carried out with milling devices. On the market there are several mill equipment such as mixer mills, planetary ball mills, pebble and attrition mills, each offers their own advantages and disadvantages. Specifically in this works the cellulose depolymerization reaction is been studied with both planetary and a mixer ball mill also to compare their behaviors and the differences that could emerge on the final reaction products. As described below, the two milling machines differs not only on the force intensity that can be exerted on the reactants but also in the way how their bowls are swung.

1.3.1 Milling devices utilized

- Planetary ball mill RETSCH PM 100

The majority of the tests on which our work is based were performed in a planetary ball mill, represented in Figure1.9. This instrument is composed by a zirconia 50ml bowl that can accommodate up to ten grams of starting material and six 25mm diameters zirconia balls. The grinding jar is arranged eccentrically on the sun wheel of the planetary ball mill and the movement direction of the sun wheel is opposite to that of the grinding jars. As it is shown in Figure 1.10, in this way the grinding balls are subjected to overlapped rotational movements and the difference in speeds between the balls and grinding jars produces an interaction between frictional and impact forces, which releases high dynamic energies. The main rotation disk of this device can spin up to 500rpm and are used for ultrafine grinding of powders or grains, but can also be used to induce mechanochemical reactions [43].

Figure 1.9 Planetary ball mill, Retsch PM 100 model.

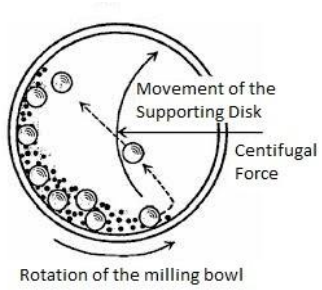


Figure 1.10 Grinding balls movement inside the Planetary ball mill bowl.

- Mixer ball mill RETSCH MM 400

The main elements of this apparatus, represented in Figure 1.11 consist in two arms each of them made to hold one capsule in a clamp, which is swung back and forth from 3.0Hz up to 30Hz. In this way the particles caught between the colliding balls, or between the balls and the capsule walls, experiencing high stresses. Moreover most of the collision energy is applied over a small contact area, represented by the surface contact between the spheres, that makes the applied energy per area extremely high. Indeed considering the high velocity of the balls in the capsules this strength is applied for a short time, and the time of relaxing is short as well leading to very high stress gradient[43]. All this factors are conducting to giving strong forces on the

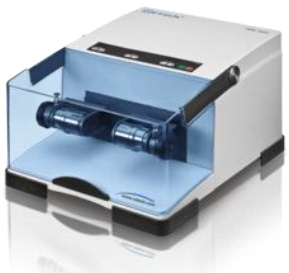


Figure 1.11 Mixer ball mill, Retsch MM 400 model.

powder that lead in a incredible particle size reduction as well as a energy site where reactions can take place.

1.3.2 Milling parameters

Conduce reactions in this kind of devices is not easy as it could seem due to the fact that there are plenty of parameters that could change the reaction results and thus it is required the best setting up to optimize the yield and selectivity of the desired products. Indeed, as it is been reported by a lot of scientists that have worked on this subject, slightly differences in apparent worthless settings can heavily alter the tests reproducibility conducing in a wrong deductions of the solid catalyst performances[44].
Therefore the main milling parameters are listed below:

- Milling speed;

  Obviously the milling speed that the grinding balls can reach inside the jar is one of the most important parameters that influences the reaction results. As it emerge from the kinetic energy formula, the energy the milled materials undergo is square correlated to the velocity and direct proportional to the mass of the milling media.

$$E_k = \frac{1}{2}mv^2$$

  Are exactly the energies that the milling media engrave on the milled materials that permit the reagents to overtake the activation barrier energy and drive them towards the products, becoming in this way key parameters for our purpose. Furthermore the mechanical motion is mandatory to create the right close contact between the substrate and catalytic reactive sites for the occurring of the chemical reaction and of course, higher forces are able to create better interactions.

- Temperature;

As could expected the overall amount of energy produced by the milling media is purchased by the reagents but a proportion of that is released as heat, that increases the temperature inside the grinding jar, which may be beneficial or detrimental for the desired reaction. Especially in our kind of work, as it is explained by Rinaldi et. al.[45] at the beginning of the water soluble products formation, a cake starts to storing on the wall side of the grinding vessel, resulting in a barrier for the heat exchange with the outside environment. Indeed the product layer that is formed on the vessels side during the reaction can be considered as a fouling coefficient for the heat conduction, and as it is yet known highly reduce the heat dispersion of the whole system. Particularly, as Figure 1.12 can confirm, this phenomenon also occurs in our planetary ball mill vial under the milling conditions utilized. Hence to prevent to reach too high temperatures inside the milling bowl, that could conduct in a products decomposition, it is needful to add some grinding breaks in order to cool down the system. This latter is an important parameter as well, especially for the industrial productivity point of view, because will be must optimize both the time extent of the breaks as the milling time after that will be necessary stop the machine to avoid the incipient products decomposition.

Figure 1.12 Water soluble products cake formed inside the planetary ball mill bowl.

- Grinding material and size

The seller companies offer plenty different varieties of grinding vessels and balls, create specifically to permit the best milling conditions for every kind of substances. Indeed the grinding items could be purchased in a multitude of materials such as agate, corundum, tungsten carbide, zirconia and several varieties of steel or polymers. It is a basal requirement that the grinding items must be harder than the milled substances otherwise no successful reaction occurs and, worse than that, all the grinding elements result abraded after the milling, no more useful for any purpose. Furthermore the materials of the grinding bowl and balls which they are constructed could conduct in the possibility, or not, of some contaminations on the milled products or on the catalyst performances that in both ways influence negatively the

scientific deductions. In accordance with these explanations, in our work are been chosen items totally inert and harder than the starting substrate, the expected products and the studied catalysts, such as made of zirconia, as it is depicted in Figure 1.13. The absolutely catalytic inefficiency of the grinding materials used was verified by a blank reaction performed before every tests, milling cellulose in absence of any catalyst. Additionally the size ratio between the balls to grains, or milled powder, determine the fineness of the end products. If the starting size of the sample material is bigger than the milling balls used, the milling balls will have the tendency to glide off the surface of the sample material and not mill it very well. The final fineness of the milled product, is determined by the size of the milling balls used and as a very general guideline, the final fineness achievable is $1/500^{th}$ of the milling ball diameter. Among the milling specialist it is common use larger milling balls diameters to effect the initial particle size reduction, before changing to a smaller balls diameters. Once a final particles dimension of around 20μm has been reached, the powder particles will become attracted to each other and start to stick or agglomerate together. To overcome these attractive forces in-between the powder particulates a barrier needs to be introduced and this barrier is in the form of a liquid. Obviously the liquid used must be compatible with the sample materials. Ideally water can be used, or a solvent with a low vapor pressure, such as isopropyl alcohol, in the way that can be easily dismissed once the grinding is completed. In our work all the experiments were conducted using single size grinding balls to prevent the crushing of the smaller balls because of the impact with the bigger ones.

Figure 1.13 Grinding items of the mixer ball mill used made of zirconia.

- Amount of free volume

The grinding bowls have useful working capacities of just under half their volume. For example a 250 milliliters grinding bowl has a useful working capacity from 30 to 125 milliliters, with the remaining volume being occupied by the milling balls and their movement. In order to have efficient impacts, there must be sufficient space amount for the balls to move freely in the milling vial. Indeed if the vessels is filled

significantly with the starting material, the impacts will be less energetic. Contrarily lower free volume available means decrease the free path of the balls, resulting in more collisions per unit time. Therefore, more energy is exerted on the reagents, allowing for faster crushing. Moreover if larger amount of free volume is present lower quantities of materials are produced. Hence the amount of free volume is a parameter that should be accurately optimized if the process will scaled up in a industrial plant but for our task to study the cellulose depolymerization reaction, a constant amount of ten grams of overall reactants was charged in the planetary ball mill and four grams in each capsule of the mixer ball mill to have the same milling conditions.

- Water quantity

Since water is one of the main reactants for the cellulose depolymerization, its amount inside the milling system, both as reagents moisture as if physically added, is obviously another parameter that could influence the yield and selectivity of the reaction products. The moisture content of the microcrystalline cellulose purchased was determined by thermogravimetric analysis (TGA), that had reported 6% wt humidity. This corresponds to a glycosidic bonds to water ratio of about 1:2 showing thus in a enough stoichiometric ratio for the studied reaction. For the same reason TGA were conducted on the solids catalyst tested. In addition to the water chemical importance for the hydrolysis reaction, the physical aspects should be considered since a liquid inside the grinding bowl buffers the mechanical forces exerted on the substrate. As a confirm of this behavior, when some milliliters of water were added in the mixer ball mill no product was detected. On that account in our experiments, no water neither other liquids were added in the milling devices in order to achieve the strongest possible depolymerization rates.

1.4 CATALYSIS

Catalysis is the branch of chemistry that studies the reaction accelerations performed by the addition of small quantities of compounds so called catalysts. Moreover the definition of catalysts requires its none consumption, or at least in a really tiny amounts, during the reaction occurring, thus at the end of the chemical process it can be recycled for another reaction cycle. Although catalysts are not consumed by the reaction itself, they may be inhibited, deactivated, or destroyed by secondary processes so sometimes, depending on the chemical and physical environment where they are exploited are required regeneration steps or their replacement. The acceleration phenomenon can be explained by a change in the reaction pathway which leads to a change in activation energy of the process and thus influence only its kinetic expect but not the thermodynamic equilibrium. Indeed in the presence of a catalyst, less free energy is required to reach the transition state, but the total free energy from reactants to products does not change. Theoretically a chemical reactions could be always performed both with the presence or absence of a catalysts, only could take really long time to occur if this supporting species is missing. Catalysts not only provide reaction acceleration that means major plant productivity but also lower operational temperature and pressure thus facilitating the whole process and requiring cheaper equipments. In some cases, such as zelolites, they also prevent the formation of side and consecutive products, reducing in this way the separation costs and the waste disposal. Hence during the last century this chemistry field is been widely and deeply studied to improve the economic and environmental benefit of the chemical industries. The catalyst world market turnover was $13 billion in 2008. Furthermore, more than 80% of the installed chemical processes since 1980 are based on the use of catalysts[46]. Catalysts can be classified as heterogeneous or homogeneous, depending on whether a catalyst exists in the same phase as the substrate. Homogeneous catalysis covers the field of soluble catalysts which are therefore dispersed in the reaction solution whereas the catalyst in a heterogeneous process is in a different phase to that of the reaction solution. Despite the typically lower activity caused by utilizing a less-dispersed catalyst, a heterogeneous catalyst has, compared to a homogeneous catalyst, the advantage that the separation of the catalyst at the end of the reaction step is significantly easier and thus could be regenerated and reused for another reaction cycle, thus reducing the overall material utilization and the waste disposal.

1.4.1 Heterogeneous catalysis

In this work are compared catalytic activities of different solid acids toward the cellulose depolymerization. Were selected acid catalysts, both Bronsted and Lewis species, because as explained in the "reaction mechanism" paragraph acid compounds are required for the hydrolysis of the glycosidic bonds of cellulose. Solid acids rather than the classic electrolytic liquid acids exhibit plenty of advantages for the green chemistry approach. Rigorously, due that the cellulosic substrate is also solid as the catalysts used, the studied reaction should be classified as homogeneous catalysis, but due that the utilization of a solid catalyst leads to the same advantages and drawbacks that usually are present in a heterogeneous catalysis we refer to this reaction as a heterogeneous system. For instance, as in the heterogeneous catalysis, one of the main limit to overcome is the substrate approaching and interaction with the catalytic species. Moreover if the sugar products, obtained from the cellulose depolymerization, want to be available for further chemical transformation, must be recovered with the help of water that produce an aqueous solution where the insoluble catalysts is not dissolved, thus allowing an easy separation of the reaction products from the catalysts, as occur in the heterogeneous catalysis. One of the main advantages that posses a solid reactant is the less hazardous behavior due to its physical state features. Patently a solid cannot exhibit any vapor pressure so doesn't release any toxic vapors like hydrochloric acid or hydrofluoric acid and it is less complicated to handle due to its no possibility to have a proper concentration. Furthermore solids not exhibit vapor pressure, or however in a negligible amount, thus cannot posses flash point like the most commonly organic solvents exploited by the chemical industry. This shows a enormous benefit for the chemical industries during the storing, shipping and reaction operations. For instance one of the acid that was tested is kaolinite, a clayey compound that could be found in nature in the deep bed of the rivers. Thereby a less specialized personnel can be exploited for this kind of reaction also in huge magnitudes as the chemical plants. Furthermore the excepted products of the hydrolysis reaction are sugars or longer oligosaccharides that until five units of monomer should be totally soluble in water. This is translated in huge advantage for the chemical process because once that the reaction is been conducted the overall product amount can be recovered with an water extraction and thus, the unconverted starting material together with the solid acid can be recycled and reused for a further reaction. More than that if the solid acid doesn't release any protons, no neutralization

process must be conducted on the end product and in this way more chemicals utilization could be saved, increasing the profit, the working safety, and the environmental impact of the overall plant. On the other hand working in total absence of any solvent leads in a serious disadvantages for the reaction developing. Indeed in solid–solid system arise a barrier for the mass diffusion transport required for the useful impact between the substrate and the catalytic sites. This drawback is cleverly overtook by the mechanochemistry since the milling at high frequencies is able to produce ultrafine particles, both as the substrate as the catalyst. Hence, in the ultrafine environment, the reactive sites responsible of the reaction occurring are more exposed and available to produce efficient contacts, moreover assisted by the mechanical forces, that drive the reagents toward the desired products.

1.4.2 Solid acids tested

To compare the activity of some catalyst toward the cellulose depolymerization were chosen solid acids with some common features or differences such as the functional group responsible of the catalytic site, the acid strength and other various physical properties. This particular selection has permitted to draw conclusions on how the solid-solid reaction works and which catalyst properties lead to the best reaction yields and conversions.

- Amberlyst 15 dry hydrogen form

Amberlyst 15 is an acid resin composed by a main polymer chain of polystyrene where some inclusions of divinylbenzene as monomer permit to built several cross-links inside the resin web structure, thus making this solid insoluble in water. The amount of divinylbenzene monomer during the copolymerization process influence the resin reticulation degree that is responsible of some typical parameters like the ion exchange capacity or the mechanical resistance, both extremely important for the catalytic purpose in the milling system. The ion exchange capacity is defined as the total amount of the available exchanging protons per gram of the polymer. As in the most of the acid resins, in this kind of catalyst, the acidity is due to the presence of several sulfonic groups on the aromatic lateral side of the main chain[47], shown in Figure 1.14 A. Amberlyst 15 dry exhibits >0.0047 eq/Kg of exchange capacity and it appears as a opaque black beads, Figure 1.14 B, in the range of 0.300 to 0.425 mm size with an average porous diameter

of 300 Å and surface area of 53m^2/g[48]. Apparently the porous size of this catalyst should appear as a limit for the desired reaction due that the majority of the acid sites are situated inside the porous cavities rather than on the external particle area and that the cellulosic starting material, being a polymer constituted by an average vaule of 225 units, obviously has larger dimensions that prevent its contact with the catalytic sites. For best understanding one glucose monomer has a molecular diameter around 10Å. With this knowledge seems impossible that this kind of resin can work efficiently as a depolymerization catalyst for cellulose. The inefficiency of the system is confirmed by a blank test conducted stirring a mixture of 1:1 in weight of amberlyst 15 and microcrystalline cellulose in water at 25°C for 1h. As expected no one product is resulted from the hydrolysis reaction in these conditions.

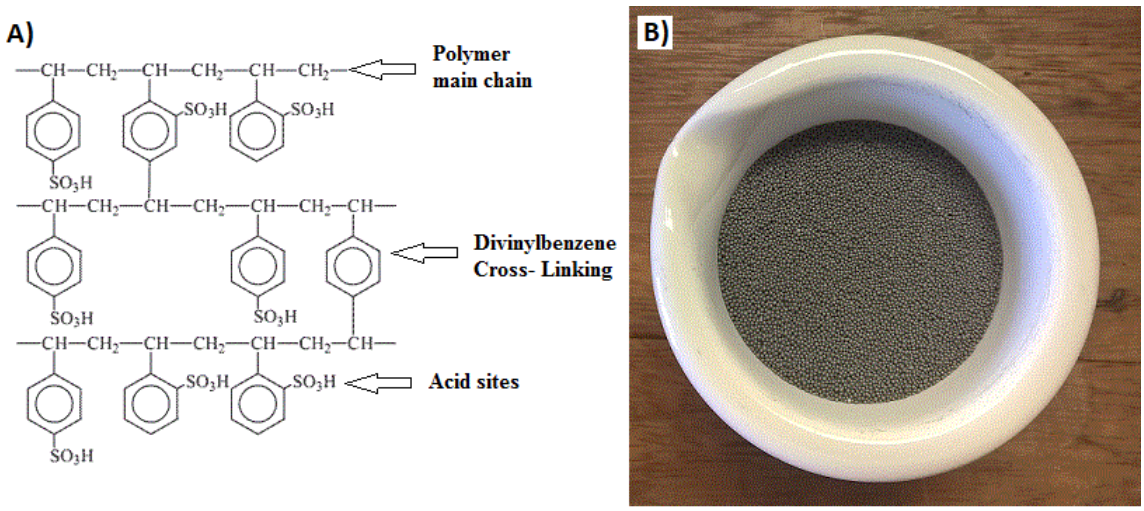


Figure 1.14 A) Cross-linked network of the Ambelryst 15 whit its acid functional groups. **B)** Macroscopic appearance of Ambelryst 15 beads before the grinding.

Nonetheless the mechanical treatments applied on the reactants deeply alter not only the cellulosic substrate conformation but also the catalyst features. As already described the milling process can produces particle sizes of dozens of µm. This morphology changing extremely increases the surface area of the catalyst particles that expose, in this way, a remarkable larger amount of acid sites on its external area, making possible the interaction with the substrate. The physical resin changes are confirmed by that after five hours of milling at 500rpm in the planetary ball mill the polymers particles are so fine and so too light that cannot be separated from the insoluble product fraction with a centrifuge treatment at 4350rpm. Or on the other hand if the sample was milled at 500rpm for ten hours almost the total amount of the polymer became soluble producing a heavy dark solution, as a confirm that the acid site on external area of the particles are

increased and that the polymer reticulation is broken, leading the solid from a starting insoluble beads to an end polar soluble powder. This acid resin could absorb some moisture from the external environment thus resulting in a source of extra water inside the reaction system. To verify its humidity content a TGA was conducted on a few milligrams of this compound, resulting in 0.8% of moisture over the total weight of the sample. Considering also the instrumental error of the technique the water content in the acid could be considered negligible. Thanks to this all the resin weight charged in the ball mill can be attributed to a true value of solid acid amount and furthermore, if different quantities of this catalyst are tested don't lead in a variation of the molar ratio H_2O/AGUs. In this way when ambelryst 15 was used as catalyst the only water amount present in the system was due to the cellulose moisture content and thus resulting in a stoichiometric ratio of 2:1.

- Para toluen sulfonic acid

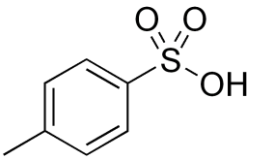


Figure 1.15 Para-toluen- sulfonic acid

para-toluene sulfonic acid, or p-TSA in its abbreviated name form, is a strong organic acid with a fusion temperature of 106°C and thus it appears at room temperature as a white fibrous solid. It shows up a solubility in water about 0.67g/ml and thus result not the best catalytic system for our purpose[49]. Indeed, with the water extraction process that was used, it cannot be separate from the end products resulting impossible to reuse for a further reaction. In spite of this its study was worth to be performed for the similar chemical structure, shown in Figure 1.15, that it shows compared to amberlyst 15. Indeed the catalytic activity of this compound is due to the same acid sulfonic group of the polymeric resin. The difference between the two system mainly reside that, in the latter, the sulfonic group could stand also on the orto position of the aromatic ring but this shouldn't highly influence the acid strength. Indeed the acidity of this both organic group it is due mainly to the possible dislocation of the produced negative charge on the three oxygens of the sulfur atom and inside the aromatic ring itself. Theoretically this acid should be more active than its resin form because, in this latter, the majority of the acid sites are hidden inside the porous of the netting structure, resulting less available for the contact with the cellulosic substrate reactive sites. Unfortunately it is difficult purchased the compound in its anhydrous form and thus

was used its hydrate form with the presence of one water molecule each molecule of acid. This lead to introduce an extra water amount in the reaction system. Due to this when 0.2 equivalents of this solid acid were used, a molar ratio of H_2O on AGUs equal to 2.7:1 arose. This result in the highest H_2O to AGUs ratio that is used in the milling but not highly differ from the 2.35 water ratio arisen when the largest amount of amberlyst 15 was used.

- Kaolinite

Kaolinite, ($Al_2Si_2O_7 \bullet 2H_2O$) is a natural clay mineral derived from the weathering of feldspar minerals found in rock such as granite. It belongs to aluminosilicate group and consists of octahedral aluminum structures (as AlO_6 units) layers covalently bound to tetrahedral silicon structures (as SiO_4 units) layers, in a ratio equal to 1:1. Each aluminum structures layer is linked with one silicon structures layer because every aluminum octahedral has one oxygen atom in common with one silicon tetrahedral. In this way one sheet of clay is formed. In the overall kaolinite structure, illustrated in Figure 1.16 the divers clay sheets are held together through hydrogen bonds between one proton present on each oxygen atom of one side of the Al layer and one oxygen atom on the following Si layer. As a consequence of this structure, the silica/oxygen and alumina/hydroxyl sheets are exposed and could interact with different kind of chemical substrate, such in this case the cellulose glycosidic bonds[44]. Moreover due to the mechanical forces exerted on the material, the hydrogen bonds that held together the clay sheets are easily broken down producing several single sheets. This conduce in wide superficial area where all the active sites can interact with the glycosidic oxygens of cellulose leading in the cleavage of the polymeric linkage. Furthermore once the kaolinite structure is fragmented resulting in one sheet form, the hydrogen bonds between one sheet and another are broken and the protons present on each oxygen atom on the aluminum structure side are available for new Van der Waals linkages.

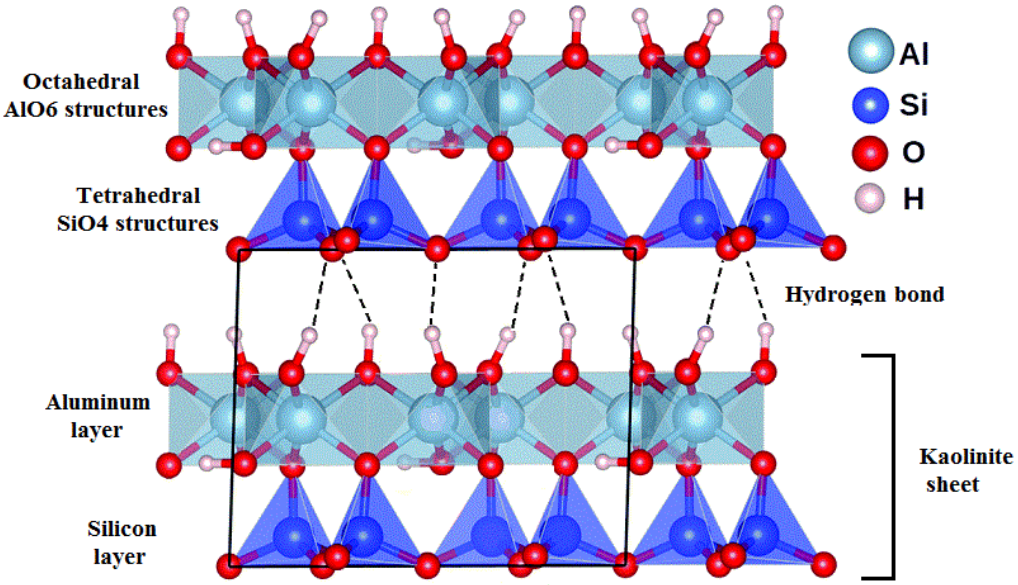


Figure 1.16 Kaolinite physical conformation.

- Aluminum Phosphate

Aluminum phosphate ($AlPO_4$) is a chemical compound that shows framework structure similar to zeolities and thus is used for industrial purpose as catalyst or molecular sieve. As zeolities could be found in different frameworks shape originating a class of compounds known as “ALPOs”. Nonetheless each structure is composed by alternating AlO_4 and PO_4 tetrahedrons, that varying their orientation form different-sized cavities. Just because this solid shows a framework shape and that contains aluminum atoms was chosen as catalyst for our reaction to have a comparison with kaolinite. Due that was utilized its anhydrous form the total amount of water present in the system when this catalyst was utilized comes only from the cellulose moisture, thus resulting in a H_2O-AGUs ratio equal to 2:1.

CHAPTER 2: EXPERIMENTAL SECTION

2.1 REAGENTS

All the reactants utilized in this experimental work: microcrystalline cellulose, α-cellulose, HCl 37%, kaolinite, amberlyst 15 anhydrous form, aluminum phosphate, para-toluen-sulfonic acid; were purchased by Sigma Aldrich.

2.2 ACID IMPREGNATION OF MICROCRYSTALLINE CELLULOSE

Since acidulate cellulose was chosen as a benchmark for the following activity catalyst comparison, the acid impregnation process was accurately optimized as reported in the "optimization of the cellulose acid impregnation" paragraph, described in the results and discussion chapter. This study was useful to set the parameters of the acid impregnation processes that lead to a constant value of the acid equivalents number on cellulose, in order to have a reproducible substrate in every milling tests.

Therefore, in every acid impregnation process, 10 grams of cellulose were added in a 250ml glass flask with 150ml of diethyl-ether and 1.5 ml of HCl 37%. After one hour of stirring at 25°C the resulted suspension was filtrated with a Wathman paper filter and the acidulate powder was left dried in air for half an hour. The acid impregnation yield and the amount of acid resulted on the cellulose substrate was tested with a basic titration utilizing a solution of sodium hydroxide 0.02M and phenolphtalein as colorimetric indicator. Usually 0.5 grams of the acid product were suspended in 100ml of water and titrated with 30ml c.a. of basic solution under magnetic stirring.

2.3 MILLING PROCESS

2.3.1 Planetary ball mill

The reactions were conducted in a Retsch planetary ball mill 100 at the milling speed of 500rpm with six 15mm diameter balls and a 50ml bowl. Zirconia was used as grinding material, both for the bowl and balls. The grinding bowl was charged in each test with an overall amount of reagents (cellulose plus the solid acids) equal to ten grams. Moreover, well-known that the grinding increase the temperature inside the reaction system, time breaks of ten minutes were performed every thirty minutes of milling in order to avoid reagents and products overheating.

2.3.2 Mixer ball mill

For the tests conducted in this kind of device was chosen a Restch MM 400 model. Each capsule was charged with four grams of reagents plus four 10mm diameters zirconia balls. The milling capsules were constituted of zirconia as well as the grinding balls. Each experiment was conducted at the maximum frequency of 30Hz. Furthermore after every fifty minutes of grinding a time break was required until the capsules have reached again the room temperature.

2.4 SOLUBILIZATION AND CENTRIFUGATION STEP

In order to obtain detectable products of the milling process the resulting powder was totally discharged from the grinding vial and solubilized in water. Depending on the cellulose-solid acid ratios charged in the mill, amounts from 2 to 4 grams of powder product were added in a 100ml round glass flask with 40ml of water. Thus the suspension was stirred at 1500rpm for one hour at room temperature. Further 10ml of cold water were used, once the solubilization step was complete, to wash the glass flask and collect the remaining sample. Thus the resulted suspension was centrifuged at 3450rpm for 30minutes in order to separate the sugar solution from the insoluble products. In this way the solution obtained, previously adequate dilutions, was ready for the HPLC-ELSD or ESI-MS analysis.

2.5 ANALYTICAL TECHNIQUES

2.5.1 HPLC – ELSD

The HPLC is a chromatographic method that permits to separate a complex mixture solution in its solute components thanks to their different polar affinity to the static phase or to the mobile phase. Thus, according to the polarity, charge and dimension of the compounds eluted through the column, they are differently withheld by the static phase and so separated. Once the compounds are separated they are individually driven toward the detector where are quantified. Thus in order to determine glucose and cellobiose yield a high performance liquid chromatography system was used and performed on Agilent 1200 infinity series. The separation was carried out with Agilent Hi-Plex Ca 300x7.7mm column with isocratic condition of water as mobile phase at a flow rate of 0.750 ml/min at 80°C. In this column the static phase consists of sulfonated crosslinked styrene-divinylbenzene copolymer gel impregnated with various metal cations, especially calcium[1]. Afterward the sugars investigated were analyzed with an evaporative light-scattering detector (ELSD) and identified by comparison of retention time with those of the corresponding reference compounds. The amount of products were quantified using an external calibration curve and a sorbitol solution as internal standard.

2.5.1.1 Evaporative light-scattering detector

The ELS detector is a chromatographic detector that responses to all compounds that are, at the analysis conditions, sufficiently non-volatile relative to their mobile phase. Therefore it must be selected an HPLC mobile phase that could easily and quickly evaporate and desolvate. Obviously solvents with relatively low boiling point and low viscosity are preferred such those commonly used in the HPLC analysis like acetonitrile, THF, water, methanol and ethanol. This detector is especially created for compounds that don't present chromophores functional groups and so cannot be quantified by UV/VIS detector such carbohydrates, sugar alcohol, and others organic molecules, or for substances that aren't able to well ionize for the MS analysis. This device can be coupled with other type of detectors like absorbance detector but it must been the last of the series due that it is a destructive detector. ELS detection can well works both with isocratic or gradient elution with a wide variety of mobile phase and additives.

The operation principle of ELSD mainly consists of three consecutive processes: nebulization of the chromatographic effluent, evaporation of the mobile phase, and detection of the light scattered by the non-volatile residual particles.

- Nebulization

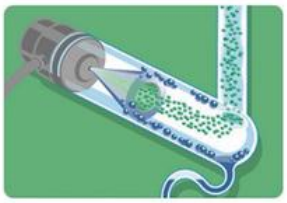


Figure 2.1 Nebulization process of ELS detector.

In the first step of ELS detection mechanism, the effluent from a chromatographic column enters in a nebulizer where it is transformed into an aerosol as illustrated in Figure 2.1. These nebulizers create a high flow of carrier gas, usually nitrogen, over the liquid surface producing a high amount of droplets with remarkably uniform size. Since the amount of the light scattered is a function of the particles radius, distribution and average values of droplets diameter are considered to be very critical parameters, which strongly influence detectability, sensitivity and repeatability of the ELSD methods. The formation of uniform, reproducible and stable aerosols depends on the relation of the nozzle diameter and the flow rates of mobile phase and nebulizing gas. Indeed one of the parameters that can be better set up in this process is the carrier gas flow versus the chromatographic effluent flow rate that can be controlled thanks to the concentric flow shape of the nebulizer. High gas flow produces small droplets, requiring less heat to evaporate the solvent. Conversely, low gas flow produces large droplets, requiring more heat to evaporate the solvent. This parameters must be accurately optimize since narrow distribution of droplet size is a requirement for good repeatability and ELSD sensitivity[2].

- Evaporation

Figure 2.2 Evaporation process performed in a drift tube during an ELS detection.

The evaporation of the mobile phase is performed in a heated drift tube, shown in Figure 2.2, and here the size of the aerosol droplets is reduced. Ideally, the purpose of this stage is to completely vaporize the mobile phase, without any analytes loss due to evaporation or thermal decomposition. Evaporation is also a function of time and pressure but the main parameters that, in this step, could highly influence the analysis results is the temperature which the drift tube is kept. Indeed the completeness of the mobile phase evaporation and the extent of loss of analyte is mainly determined by the evaporation temperature, which should be selected in accordance to the mobile phase and analyte volatility. Inappropriate selection of the evaporation temperature results, in case of low temperature, in an excessive noise or baseline with spiked sharp peaks, or in case of high temperature, in reduced sensitivity. Apart from the analyte loss, high evaporation temperature causes rigorous solvent evaporation, which destroys uniformity of particle size, and favors the formation of liquid rather than solid particles. Both effects result in decrease of ELSD sensitivity. In particular, when analyzing semi-volatile substances with low boiling points, the intensity of the signals obtained varies greatly with this set temperature. If it is too high, semi-volatile substances may be partially or completely evaporated together with the mobile phase; consequently, and the quantity of minute solid particles that scatter light may decrease. In such cases, decreasing the set temperature of the drift tube increases the number of target substance particles present after evaporation, and makes it possible to attain a higher signal intensity. The evaporation temperature is usually set between 30◦C and 100◦C. Efficiency of chromatographic separations does not appear to be affected by the length of the evaporation tube, providing that an appropriate level of vacuum is applied at the end of the flow path in order to establish a stable flow of liquid and solid aerosol[3].

- Detection

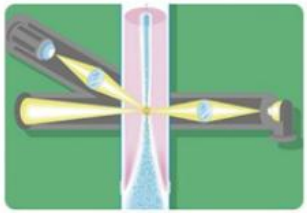


Figure 2.3 Detection process of the ELS detector.

The aerosol, after the evaporation process, ideally composed by solid particles of analyte, enters the optical cell and passes through a light beam as shown in Figure 2.3 The detector measures the scattered light at 60° relative to the excitation beam in order to minimize polarization effect and stray light. Since scattering and not absorbing phenomenon is intended to occur when the light interacts with the analyte particles, a tungsten filament or halogen lamp that produces a continuous spectrum of wavelengths, rather than a monochromatic laser emitting diode, is favored as a light source. The scattered light is captured by a photomultiplier or a photo diode, providing the output signal. The particle sizes determined how the light is scattered and the stronger the scattering, the more intense the final signal on the ELS detection chromatogram. The dry particles diameter D produced in the drift tube is related to the droplets diameter D_0 produced by the nebulizer and the concentration c of the eluted flow according to the following equation:

$$D = D_0 \times \sqrt[3]{\frac{c}{d}}$$

Where d represent the density of the dry analyte. In this way the scattered light that is a function of the dry particle diameter D depend on the concentration of solution analyzed and so is rough measure of the mass material represented by the chromatographic peak. But an appropriate calibration curve is still required due that not all the particles diameters produces the same amount of scattered light. Indeed light scattering processes are classified in two types: elastic scattering, in which the scattered radiation has the same frequency as the incident radiation, and inelastic scattering, in which the scattered radiation resulted has a different frequency. In ELSDs, inelastic scattering is considered to be negligible and it is not further examined. Depending on how the light is scattered by the particles, elastic scattering is classified in three types, Rayleigh, Mie and Refraction-reflection, shown in Figure 2.4, and this depends on the dimension ratio between the dry particles diameters D and the wavelength of the incident light beam λ. As already preannounced the amount of light scattered is function of the particle dry

diameter, with different order of magnitude, depending on which kind of scattering occur.

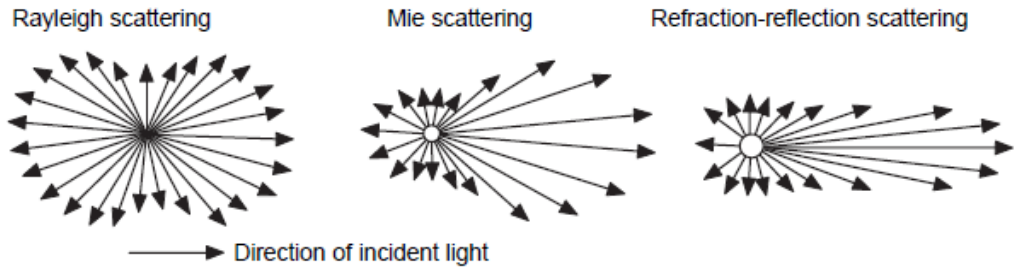


Figure 2.4 Three types of elastic scattering produced depending on the dimension ratio between the particle diameter and the wavelength of the incident beam light.

- Rayleigh scattering occurs for the smallest particles when $\frac{D}{\lambda} < 0.1$, in this range the light scattered is proportional to D^6 and consequently the light scattered is proportional to the square of the eluted solution concentration *c*.
- Mie scattering occurs when $0.1 < \frac{D}{\lambda} < 1.0$, the light scattering is proportional to D^4 resulting dependent of $c^{4/3}$.
- Refraction-reflection phenomenon appear for the larger particles with $\frac{D}{\lambda} > 1.0$, the scattered light result as a function of D^2 with an exponential dependence from the solution concentration equal to $\frac{2}{3}$.

Actually, in most cases, more than one scattering mechanisms occur in the ELSD optical cell, due to a variations of the aerosol droplet diameter, which is dependent on the nebulization and evaporation processes, the polychromatism of the light source and dependence of the average droplet diameter on the sample concentration. It is this variance that prevents linearity in ELS detection calibration plots over more than one order of magnitude. Indeed in most applications, a non-linear response has been observed for the ELSD. The area of the chromatographic peak (*A*) appears good correlation with the analyte mass (*m*) according to the exponential relationship:

$$A = a \times m^b$$

where *a* and *b* are coefficients depending on the ELSD instrumentation and on nebulization and evaporation processes such as flow rates of the nebulization gas and mobile phase, composition of the mobile phase and evaporation temperature. The contribution of refraction to the light scattering process suggests that the scattering response is a function of the analyte refractive index, which has been

reported to be the reason for the approximately constant ELSD response factor for molecules with similar structure[4].

As previously mentioned the ELS detector is favored for analyze compound that aren't detectable whit UV/VIS system but also RI detector could be an opportune solution for this kind of analytes. For this reasons are following listed some advantages and disadvantages when ELS detector is used. Firstly, since the solvent is evaporated in the drift tube, the ELS analysis is not affected by the interference due to the solvent peaks as could occur in the RI chromatogram. Moreover RI detector cannot work with a gradient elution condition because of fluctuations in the baseline caused by changes in the refractive index of the mobile phase, so is not suitable for the analysis of a multiple components complex mixtures. Whereas with ELSDs, baseline fluctuations do not occur in gradient elution, meaning this method can be used to perform the efficient, high-sensitivity analysis of multiple components. Furthermore ELS detectors don't require time to allow for the instrument and the baseline to stabilize, they are not easily affected by changes in the ambient temperature, and they are 5 to 10 times more sensitive than RI detectors. ELS detection cannot produce negative peak as the RI detection that usually complicate the determination area value. On the other hand ELS is destructive technique because the analyte is sacrificed to generate the scattering solid particles. Ideally, therefore, the ELS detector should be the last in a series. Any particles can interfere with the sample signal, such particulates in poor-grade chromatographic solvents or transported by the carrier gas, because the detector response equally to all particles with same diameters causing problematic background noise. ELS detection cannot analyze compounds whose volatility resembles that of the mobile phase[3].

2.5.1.2 Glucose and cellobiose calibration curves construction

In order to have a real correlation function between the area of chromatographic peaks and the analyte concentrations a calibration curve with standard solutions was constructed for glucose and cellobiose. Furthermore this previous injection was useful to determine the retention time of the sugars searched that result in a value of 10.8 minutes for glucose and 9.0 minutes for cellobiose. Thus bigger oligomers seems to have less affinity for the stationary phase of the column due that they are eluted before than the smaller ones. Unfortunately calibration curve of oligomer longer than two AGUs cannot be constructed because standards of that compounds are not available on the market. Moreover in all standard solutions and samples injected was added a solution of internal standard. An internal standard is useful because prevent the fluctuation of the detector response due to same external environment parameters changes that normally occurs when a long period work is developed. In this way the same known number of internal standard number of moles is added in each sample producing the relative chromatographic peak area. Afterwards a function of moles of sample on moles of standard versus area of sample on area of standard is constructed. The internal standard chosen must be stable during time and with the HPLC column temperature, suitable with the HPLC eluent, mustn't react with the compounds analyzed and must be soluble and inert toward the sample solvent. Obviously the internal standard cannot be one of the compounds that are want to be quantified, must have different retention time from them to be well recognized and lie in a concentrations interval suitable with those of analytes. For the purpose of this work sorbitol is a molecule that satisfied all these requirements and thus can be used as internal standard showing a retention time equal to 20.5 minutes. For both sugar analytes standard solutions were prepared in several concentration by subsequently dilution from the same mother solution. In particular were prepared sugar solutions of 0.025%, 0.050%, 0.100%, and 0.200% in weight using water as solvent. Each calibration sample was injected with 0.5ml of standard solution and 0.1ml of 0.50% in weight of sorbitol as internal standard. Thus the resulting calibration curves are plotted in Figures 2.5 and 2.6.

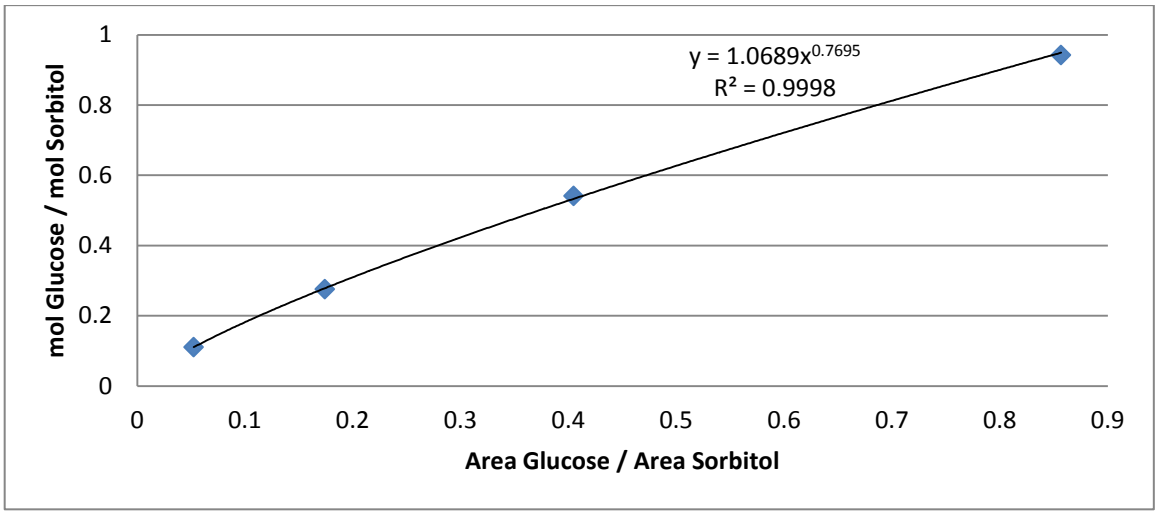


Figure 2.5 Glucose calibration curve.

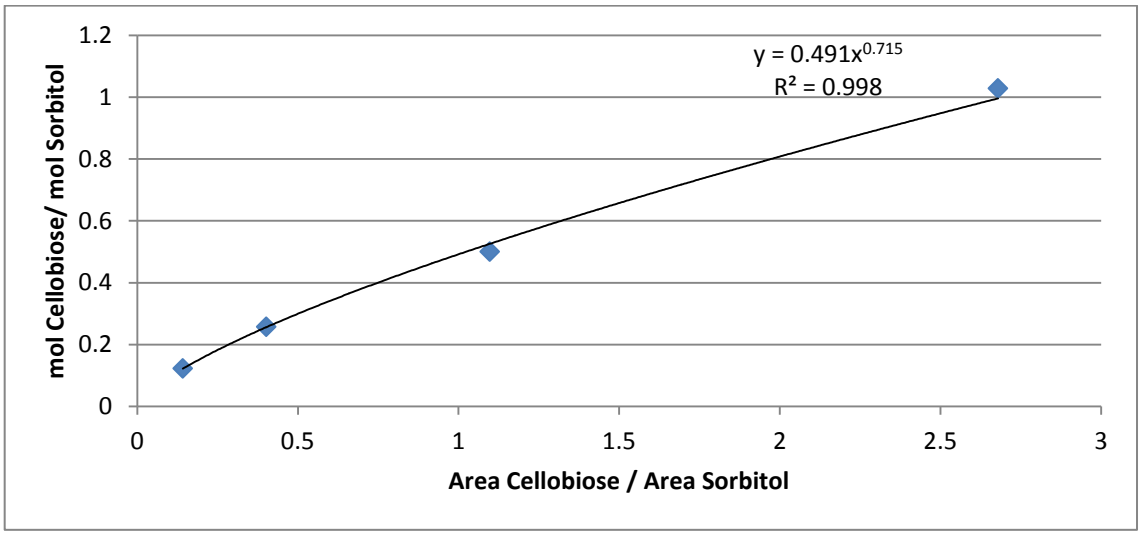


Figure 1.6 Cellobiose calibration curve.

Both two sugars calibrations show an exponential curve tendency meaning that for both compounds the concentrations interval selected contain all the three types of particles sizes that produce different light scattering. Comparing the exponential factor of the trends, that for both is near the value of 0.75, with the single ones of each kind of scattering, 2 for Rayleigh scattering, $\frac{4}{3}$ for Mie scattering and $\frac{2}{3}$ for Reflection- refraction, can be deducted that the average diameter of the particles analyzed produce an average scattering closer to the reflection- refraction rather than the other two type of scattering. Thus the dry particles analyzed seems to have an average diameter greater than the wavelength of the light source utilized, that if it is a tungsten lamp, the maximum emission of the polychromatic light is 0.35 μm. Indeed, with this kind of detector, particles with radius smaller than 100nm are usually not detectable. For both trends the

R^2 value is remarkably near to the unity, meaning that the standard solutions were well prepared and that the future sugar determination will be accurate under the analytical point of view.

2.5.2 Mass-Spectroscopy

In order to understand which products were obtained from the depolymerization process, the aqueous solution resulted after the solubilization step was injected in a liquid chromatography separation system followed by a mass-spectroscopy analyzer instrument. In this way the analyzed solution was firstly separate by a liquid chromatographic system thanks to the different affinity that its components show toward the stationary phase of the column or toward the mobile phase, thus emerging in different chromatogram peaks. After that, as soon as each fraction of the eluent was ejected from the column, depending on its retention time, was injected in the following ionization chamber for the mass-Spectrometry detection. In this way could be detected the product mass distribution of every chromatogram peak. For the mass-spectroscopy analysis was utilized a bruker amazon SL instrument.

2.5.2.1 Mass-Spectroscopy overview

Mass-spectrometry, MS, is an analytical technique where ionized chemical species can be separated and detected according to their mass to charge ratio. Thus knowing that both monovalent and multivalent ions are produced from the analytical sample, could be easily determined the masses of the compounds injected. Indeed the instrument return a mass-spectrum where is plotted the ion signal as function of the mass to charge ratios. Typically can be analyzed solid, liquid or gas samples that are ionized with different source of energy like electron beam or plasma environment that, depending on its intensity, are classified under soft or hard ionization methods. Hard ionization techniques such electron ionization, EI, or inductively coupled plasma, ICP, exert on the sample larger amount of energy that together with the ions production, fragment the molecule injected. Soft methods are less likely to break down the chemical species during the ionization process due to the lower residual energy that remain on the subjected molecule. Depending on the purpose of the analysis the first or the second ionization

method could be chosen. For instance hard ionizations are preferred when the molecular structure of a unknown sample want to be determined because the fragments are indicative of its functional group but obviously can't return information on their position. Indeed commonly, for this kind of analysis, the MS results are always compared with an NMR spectra to better understood the molecular structure of the sample. On the other hand soft ionization could be used to determine the compound distribution of a multicomponent sample, especially with high molecular weight such polymer or other macromolecule[5].

The analyzer part of the spectrometer contains electric and magnetic fields, which exert forces on the ions produced traveling through these fields. The speed of a charged particle may be increased or decreased while passing through the electric field, and its direction may be altered by the magnetic field. The magnitude of the deflection of the moving ion's trajectory depends on its mass to charge ratio. Lighter ions get deflected by the magnetic force more than heavier ions. To do that are available several instrument each using different physical phenomenon with their advantages and disadvantages. For example time-of-flight (TOF) analyzer uses an electric field to accelerate the ions through the same potential, and then measures the time they take to reach the detector. If all the particles have the same charge, the kinetic energies will be identical, and their velocities will depend only on their masses. Ions with a lower mass will reach the detector first. Quadrupole mass filter uses oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency quadrupole field created between four parallel rods. Only the ions in a certain range of mass to charge ratio are passed through the system at any time, but changes to the potentials on the rods allow a wide range of m/z values to be swept rapidly, either continuously or in a succession of discrete hops. A magnetically enhanced quadrupole mass analyzer includes the addition of a magnetic field, either applied axially or transversely. This novel type of instrument leads to an additional performance enhancement in terms of resolution and sensitivity depending upon the magnitude and orientation of the applied magnetic field. Moreover there are other analytical systems like the quadrupole ion trap in its different geometrical shape or the ion cyclotron resonance[6]. After the analyzer instrument is situated the detector, a device capable to detect charged particles, like an electron multiplier, that return different signals depending on the extent of their deflection and proportional to the relative abundance of the ion recorded. Other models of detectors are commercially available as the faraday

cups or the microchannel plate detector, commonly used in modern instrument. Our samples were analyzed with a Bruker Amazon SL ion trap mass spectrometer that generates ions thanks to the electrospray ionization technique. Each solubilized sample were diluted 100 times and 10µl were injected.

2.5.2.2 Liquid chromatography in the Mass-Spectroscopy instrument

For the water soluble product separation was used a C18 reverse phase column, (water acquity, UPLC BECH C18,7 µm 2.1x100 mm) kept at 40°C in a gradient elution mode. Indeed the eluent phase was constituted by two different solutions: 0.1% of formic acid in water and 0.1% formic acid in acetonitrile. During the retention the aqueous phase decrease from 95% to 5% of the total eluent for the relative increment of the organic phase. A commonly diod array detector was used for the detection of the eluted compounds.

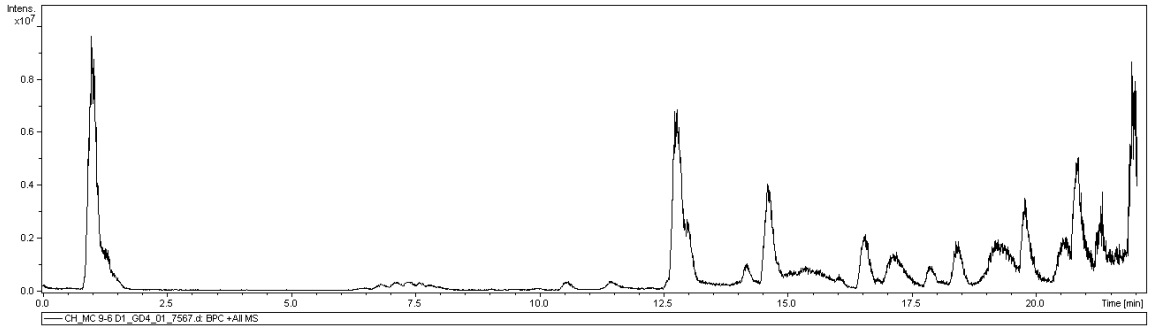


Figure 2.7 Classic chromatogram obtained from the injection of a milled sample in the ESI-MS instrument.

Figure 2.7 shows a classic chromatogram that was obtained from the elution of a solution of one sample discharged from the mill and subsequently solubilized. As it can seen is not a perfect separation due that the product mixture is really complex, but with the instrumentation and conditions available is the best result that could be obtained. Nonetheless, as explained in the following "Results and discussion" chapter, this chromatogram could be used for the quantification of the total soluble products obtained by the depolymerization reaction performed in the milling device.

2.5.2.3 Electrospray ionization technique

Most commonly the electrospray ionization (ESI) method is used when the MS analysis, such in this case, is coupled with a previously LC separation system. The sample liquid flow, coming from the LC separation, is injected in a capillary and must contain a certain amount of ions. For this reason 0.1% of formic acid was added in the LC eluent. At the end of the capillary an high voltage is applied, in this way the charged liquid is modified until the creation of a Taylor cone that, once the superficial tension of the solution is overcome, could easily generate an areosol. To facilitate the nebulization process usually heated inert gas, such nitrogen or carbon dioxide, are mixed to the sample flow. Usually the capillary is heated to facilitate the solvent evaporation that is required to decrease the droplet size. Indeed after the droplets radio has reached a certain value, known as the Rayleight limit, the particles are unstable due to the electrostatic repulsion of like charges that become more powerful than the surface tension, responsible to hold the droplet together. At this point the droplet undergoes in Coulomb fission, whereby the original droplet explode creating many smaller, more stable droplets. The new droplets undergo again in desolvation and further successive Coulomb fissions until ion species of the anylites are formed, as Figure 2.8 represent. In this way are formed ions by addition of hydrogen cation and denoted $[M + H]^+$, or of another cation such as sodium ion, $[M + Na]^+$, or by removal of a hydrogen nucleus, $[M - H]^-$. Moreover multiply charged ions such as $[M + nH]^{n+}$ are often observed and the resulting mass-spectrum is more difficult to interpret[7]. Due to the presence of formic acid in the eluent phase, the electrospray used for our tests operate in a positive mode, which means that the number of charged species reflect the number of basic sites on a molecule that can be protonated at low pH. The electrospray ionization is especially useful to analyze macromolecule species because it overcomes the propensity of these to incur in some fragmentation process that usually occur with other ionization mechanisms that exert more energy on the sample, such the electron ionization[8]. In this way the sugar oligomers detected could be attributed only to the depolymerization milling process and not to the analytical technique used, making reliable the products distribution obtained.

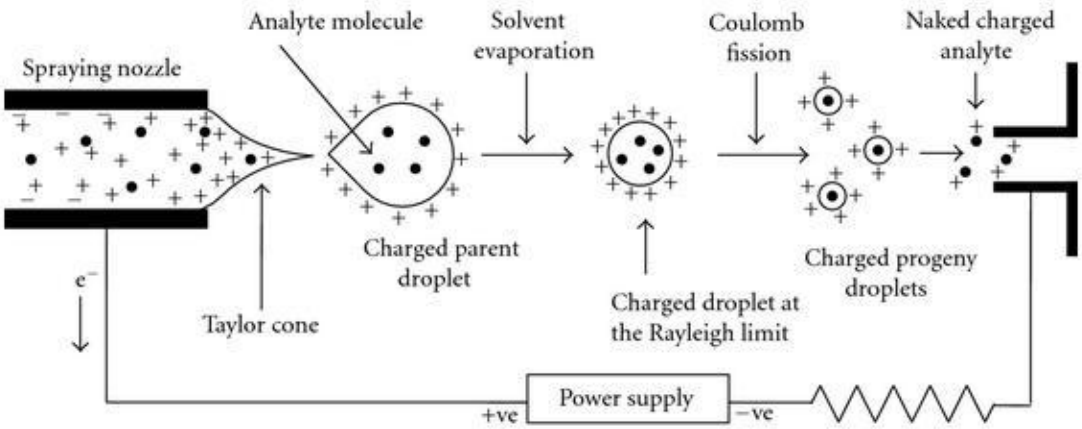
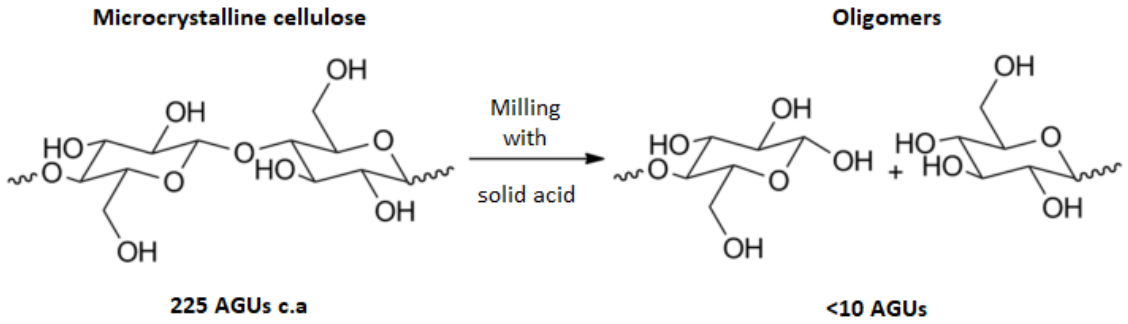


Figure 2.8 Electrospray ionization scheme: formation of charged analytes from the injected aqueous solution.

CHAPTER 3: RESULTS AND DISCUSSION

The purpose of this work was to explore the possibility of using a mechanochemistry process with solid catalysts for the depolymerization of cellulose (Scheme 1). The principal product expected by this reaction was glucose, deriving from the break of the β-1,4-glycosidic bond of cellobiose, plus longer oligomers coming from the not perfectly extent of the depolymerization process. Moreover, since an acid species is present in the reaction environment, some side reactions could occur resulting in undesired products, such as some sugar fragments of the whole cellulosic polymer chain, that however, cannot be related to any oligosaccharide structure.



Scheme 3.1 Cellulose depolymerization assisted by mechanical forces and acid catalysis.

Since during this work it was important to obtain, at the end of the solid depolymerization reaction, products in a suitable form that permit their qualitative and quantitative determinations an experimental methodology of the overall process had to be developed. This involves the selection of the best cellulosic substrate to be treated, the setting of a catalytic benchmark on which compare the solid acids performances, the tuning of the milling parameters and the establishment of a method to transforming the solid products in a physical state available for its analytical determinations, without affecting its conversions and yields arisen from the milling process. Moreover, since the classical analytical techniques resulted not suitable to obtain reliable conversion values, an analytical method was developed to determine the total amount of water soluble products at the end of the milling process, that it was considered in this way as the conversion value of the studied reaction. Thanks to these preliminaries studies, it was possible to compare the catalytic performances of the solid acids under investigation, confronting water soluble products conversion and sugar yields resulted from the grinding in the planetary ball mill. Furthermore analyzing the results obtained could be

deduced important information regarding the dependence of the depolymerization rate on some catalytic features such as the number of the acid sites or the distribution effect. Moreover it was possible to understand trough which mechanism the cellulose depolymerize toward shorter oligosaccharides in reactions assisted by mechanical forces. At end of this work the same topics were addressed in the mixer ball mill to study its performances and compare them to the ones of the planetary ball mill.

Thus this “results and discussion” section tackle and deepen the following topics:

1) Development of the mechanochemistry process for cellulose depolymerization;
2) Development of a method to measure the water soluble products (WPS) using the ESI-MS technique;
3) Study of the reaction using the Planetary ball mill;
4) Study of the reaction using the Mixer ball mill;

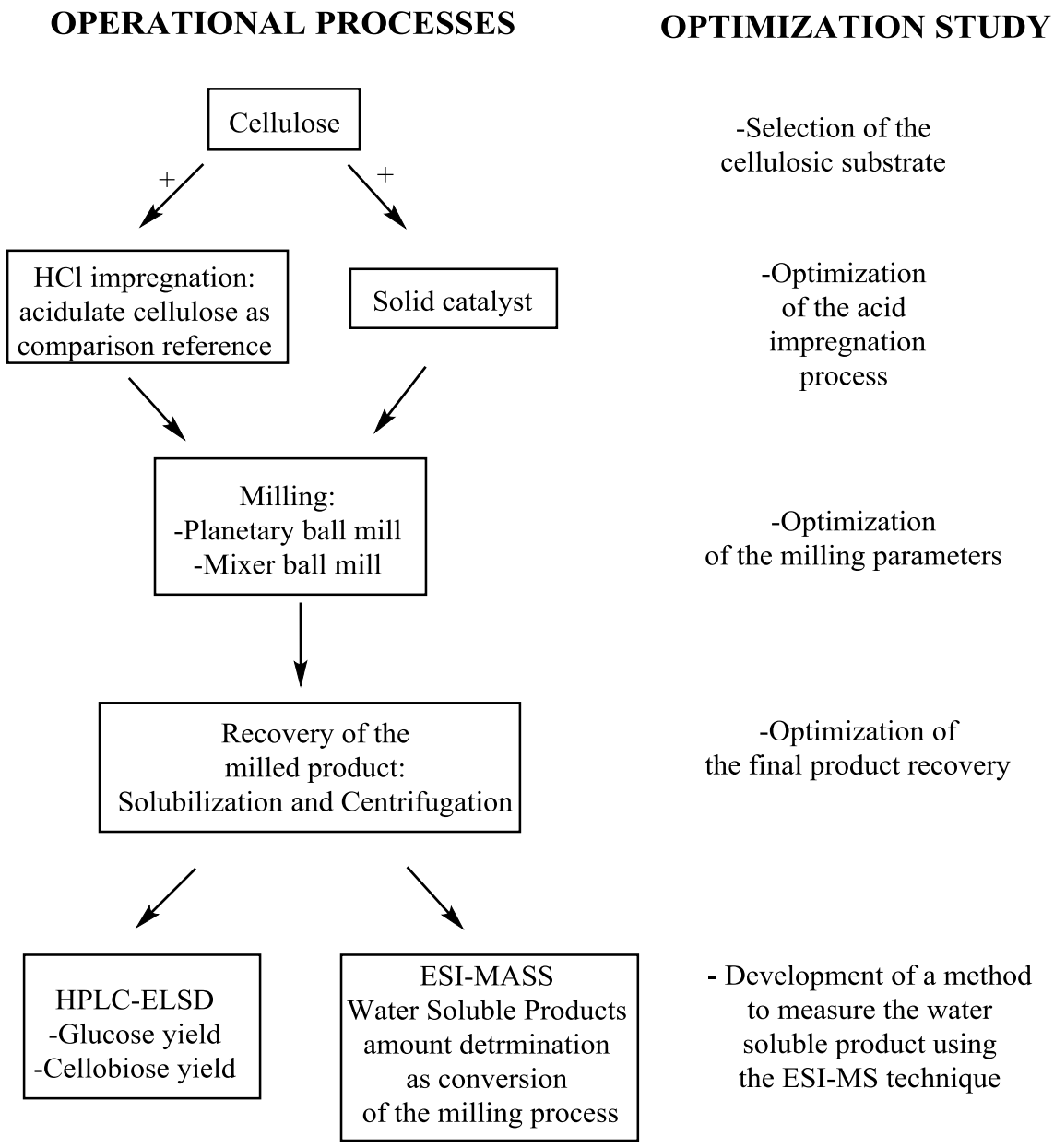
3.1 DEVELOPMENT OF THE MECHANOCHEMISTRY PROCESS FOR CELLULOSE DEPOLYMERIZATION

In this paragraph is reported the operations sequence constructed and optimized in order to develop a correct experimental methodology to depolymerize cellulose trough mechanochemistry and to obtain detectable products at the end of the reactions. Since in nature and on the chemical market are present plenty of different kinds of cellulosic substrates, in term of chemical composition and physical morphology, the first step was to choose the best starting material on which conduct the depolymerization reaction. Subsequently was set a catalytic benchmark on which compare the performances of the solid acids under investigation, that with the help of literature was identified in the unusual acidulate cellulose substrate, obtained with an acid impregnation process[1]. Because the number of acid equivalents, resulted on the cellulosic substrate with the acid impregnation technique, is a key parameter on the depolymerization rate, this process was deeply studied too. Furthermore the resulting product of the milling process is an homogenous solid mixture composed by the depolymerized compounds, the unconverted cellulose and the grinded solid catalysts. Hence the powder discharged from the milling device was solubilized to obtain an aqueous solution that was afterward centrifuged in order to separated the soluble products from the insoluble particles.

Thus in this section are discussed all the optimized operational steps that have permitted a reliable comparison of the solid acids studied:

1) Selection of the cellulosic substrate;
2) Acidulate cellulose as reference of the catalysts comparison;
3) Optimization of the milling parameters;
4) Optimization of the final product recovery;
5) Identification of the product of the reaction;
6) Study of the oligosaccharides solubility.

To better understand the whole procedure followed to transform the cellulosic substrate into water soluble products, thus permitting their quantification and then the catalyst performances comparison, is reported in Scheme 3.2 the operational process scheme with aside the optimization studies performed for each step of this experimental methodology.



Scheme 3.2 Scheme of the developed operational process and the relative optimization studies.

3.1.1 Selection of the cellulosic substrate

Microcrystalline cellulose is a white powder obtained from the previously mild hydrolysis and purification of raw cellulose. Due to this earlier treatment it is composed by 100% of glucosan units with an average degree of polymerization around 225 AGUs, differing to α-cellulose that is constructed by 76% of glucans and 16% of xylans appearing with its granular and fibrous shape as a heterogeneous solid. The physical different morphology of the two cellulosic substrate is evident in Figure 3.1.

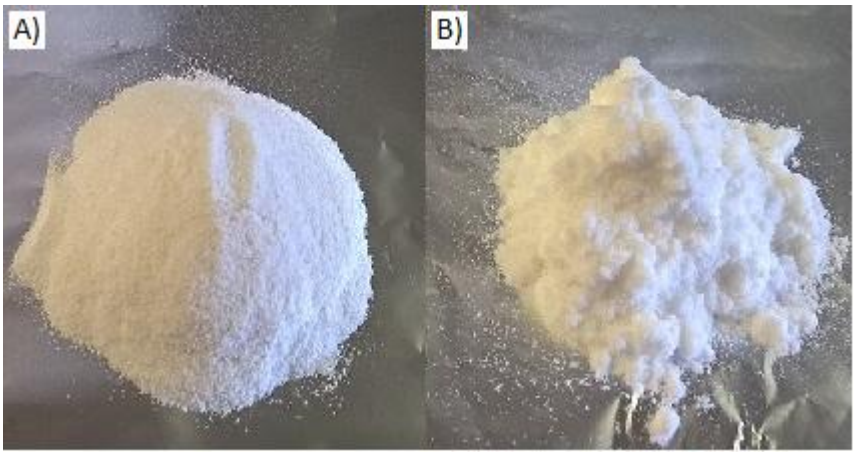


Figure 3.1 A) Microcrystalline cellulose; **B)** α-cellulose.

In literature can be found experimental works carried out with both these two cellulosic compounds[1,2] and so, to select the best substrate, a preliminary grinding treatment was performed on these substances with a planetary ball mill. Due that the most complex catalytic reaction to conduct is the one with acidulate cellulose, the preliminary tests were performed with this singular type of catalytic system. In particular 10 grams of acidulate cellulose with 0.2 acid equivalents were introduced in the milling bowl and grinded for 10 hours. Afterwards the powder products were analyzed with the ESI-MS and HPLC-ELSD techniques. If α-cellulose was used as starting material discharge the mill and hydrolyze the grinded powder require lot of efforts that most of times means obtain irreproducible results. For example Figure 3.2 shows the milled powder discharged from the mill and, as it can seen, it is a sample totally heterogeneous. Indeed were present darker larger agglomerations that could be attributed to the reagent fraction that was converted in sugar oligomers and paler finer particles probably representing the unreacted starting material. Furthermore these agglomerations are really hard and could encased some other particles that, in this way, cause a non homogeneous solubilization or

determination errors on the water soluble products conversion. Indeed, when α-cellulose was used as substrate, the overall powder discharged from the planetary ball mill was further inserted in the mixer ball mill for five minutes at 10Hz with 2mm diameter zirconia balls to homogenize the whole sample, hoping that the low milling frequencies don't alter the depolymerization extend. On the other hand microcrystalline cellulose appears as a homogeneous white powder with a regular dimension distribution of its granules. It is enormously heavy than α-cellulose and thanks to its granular physical morphology could be easier handled. Moreover when it was grinded its conversion in soluble products has anyway let in sticky layer formation on the side wall of the milling bowl but easier to remove than the one formed with α-cellulose. Furthermore once this layer was collected it resulted again in a homogeneous granular shape product. The yield in glucose, cellobiose, and WSP obtained using the two substrates was almost equivalent. The only difference arisen was that when α-cellulose was used, slightly lower yields were produced, probably due to the smaller amounts of AGUs present in this substrate, but must be considered that, for the reasons previously described, reproducible values are quite impossible to obtain. Moreover also reproducible values of acid equivalents arisen from the impregnation step were more difficult to obtain with α-cellulose, probably due to its more heterogeneous morphology. Thus catalytic activity results could be hardly considered truthful because this cellulosic substrate not always shows the same acid content. To conclude, working with α-cellulose instead of microcrystalline cellulose leads to more complex products in terms of chemical composition and physical features that conduce in harder and less accurate analytical products determination. Hence the following cellulose depolymerization study was realized with microcrystalline cellulose as starting material whereas α-cellulose was neglected.

Figure 7.2 acidulate α-cellulose milled 10 hours in the planetary ball mill at 500 rpm.

3.1.2 Acidulate cellulose as reference for the catalysts comparison

According to Rinaldi and co-workers cellulose impregnated with an electrolytic acid like HCl or H_2SO_4 conducts in 100% of water soluble products in two hours of milling at 800rpm, proving in this way the best catalytic systems for depolymerize cellulose in useful products[1]. For this reasons in our work acid impregnated cellulose was selected as benchmark to understood the catalytic performance of the successive studied solid catalysts. For the acid impregnation step HCl was used, rather than H_2SO_4, for its less hazardous behavior and its non-oxidant property that could conduct in a shortly decomposition of cellulose after its impregnation. Indeed, as reported by Rinaldi and co-workers, after two days aging at room temperature under air, the impregnated powder starts to became dark gray and decompose quantitatively in a black solid after seven weeks[3]. Anyway, to avoid the incipient decomposition process and to have more reproducible results, the acidulate cellulose was charged in the planetary ball mill as soon as the impregnation step was concluded. The general procedure followed to impregnate cellulose with an electrolytic acid is reported in the experimental section as "Acid impregnation of microcrystalline cellulose".

3.1.2.1 Optimization of the cellulose acid impregnation

Since, during the milling treatment, the acid catalyst presence is a mandatory requirement for the cellulose depolymerization occurring, the acid impregnation step was studied and optimized in order to have the same catalytic reference for every test performed. Hence thanks to a basic titration analysis was determined the acid equivalents number resulted on the cellulosic substrate after the impregnation step. With the acid impregnation procedure described in the experimental section substrates with 0.20 acid equivalents could be obtained with a really strictly reproducibility for every impregnation process. Furthermore this reagents ratio leads in the best acid load on the substrate for the depolymerization purpose. Indeed, as illustrated in Figure 3.3, if an acidulate cellulose with a 0.15 acid equivalents was charged in the planetary ball mill, lower yields in glucose and cellobiose were obtained. On the other hand, if an impregnated cellulose with an acid load of 0.25 equivalents was charged in the planetary ball mill, after five hours of milling the resulting product was a dark layer hard stuck on the wall side of the mill. It requires almost forty minutes to be totally detached also with the help of boiling

water and mechanical forces. The MS-ESI and HPLC-ELSD analysis of this sludge has reported a complex mixture of chemical products but no increases in glucose or cellobiose yield. Probably the dark color of the muck could be attributed to the side production of some carbonaceous compounds resulting from the not selective breaking of the β-1,4–glycosidic bond. For the hard efforts with this non homogeneous product must be treated and the analytical difficulties that arise from this higher acid load, was concluded that it isn't the best condition to conduct the milling both in lab scale as industrial scale.

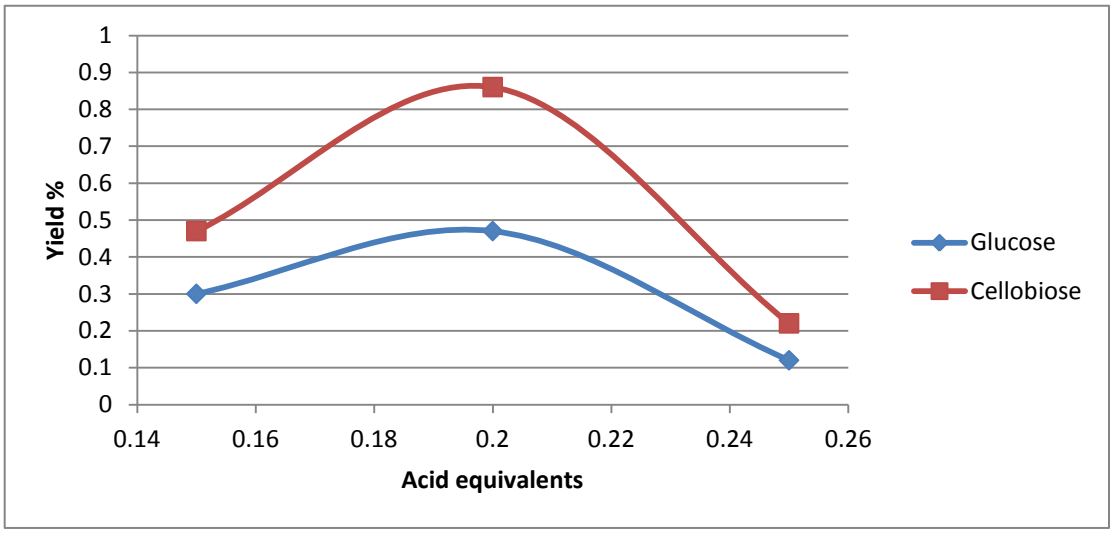


Figure 3.3 Glucose and cellobiose yield in function of the acid equivalents resulted on the cellulosic substrate.

The several impregnation processes conducted show that there is a direct tendency between the acid equivalents resulted on the cellulosic substrate and the concentrated acid amounts added in the stirred flask. For instance, as can be seen in Figure 3.4, 0.75ml of HCl 37% lead in 0.10 acid equivalents against the 0.25eq that were arisen if 2.0ml were added. In this way was also confirmed that the impregnation yield, moles of acid held by the substrate on the moles of acid introduced, is independent from the acid amount insert in the flask and so the not full impregnation isn't due to cellulose limited ability to restrain acid amount. Indeed with the different acid volumes insert, the impregnation yield arisen show always an average value around 70%. Hence the 30% of the acid utilized remains in the diethyl-ether suspension that thus could be reutilized for a further impregnation process if this operation will be industrially scaled-up. However for

ours lab tests, in order to have the exactly same acid strength of the cellulosic substrate, the acid impregnation process was conducted every time with fresh starting materials.

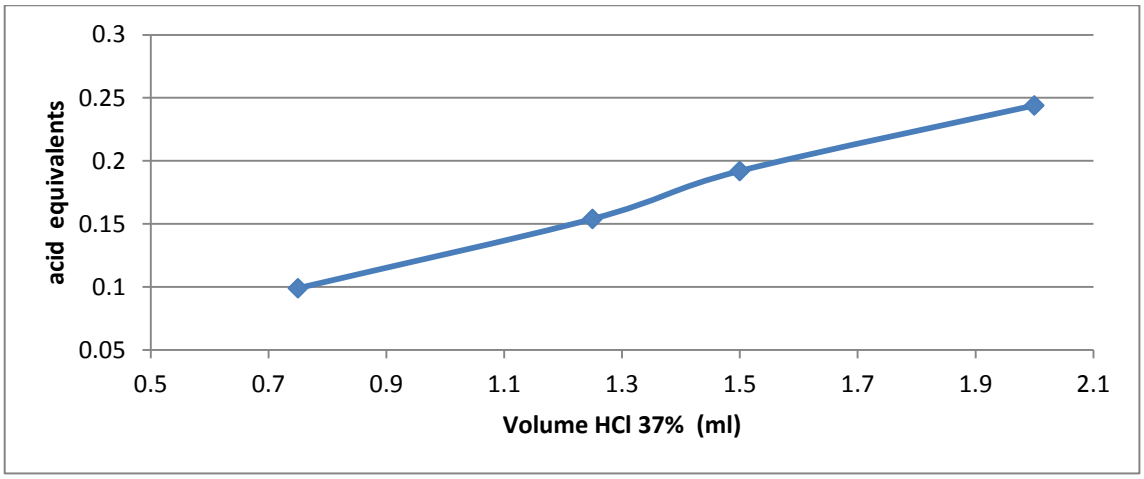


Figure 3.4 Acid equivalents resulted on the cellulosic substrate in function of the HCl milliliters insert in the glass flask during the acid impregnation process.

On the other hand Figure 3.5 suggests that the acid impregnation yield is strictly dependent on the stirring time the suspension is submitted instead of the acid amount insert in the flask. Indeed if the same amount of 1.5ml of HCl is loaded in the ether suspension and stirred for forty minutes instead of one hour, 0.15 acid equivalents against 0.20 eq were arisen, so in twenty minutes of stirring the yield was increased from 53% to 70%. Thanks to this result longer impregnation process were conducted in order to restrict the HCl amounts used but the increment in acid equivalents doesn't show a linear trend, suggesting that a 100% of impregnation yield requires too long times to be achieved. In this way emerge that the cellulose ability to restrain some acid molecule depends on the acid concentration of the solution where it is suspended.

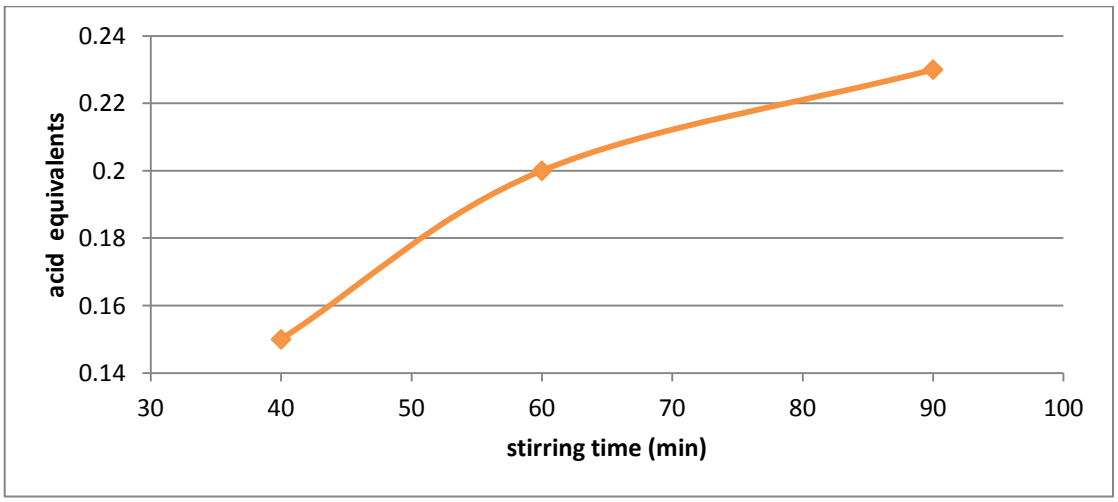


Figure 2.5 Acid equivalents resulted on the cellulosic substrate in function of the stirring time of the acid impregnation process.

Not only the volume of the concentrated acid insert in the glass flask or the stirring time influence the cellulose impregnation yield, but also the kind of the substrate subjected to this process. Indeed α-celluose results more able to hold electrolytic acids rather than microcrystalline cellulose if both were treated with the same conditions. Probably because the first, for its partially heterogeneous monomer composition, has a less crystalline fraction that leads in a more open structure, that in this way, can easier accommodate acids amount. This short but efficient study on the HCl impregnation suggests that would be worth to deeper investigate all the parameters that lead to the best acid impregnation yield if this method will be chosen as a industrial depolymerization process. On the other hand our purpose was to compare the activities of different solid acid as catalysts for the cellulose depolymerization reaction and for this reason was chosen to load the substrate with the same procedure described as acid impregnation of cellulose for every further impregnation step. Moreover in order to verify if during the milling the heat released by the attrition force could cause losses of HCl from the cellulose, a titration was conducted on the product as soon as it was discharged from the mill. With this quickly checking test is been proved that the substrate doesn't loss any acid amount during the milling process.

3.1.3 Optimization of the milling parameters

As already reported in the mechanochemistry introduction are present plenty of different milling parameters that could influence the chemical and physical products composition at the end of the grinding process. Thus were selected conditions that lead to the best depolymerization rate with the maximum selectivities of the desired products and were kept constant for every milling treatment.

3.1.3.1 Optimization of the Planetary ball mill parameters

As grinding material, both for the bowl and balls, was selected zirconia in order to be harder than the substrates charged, totally inert toward the reaction studied and not capable to insert any contaminant compound. The grinding bowl was charged in each test with a overall amount of reagents (cellulose plus the solid acids) equal to ten grams in order to have roughly a constant value of free volume to avoid discrepancies on the reaction performances due to this parameters. Because the purpose of this work is to

obtain the best cellulose depolymerization rate, and is known that it is deeply correlated with the energies introduces in the milling devices, the reaction was performed with the highest milling power, 500 rpm. Due that the presence of some solvents inside the grinding bowl buffers the mechanical forces exerted on the solid reactions, the grinding was conducted in totally dry conditions. In particularly no water must be added due that the moisture of the solids used, checked with TGA analysis, is sufficient to induce stoichiometric values of this reagent. Moreover, well-known that the grinding increase the reagents temperature, breaks of ten minutes were performed every thirty minutes of milling in order to cool down the reaction environment to prevent, in this way, some products decomposition. Theoretically, thanks to this heeds, no more than 40°C should occur inside the milling bowl and thus all the products subsequently detected can be estimated as result of the milling process and of some degradation reactions. Nonetheless as "milling time" it is understood only the time when the device was actually in grinding mode, not calculating the grinding breaks.

3.1.3.2 Optimization of the Mixer ball mill parameters

As done in the planetary ball mill it must be selected a constant load in order to have the same amount of free volume inside each capsules during every experiment. It must be considered also that not the whole milling vial volume can be filled with reagents and balls because same free space is necessary for a successful grinding. Thus each capsule was charged with four grams of reagents plus four 10mm diameters zirconia balls. The milling capsules were constituted of zirconia as well for the same reasons illustrated in the planetary ball mill optimization. Each experiment was conducted at the maximum frequency of 30Hz. After every fifty minutes of grinding a break was required until the capsules had reached again the room temperature. Also in this circumstance as milling time is considered only the actually time of grinding.

3.1.4 Optimization of the final product recovery

Once the milling was completed the powder product was totally discharged from the milling vial and solubilized in water in order to have detectable samples. Depending on the cellulose-solid acid ratios charged in the mill, amounts from 2 to 4 grams of powder product were added in a 100ml round glass flask with 40ml of water. Thus the suspension was stirred at 1500rpm for one hour at room temperature. Further 10ml of cold water were used, once the solubilization step was complete, to wash the glass flask and collect the sample. The larger amount of water utilized and the heavy conditions used, such the high stirring speed and the longer time of the process, were appositely chosen to be sure that all the oligosaccharides produced that could exhibit some soluble features were able to be dissolved by the liquid phase. Indeed, as it is well known, increasing the saccharide chain length it lead in more insoluble features of the oligomers and so, for the longer products, is required more time for the dissolution. Moreover, because of the milling motion of the reaction environment, some soluble oligomers could be trapped inside of some cellulosic entanglement formed during the grinding and thus prevented to get out in the aqueous solution. With the conditions applied could be supposed that all the soluble products originated by the milling are all present in the resulting aqueous solution. After that the suspension was centrifuged at 3450rpm for 30 minutes in order to separate the sugar solution from the insoluble products. In this way the solution obtained, previously adequate dilutions, was ready for the HPLC or MS analysis.

A particular study was worth to be conducted in order to verify if this solubilization step, performed after the milling on the powder product, could influence the sugars yield and distribution trough further hydrolysis reactions. Indeed must be considered that when the milled powder product was introduced in aqueous solution also a fraction of the solid acid was present, producing in this way excellent conditions to hydrolyze the sugar oligomers coming from the grinding process. To reduce this possible effect, the solubilization step was performed at room temperature even knowing that higher temperature could lead in higher conversion of water soluble products and could be able to dissolve longer sugar oligomers. Hence 50ml of 0.15% wt cellobiose solution were introduced in a round glass flask with 0.2eq of HCl or 1.5eq of amberlyst 15 and stirred for one hour at 1500rpm at room temperature. The further HPLC-ELSD analysis shows

that from this reaction no glucose molecules were formed. This is a remarkable result because means that no hydrolysis reactions occur during the solubilization step and so the overall oligomers amount and distribution detected in the subsequently milling tests is totally due only to the grinding process. Indeed for this check test was used the shorter sugar oligomer in the presence of the highest acid concentration that could be arisen after the milling. Thus if this condition doesn't lead in any hydrolysis reaction it is extremely rare that could occur with longer sugar molecules and less acid equivalents.

3.1.5 Study of the product of reaction

In order to understand in which kind of compounds the depolymerization reaction drive to, the aqueous solution obtained from the solubilization of the milled products was injected in the ESI-MS instrument after adequate dilutions. Thanks to the mass to charge ratio detected from this analysis is possible to go back to the molecules injected. To have a proper interpretation of the mass-spectrums must be taking in mind that with the ionization technique used not only protonated or deprotonated species are produced, but most of the times other metal ions, such sodium or potassium, could be present and thus attach the analytes to form charged species. In this way are detected molecules present in the original samples injected plus this kind of cations, whose their masses must be considered to well recognize the products. In Figure 3.6 is reported the classic spectrum that arise from milled samples and in order to better interpreted it, the molecular species recognized are been labeled.

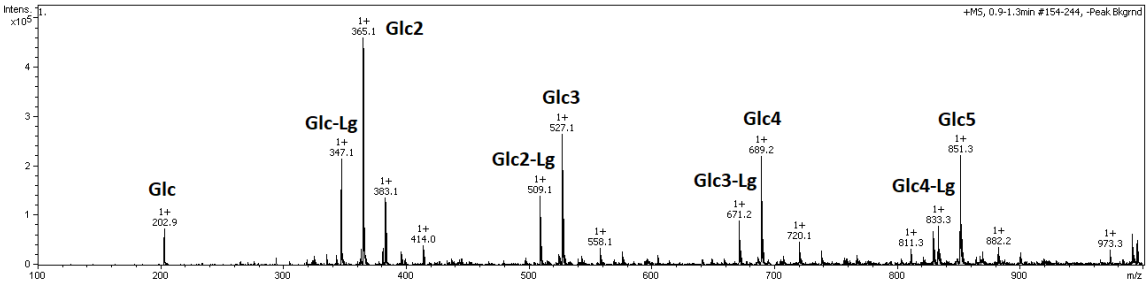


Figure 3.6 Classic Mass-spectrum of one milled sample where some depolymerization reaction is occurred producing different oligosaccharides.

In particular the depolymerization reaction occurred inside the milling devices produces suagar oligomers that are been marked with the acronym of the monomers that they are made of. For instance **Glc** refers to an anhydrous unit of glucose, whereas **Lg** to a

levoglucosan unit, that is considered the dehydration product of glucose. Furthermore every label contains the number of sugar units present in each oligomer and one extra molecule of water must be added to complete the whole saccharide structure. For instance, explaining one of the more complex acronym, **Glc_4-Lg** identify an oligomer composed by four glucose anhydrous units, one unit of levoglucosan, one molecule of water plus one sodium cation from the ionization process. All the peaks that appear in the spectrums but aren't labeled are due to species that aren't been identified. Because were injected only depolymerizated cellulose and the solubilized solid acids, could be supposed that this unknown peaks are originated from some sugar or catalyst fragmentation, also if it is impossible to understand which part of their structure was dethatched. Or could be though that they are formed by reactions of the oligomers present in the aqueous solution and the compounds present in the eluent phase such as formic acid or acetonitrile. Fortunately this unknown species compose only the lower peaks, meaning that they are present in less quantities than the oligosaccharides identified.

3.1.6 Study of the oligosaccharides solubility

As already done in previously paragraph, with ESI-MS technique it is possible to understand which oligosaccharides are present in the aqueous solution resulted from the solubilization step and thus classifiable as soluble. Unfortunately must be considered that increasing the chain length of the oligosaccharides, their solubility decrease. Hence the species detected from the ESI-MS instrument could be classified in two categories, the ones totally soluble and the ones partially soluble. Whereas the ones totally insoluble aren't obviously be detected because not injected. It is known that the peaks intensity of the mass-spectrum at every m/z value are proportional to the ions amount produced and so direct correlated with the analytes injected. Thus measuring these ion peaks intensities can be performed a comparison on the products distribution. Of course this quantification is not accurate but a comparison could be anyway conducted to have a roughly estimation. Of course the products fraction that is classified as partially insoluble cannot be considered for the product distribution evaluation, because the intensities of their peaks is lower due to their reduced solubility instead of its less yield. Hence to perform a good comparison it is clear that must be chosen a border line of the glucosyl units under

which the products are classified as totally soluble and so in agree for that purpose, whereas over which, all the saccharide detected are considered only partially soluble. Unfortunately this classification cannot be done accurately with checking the solubility of standard solution because standard oligomers longer than two sugar units cannot be purchased. But thanks to the MS analysis performed are detected species until nine AGUs, having in this way a wide range in which chose the solubility border line and have anyway a lot of polysaccharides labeled as total soluble for a good estimation of the product distribution. To perform this classification was used a mass-spectrum of a sample milled twenty hours with 1.5 equivalents of amberlyst 15, represented in Figure 3.7, because is the catalytic system that shows the best performances and so where can be found the highest amount of oligosaccharides.

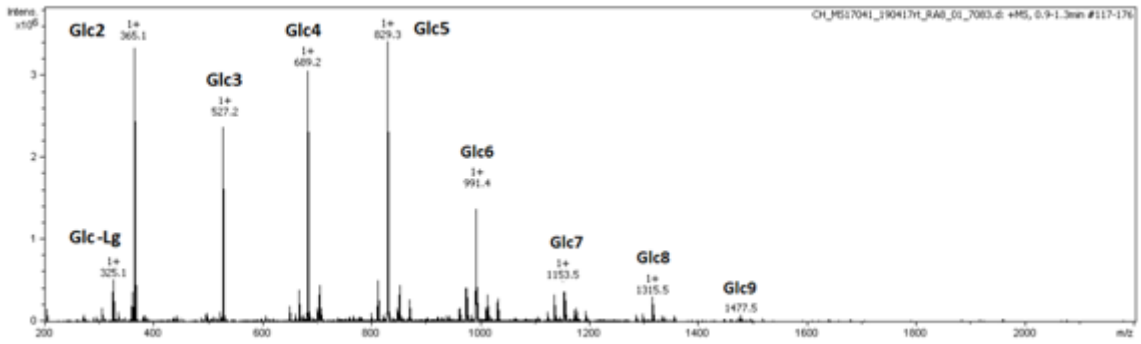


Figure 3.7 Mass-spectrum of sample obtained milling cellulose and 1.5 equivalents of amberlyst 15 for 20 hours.

As expected the peak intensities of oligomers constituted by more than five AGUs decrease progressively as the sugar chain becomes longer. Hence saccharide until five AGUs are considered totally soluble whereas the longer ones only partially soluble and not suitable for the comparison on the product distribution. For this reason in the following spectrum interpretations the m/z scale terminated at 1000m/z where only oligosaccharides until five units are detected. Furthermore with a restricted m/z scale a better resolution appear on the intensities comparison of the shorter saccharides. It must be observed that molecules longer than nine glucosyl units aren't detected and so could be expected that over that dimension all the polysaccharides are totally insoluble.

3.2 DEVELOPMENT OF A METHOD TO MEASURE THE WATER SOLUBLE PRODUCTS USIGN THE ESI-MS TECHNIQUE

Since the classical analytical techniques were resulted not suitable to determine the total depolymerization rate of the milling process, was considered as a conversion value the total amount of water soluble products arisen from the solublization step performed after the grinding. Unfortunately gravimetric analysis, weighting the unconverted solid after the solubilization step, cannot be performed because they require a drying treatment in oven that, due to the presence of acid particles among the cellulosic granules, leads in a combustion phenomenon that overestimate the conversion value. Hence a new method, exploiting the ESI- MS technique, was developed to determine the total amount of water soluble products in the aqueous solution obtained. Indeed thanks to samples classified as 100% water soluble by visual analysis, of the cellulose insert in the glass flask during the solubilization step, it was constructed a correlation between the number of AGU moles injected and the area of the LC-MS chromatogram arisen. First of all glucose and cellobiose standard solutions were injected to understand how the ESI-MS system respond to their presence. Indeed it was extremely important to obtain both their LC chromatogram and the MS spectrum of each retention peak detected. Unfortunately the LC-MS technique used for the detection leads in some inner reactions of the compounds injected, but thanks that the their reacted fraction appears always constant, a reliable quantification can be anyway obtained. Subsequently an aqueous solution of one milled sample was injected in order to understand how it acts inside the ESI-MS instrument. Hence glucose and cellobiose calibration lines were constructed with standard solutions to quantify their amounts inside the whole milled samples. Thus also the calibration line of milled samples considered 100% water soluble could be constructed and the quantitative determination can be performed. At the end of the dissertation, to check if the analytical method constructed is reliable or not, the conversion of a milling process was calculated and verified by visual analysis of the insoluble solid remained at the bottom of the centrifuged tube. For visual analysis it is understood comparing the insolubilized powder remained at the bottom of the centrifuge tube after the recovery steps, and so unconverted, to the whole solid amount charged in the mill. Nonetheless in the following paragraphs is explained and demonstrated step by step the analytical method constructed for the WSP quantification.

3.2.1 ESI-MS identification of glucose and cellobiose

In order to understand the behavior of glucose and cellobiose inside the ESI-MS instrument, 25 µg/ml of standard solution of both sugars were injected in that device. In Figure 3.8 is reported the MS – LC chromatogram of the standard glucose solution.

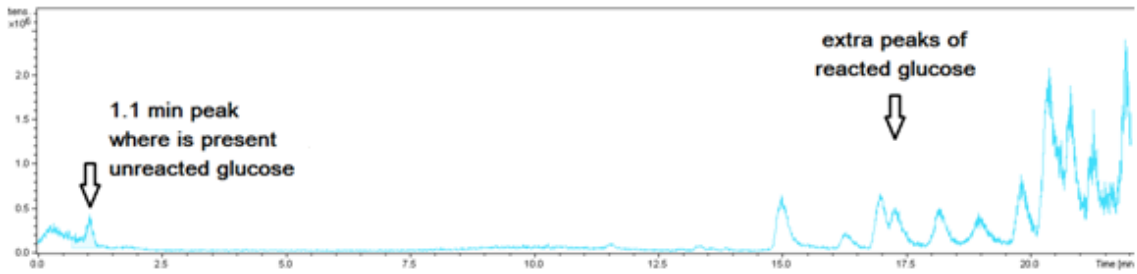


Figure 3.8 MS-HPLC chromatogram of 25µg/ml glucose standard solution where are highlighted the peaks relative to the unreacted and reacted glucose fraction inside the LC column.

Unfortunately the MS-LC chromatogram shows more than one peak, contrarily as it was expected due that only one compound was injected. Indeed only the retention peak at 1.1 min with a mass-spectrum that shows the 202.9 m/z MS signal should appear, consistent with the glucose presence. Instead a multitude of signals arise in the last five minutes of the chromatogram. The only explanation that could be attributed to those extra peaks is hypothesize that the glucose reacts with the stationary phase inside the MS-LC column or with the eluent used as mobile phase, leading in a more complex compounds with higher molecular weights, as the spectrums reported in Figure 3.9 suggest. Indeed it must be remembered that as mobile phase was used a gradient concentration of 0.1% of formic acid in water and 0.1% of formic acid in acetonitrile that could induce some unknown reactions. It was checked that no one of the masses under those extra peaks correspond to some oligosaccharides showing that no glucose polymerization occurs, not interfering with the products researched in the depolymerization process.

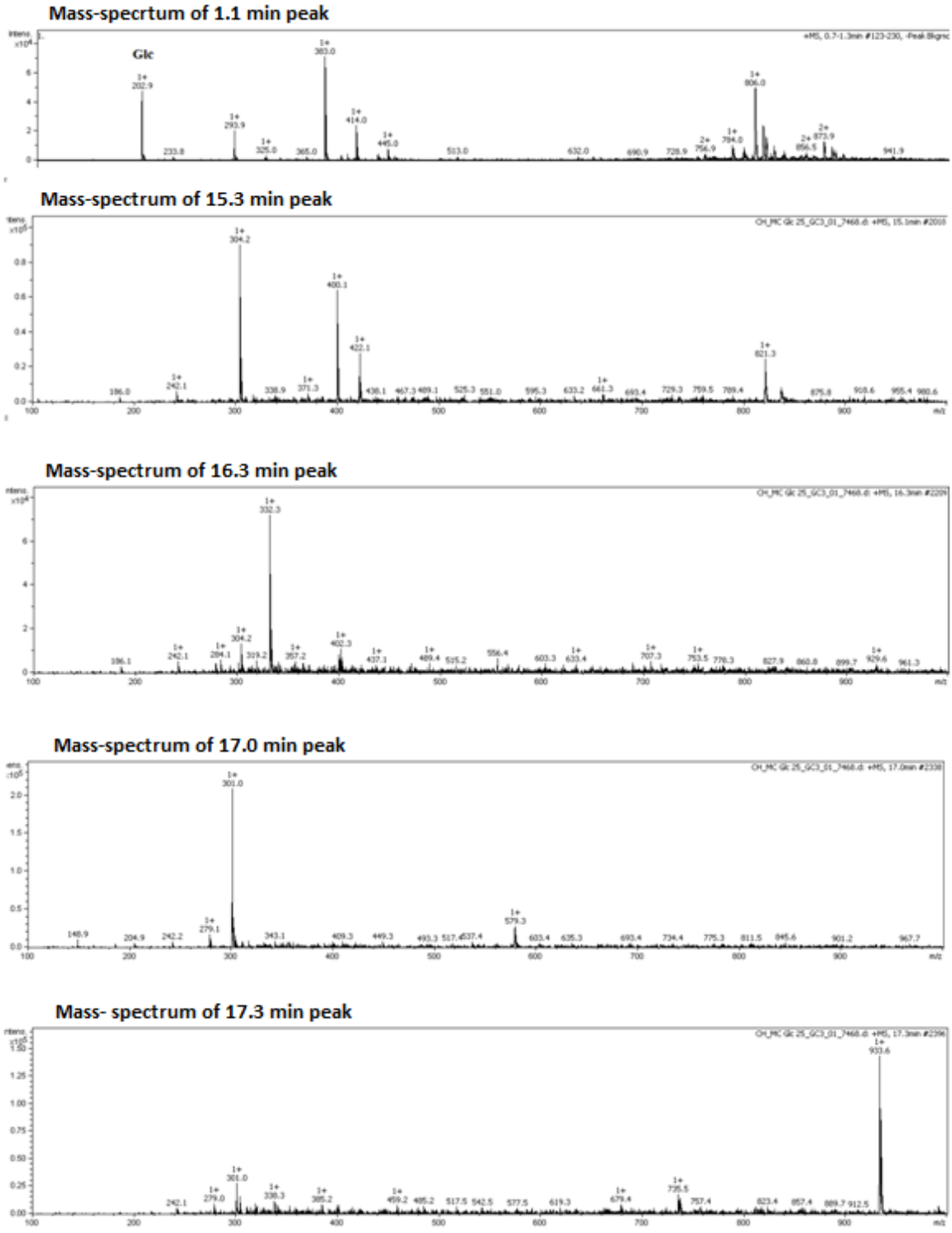


Figure 3.9 Mass-spectrums of the main chromatographic peaks resulted from the MS-HPLC separation of the 25µg/ml glucose standard solution.

The conclusion that the glucose extra peaks in the MS analysis aren't due to some instrumental mistakes, such as background over noise at the end of the retention, arise because more concentrated glucose solutions show the same multitude of peaks with the identical shapes in their MS–chromatograms.

Moreover when the cellobiose standard solution was injected only the 1.1 min chromatogram signal results from the analysis, corresponding in a spectrum peak of 365.1 m/z, reported in the Figure 3.10, in agree with the mass of the compound analyzed. Furthermore this means that cellobiose doesn't undergo in any reactions during the MS analysis as glucose. A further evidence of the glucose reaction inside the MS column is given by the comparison of the area integral values. The peak of the 25 µg/ml cellobiose chromatogram shows an area about $2x10^8$. The 1.1 min peak of the 25 µg/ml glucose chromatogram, under which the 202.9 m/z signal of glucose is situated, has an area value of $2x10^6$, whereas the value of the overall chromatogram areas is about $2x10^8$. Assuming that the detector has the same response factor for the two carbohydrates can be concluded that the 1.1 min peak of glucose is smaller than the which one of cellobiose because of its conversion in some other compounds as already explained.

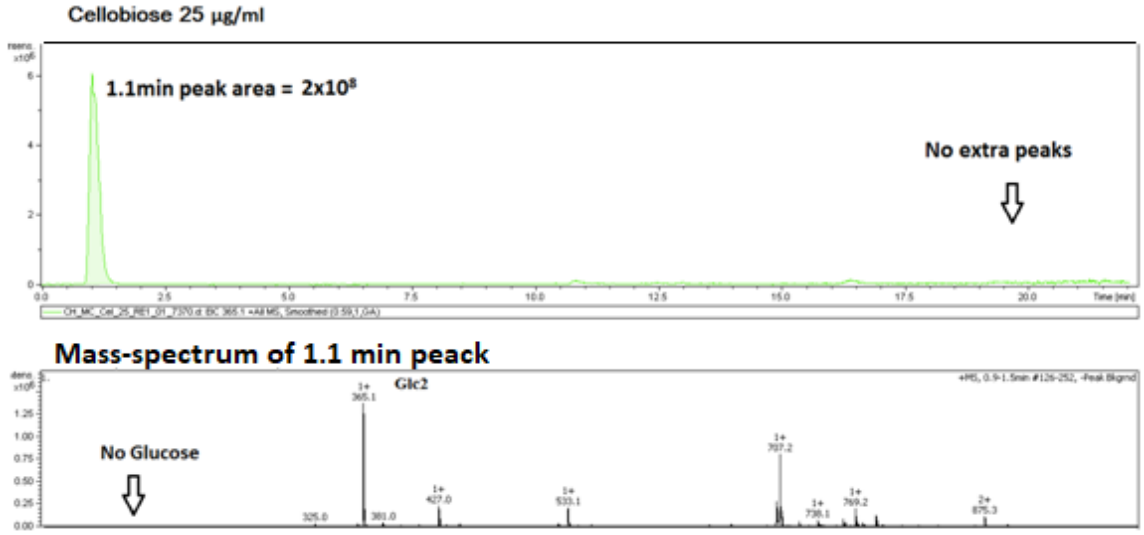


Figure 3.10 Chromatogram of the 25µg/ml cellobiose standard solution and the Mass-spectrum of the only chromatographic peak identified.

The hypothesis that glucose solution contains same impurities or reacts with the water used as solvent to prepare the standard solutions could be excluded by the HPLC–ELSD chromatogram, Figure 3.11, that shows only two retention peaks, one corresponding to the glucose and the other to the sorbitol used as internal standard for that analysis.

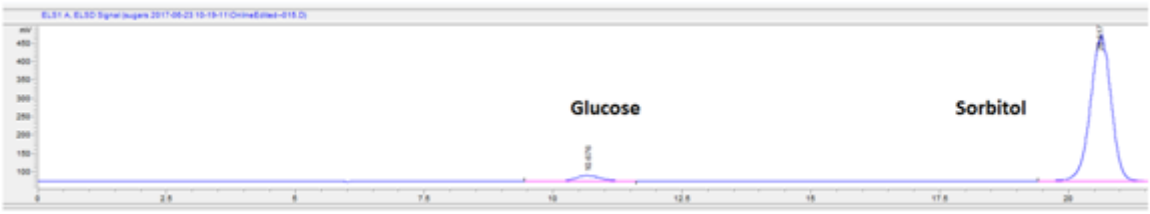


Figure 3.11 HPLC-ELSD chromatogram of the same glucose standard solution injected in the ESI-MS instrument. As can be seen it appears only the peaks relative to glucose and the internal standard sorbitol.

Moreover are reported in Figures 3.12, 3.13 and 3.14. the mass-spectrums of the 100% water soluble samples that were injected for the determination of conversion in term of water soluble products, which their concentration of glucose and cellobiose is already quantified thanks to the HPLC-ELSD analysis, and so reported with every spectrum.

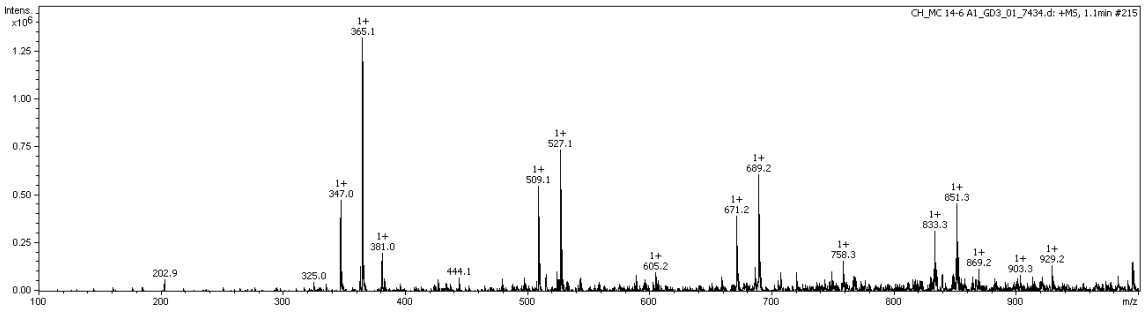


Figure 3.12 Mass-spectrum of the sample obtained milling 15 hours cellulose and 1.5 equivalents of ambersyt 15. Glucose = 18.0 µg/ml; Cellobiose = 22.3 µg/ml.

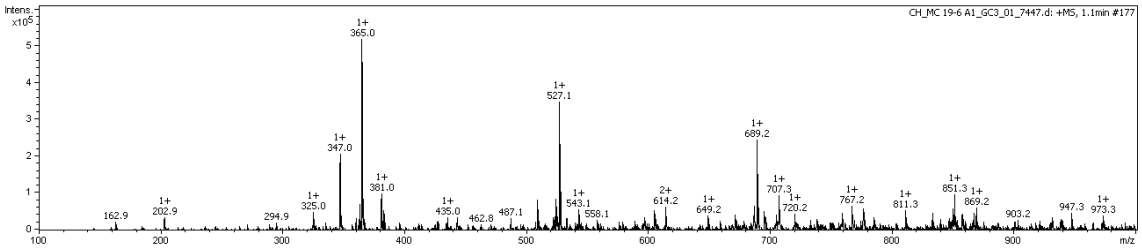


Figure 3.13 Mass-spectrum of the sample obtained milling 20 hours cellulose and 1.5 equivalents of ambersyt 15. Glucose = 11.7 µg/ml; Cellobiose = 15.1 µg/ml.

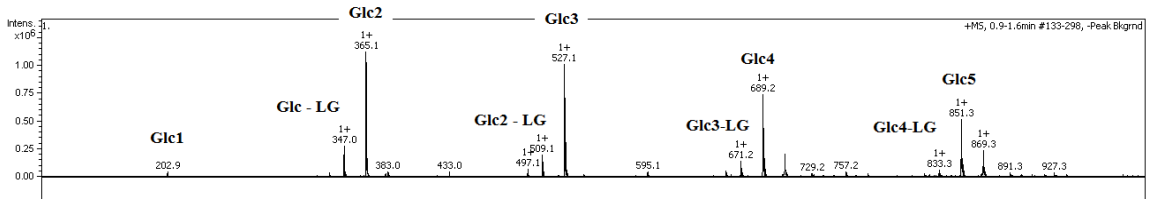


Figure 3.14 Mass-spectrum of the sample obtained milling 15 hours cellulose and 0.8 equivalents of ambersyt 15. Glucose = 15.6 µg/ml; Cellobiose = 20,5 µg/ml.

In each analyzed sample no more than 5 µg/ml arise as a gap between the glucose and cellobiose concentrations, that correspond in roughly 25% of difference from one to the other. But looking at the difference between the glucose and cellobiose signals in each sample injected, can be seen that it is a huge time bigger than 25%. This is a further evidence that under the 1.1min peak the glucose and cellobiose amount is really different though they are roughly injected in the same quantities, due to the reactions that glucose undergo inside the LC column. Indeed it is known that the signals of the mass-spectrum at every m/z value are proportional to the ions amount produced and so direct correlated with the analytes injected.

3.2.2 Study of the final reaction product

Before starting to discuss the determination of water soluble products and the Glc_{3-5} oligomers, can be useful explain how it appears one MS-HPLC chromatogram of a milled sample, Figure 3.15. Thanks to the mass-spectrums can be roughly understood the kind of compounds that are eluted at different retention times.

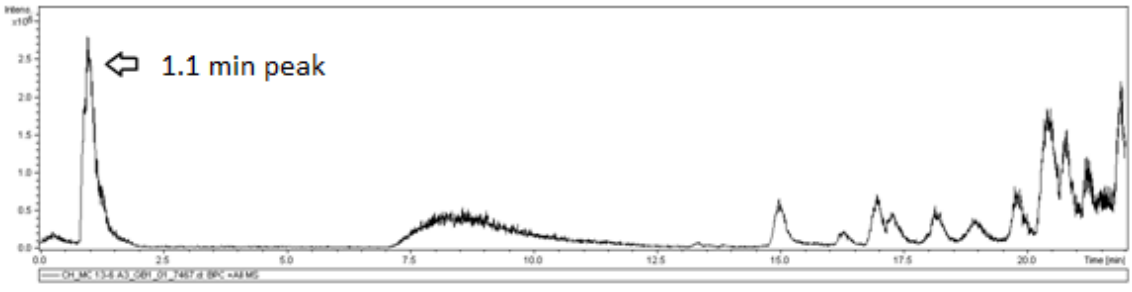


Figure 3.15 MS-HPLC chromatogram of a sample obtained cellulose and ambelyst 15.

In this chromatogram appears a high peak at 1.1min, and as the mass-spectrum shown in Figure 3.15, contains the three compounds classes that want to be determined: **Glucose**, **Cellobiose** and **Glc_{3-5} oligomers** plus some **olygosaccharides that contains also**

one levoglucosan unit (Lg), that it is the glucose dehydration product. Due that the mass-spectrum shows that the copolymers glucose-levoglucosan are in lower amount than the glucose oligomers this latter class of compounds in the following discussions are included in the **Glc_{3-5} oligomers** class, also because differ from them only for the absence of one water molecule. Figure 3.16 better represents the three classes of compounds that are enclosed in the 1.1 chromatographic peak.

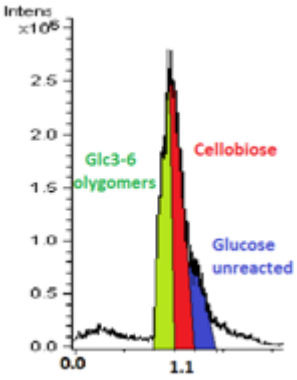


Figure 3.16 Compounds that are eluted in the 1.1 min chromatographic peak.

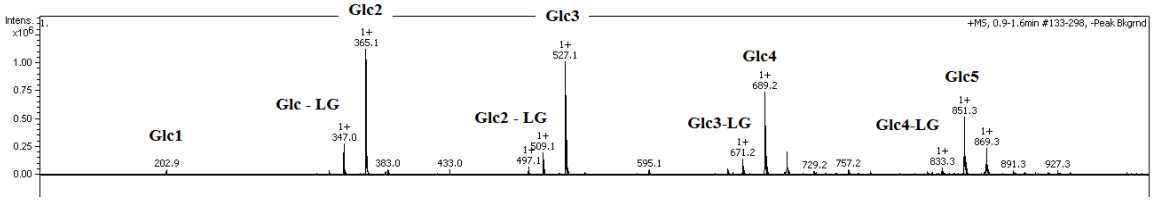


Figure3.17 Mass-Spectrum of the 1.1 min chromatographic peak showed in the Figure 3.16.

The wide chromatogram signal from 7.5min and 10min is due to the retention of the amberlyst 15 fragments. This is proved by the mass-spectrum reported in Figure 3.18 that shows a high signal at 186 m/z, referred to the fragment illustrated inside the spectrum, agreeing with the amberlyst structure. Moreover a further evidence is that this broad peak doesn't appear when samples that don't contain amberlyst 15 were injected, such as the glucose or cellobiose standards.

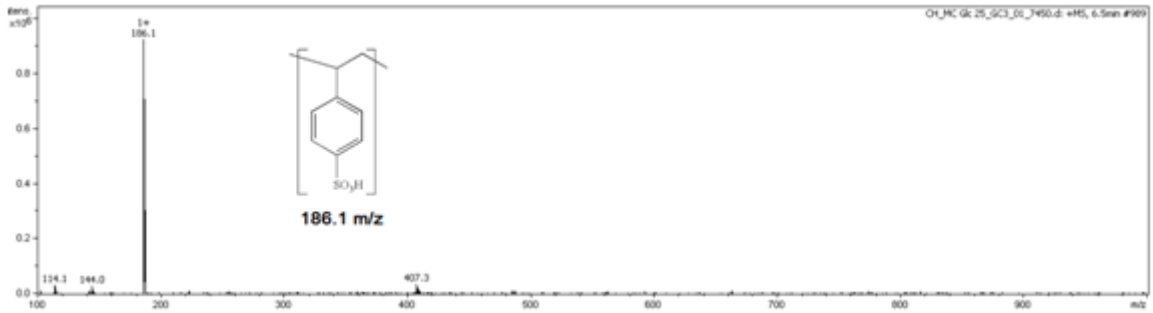


Figure 3.18 Mass-spectrum of the 7.5-10 min chromatographic peak showed in the Figure 3.16.

As already explained in the glucose mass-spectrum, the multitude of peaks in the last five minutes of retention are due to some reactions that the glucose undergoes inside the analytical instrument and with a further mass-spectrum check is been verify that any of those don't contain any Glc_{3-5} oligomers. Indeed the last portion of the milled sample chromatogram can be perfectly overlapped with the one of a glucose standard solution,

confirming again that those last peaks are originated only by compounds coming from the glucose reaction inside the LC column. With this initial control is been ascertained that the Glc_{3-5} oligomers are only incased into the 1.1min peak together with cellobiose and the unreacted glucose. Hence in order to compare the Glc_{3-5} oligomers quantity in the divers milled samples it is essential know the contribution area that the unreacted glucose and cellobiose gives at the 1.1min peak area. Thus calibrations of the 1.1min peak area against concentrations are constructed with glucose and cellobiose standard solutions.

3.2.3 Glucose calibration line with ESI-MS

Despite the reactions that the glucose undergoes in the LC-MS column is worth to construct a calibration line the glucose 1.1min chromatogram peak area against its concentration, Figure 3.19. Indeed this calibration will be useful to understand the glucose area contribution in the 1.1min peak of a milled sample, or in other words to estimate the glucose fraction that doesn't undergo in some reactions inside the LC column. For this purpose in this calibration were integrated only the areas of the 1.1min peak and not the overall chromatogram area. Understanding the glucose fraction that resides under the 1.1min peak is important to determine the cellobiose and Glc_{3-5} oligomers quantities under that peak, due that unfortunately all the sugar compounds show the same retention time. Thus the calibration line of glucose was constructed with 25, 50, 75, 100 µg/ml standard solutions prepared by following dilutions from the same mother solution and double checked thanks to the HPL –ESLD analysis.

Standard theoretical points (µg/ml)	Concentration from weight (µg/ml)	Concentration from HPLC (µg/ml)
25	25.86	19.8
50	51.44	45.2
75	76.71	79.8
100	103.13	99.7

Table 3.1 Concentration of glucose standard solution determined by weight and from the HPLC-ELSD analysis.

The HPLC–ELSD analysis was carried as check for the prepared standard solutions, but it must be considered only for the order of magnitude values, due to the fact that the HPLC–ELSD was calibrated whit a standard solutions one order of magnitude higher than the samples analyzed. Indeed the difference between the concentrations calculated

by weight and the which ones calculated with HPLC, that appear in Table 3.1, can be attributed to this reason.

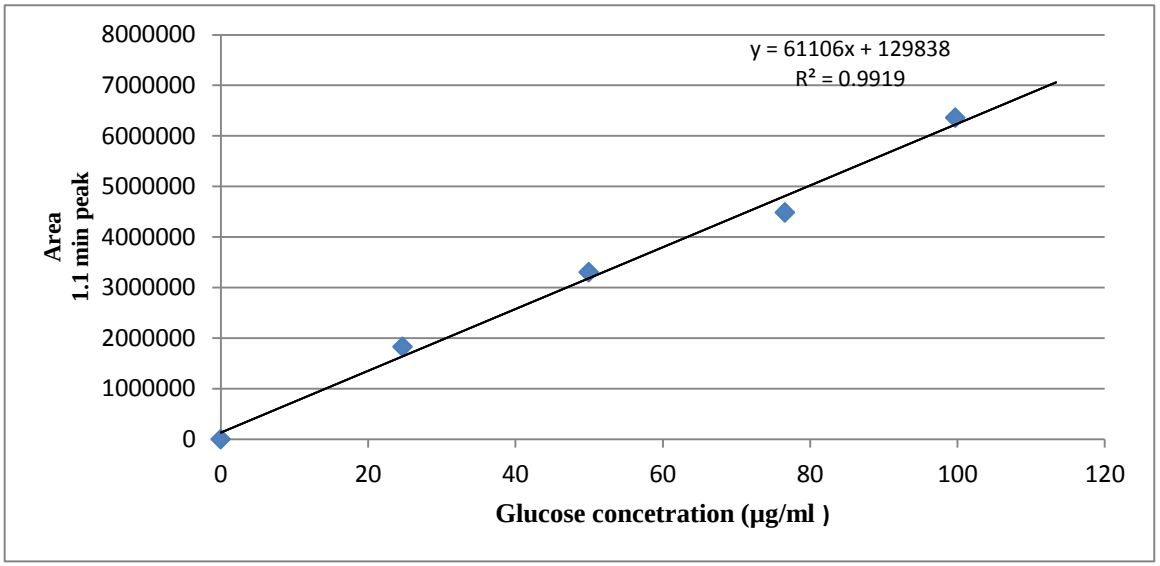


Figure 3.19 **Glucose calibration line.**

It is remarkable that despite the side reactions that occur inside the analytical instrument the fraction of glucose that results under the 1.1min peak is constant also varying the overall glucose concentration injected, as confirms the R^2 factor value of 0.991 in Figure 3.19.

3.2.4 Cellobiose calibration line with ESI-MS

For the calibration line of the cellobiose there's no need to distinguish which peak area plot against the sugar concentration, due to that the cellobiose chromatograms show only one peak each.

Standard theoretical points (µg/ml)	Concentration from weight (µg/ml)	Concentration from HPLC (µg/ml)
25	25.47	35.91
50	50.26	55.44
75	76.07	64.54
100	99.95	102.4

Table 3.2 Concentration of cellobiose standard solution determined by weight and from the HPLC-ELSD analysis.

As for glucose, the gap between the concentrations calculated by weight and the ones calculated with the HPLC–ESLD is due to the calibration range of the HPLC–ESLD, that not represents the optimum for the concentrations analyzed.

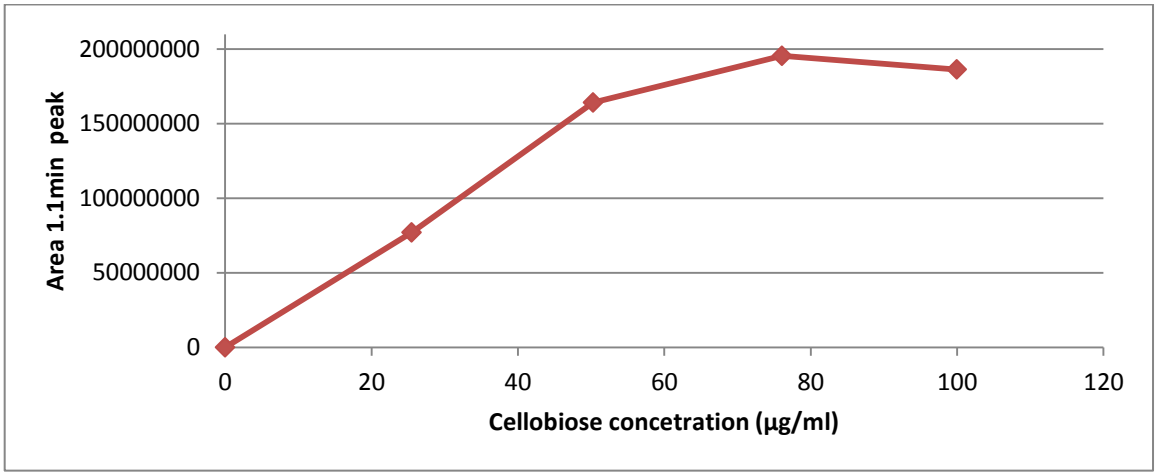


Figure 3.20 Cellobiose 1.1 peak area against the relative standard solutions concentration.

With a first glance can be seen in Figure 3.20 that the calibration line of cellobiose presents a deviation from a linear trend at high sugar concentration, in particular after 50 µg/ml the detector seems not change its response, or at least shows a very weak response compared to the which one at lower concentrations. This behavior could be attribute to a saturation effect of the detector. Indeed must considered that with the same concentration of glucose, the double matter arrive to the detector when cellobiose is injected. For the $Glc_{3\text{-}5}$ oligomers determination can be anyway useful calculate the tendency line for the first two standard points and thus obtain the linear equation, area against concentration, as reported in Figure 3.21. Also in this case the R^2 value confirms the good linear trend of the cellobiose calibration at lower concentrations. Indeed as resulted with the HPLC-ELSD analysis, higher cellobiose concentrations than 20 µg/ml aren't product in any of our milled samples and thus are completely enclosed in the linear calibration range.

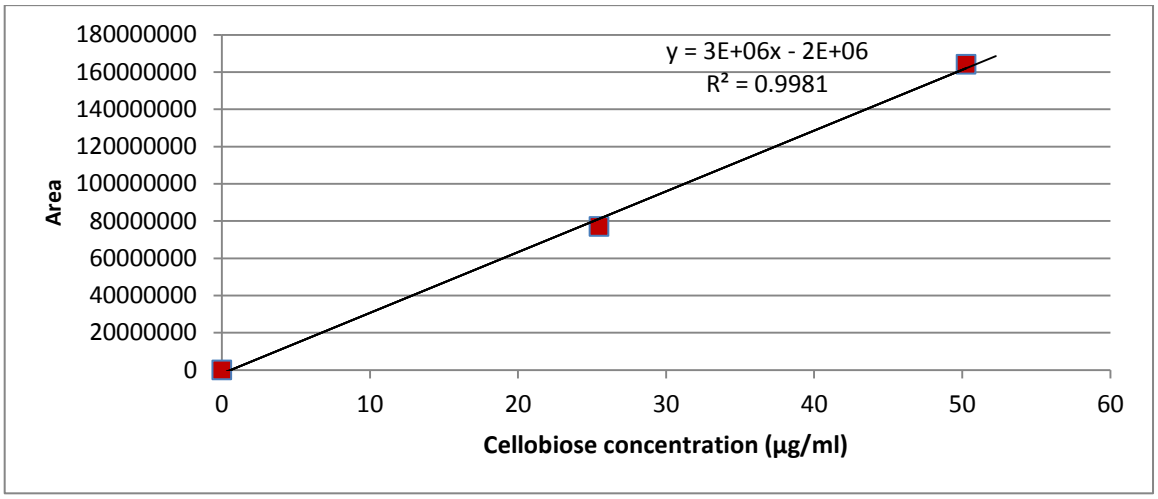


Figure 3.21 Cellobiose calibration line.

Moreover this MS analysis on the cellobiose standard solution was useful to understand the oligosaccharides behavior inside the MS system. Indeed as the spectrums in Figures 3.22, 3.23, 3.24 and 3.25 shows, no one glucose molecule was detected when cellobiose standard solutions were injected. The glucose peak should appear at 202.9 m/z, whereas the 365.1 m/z signal correspond to cellobiose. This confirms that the MS analysis doesn't break down any polysaccharides and thus every oligomers detected in the following sample analysis are the result of the milling process, agreeing with the soft ionization technique used.

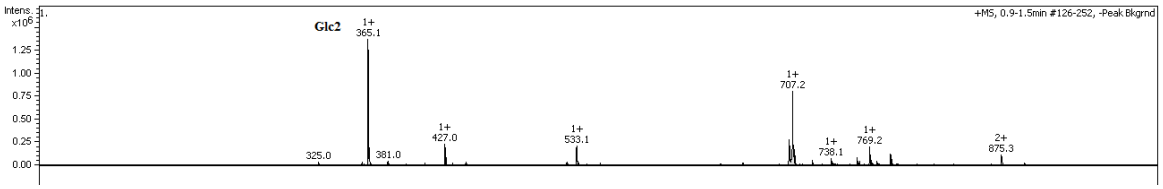


Figure 3.22 Mass-spectrum of the 25µg/ml cellobiose standard solution.

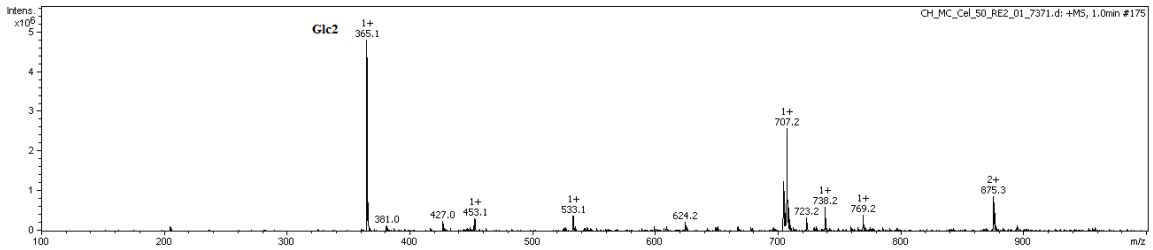


Figure 3.23 Mass-spectrum of the 50µg/ml cellobiose standard solution.

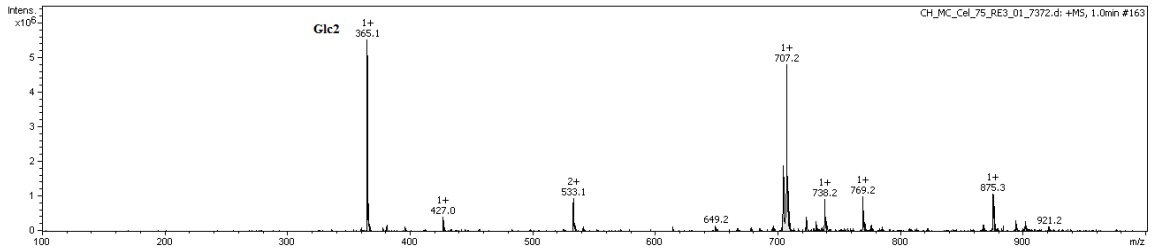


Figure 3.24 Mass-spectrum of the 75µg/ml cellobiose standard solution.

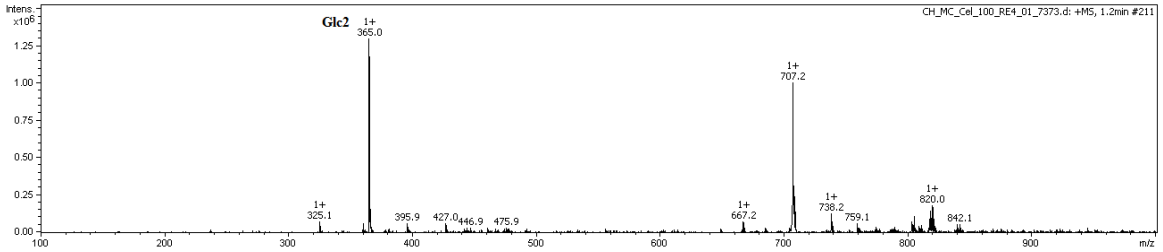


Figure 3.25 Mass-spectrum of the 100µg/ml cellobiose standard solution.

It is still unclear the 707 m/z and 875 m/z mass to charge signals appeared when the cellobiose solutions were injected because cannot be referred to any possible molecule.

3.2.5 Conversion determination as water soluble products amount

In order to know the total amount of water soluble products resulting from the milling reaction, a calibration line was constructed utilizing samples that were labeled as 100% water soluble by visual analysis, due that all the cellulose amount inserted in the glass flask was dissolved during the solubilization step.

This samples are:

- Cellulose + Amberlyst 15 0.8 equivalents milled 15h at 500rpm;
- Cellulose + Amberlyst 15 1.5 equivalents milled 15h at 500rpm;
- Cellulose + Amberlyst 15 1.5 equivalents milled 20h at 500rpm.

The surnatant of the centrifuged sample resulted after the solubilization process, were diluted 100 times and injected three times each in the ESI-MS instrument. The multiple injections of each sample were useful to understood that the ESI–MS analysis isn't much consistent in the last portion of the resulting chromatograms, because they appear different for each injection as confirms Figure 3.26.

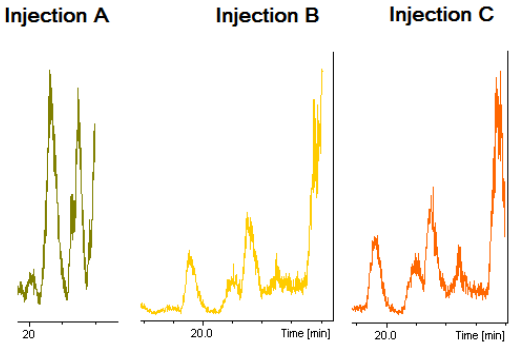


Figure 3.26 Last portions of the MS-HPLC chromatograms of the same sample injected more times.

Hence can be understood that if the overall area of the total chromatogram is integrated to be plotted against a number of moles to calculate the water soluble products amount, a large error arise depending on how the chromatograms terminate, due that the latest area are the larger contribute of the whole chromatogram. Fortunately the previously glucose calibration has demonstrated that the fraction of glucose that doesn't react inside the instrument, thus that remains under the 1.1peak, is remarkably constant also varying the glucose concentration injected. Furthermore, thanks to the HPLC-ELSD analysis, can be asserted that in the divers samples labeled as 100% water soluble, the glucose concentration is almost constant varying the time and acidity conditions of the milling process. Indeed in each of these samples the total glucose yield is about 6.5% and seems not increase if the milling time or acidity are extended, as the asymptotes in Figure 3.27 suggest.

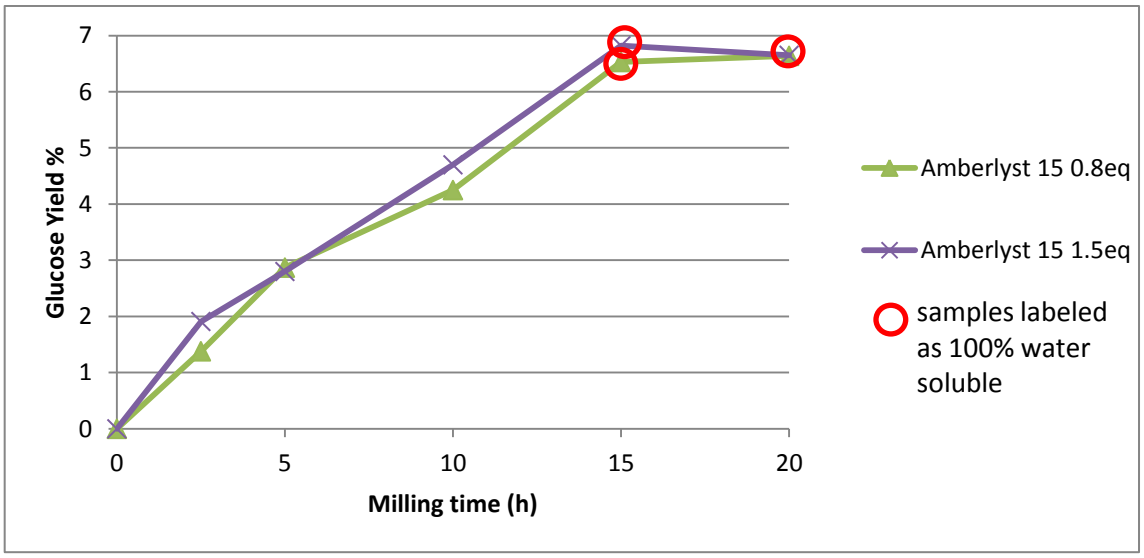


Figure 3.23 Glucose yields obtained from different tests performed in the planetary ball mill at 500 rpm with variuos equivalents number of amberlyst 15 in function of the milling time.

For these three reasons:

- the not constant area values of the latest peaks in the MS–chromatograms;
- the constant fraction of the unreacted glucose that remains under the 1.1min peak;
- and the constant yield of glucose when 100% of WSP are reached;

it was chosen to use the 1.1min peak area for each 100% WSP sample to construct the WSP calibration line instead of the whole chromatogram area. This shouldn't be conduct in a huge error in the WSP determination because, as previously described, the main Glc_{3-5} oligomers produced are incased into the integrated 1.1min peak, whereas the latest big areas that aren't considered for the integration, are due to the glucose converted into the instrument and it's known that it represents less the 6% of the total WSP amount as its total yield confirms. Thus, for the WSP calibration, only the 1.1min peak areas of each sample was integrated. Unfortunately the analysis precision is quite weak as show the area values obtained with various injections. Table 3.3

	Amberlyst 0.8eq 15h	**Amberlyst 1.5eq 15h**	**Amberlyst 1.5eq 20h**
1.1min peak AREA	77177328	49947808	34285124
	77851312	57344404	56270400
	60097776	60921796	44359508

Table 3.3 Values of the integrated area of the 1.1 min chromatographic peak arisen from the ESI-MS analysis of samples obtained using different equivalents of amberlsyt 15, injected three times each.

Despite the poor analytic precision, the average area for each sample was calculated and plotted against the molar concentration of AGUs present in the weighted cellulose for the solubilization process, considering all the dilution factors. Of course is not a proper accurate math step because not the overall amount of cellulose is converted in glucose since bigger oligosaccharides are formed, but it must be done otherwise there isn't any reference to consider the amount of cellulose solubilized for the various samples analyzed.

For instance
mass cellulose solubilised = 1.41925g

mol AGU = $\frac{\text{mass cellulose solubilised}}{\text{MW AGU}} = \frac{1.41925}{162.16}$ = 0.008752 mol

solubilized in 50 ml of H_2O = 0.175049 mol AGU/L (injected in the HPLC-ELS)

diluted about 100 times = 0.001691134 mol AGU/L (injected in the MS-ESI)

that emerge an average area value of 71708805

Thus was plotted the molar concentration of AGU (mol/L) injected against its area values in order to obtain the calibration equation.

Figure 3.28 shows that this calibration method, calculating the AGUs moles number of a 100% soluble sample versus its area, could be considerate quite reproducible because not high differences arise between calibration lines built with samples milled in different conditions of time and acidity. In this way a calibration line was constructed resulting in a average equation of $y = 3{,}67 \times 10^{10}\, x$;
where "x" correspond to the total AGUs concentration injected and "y" the 1.1min peak area.

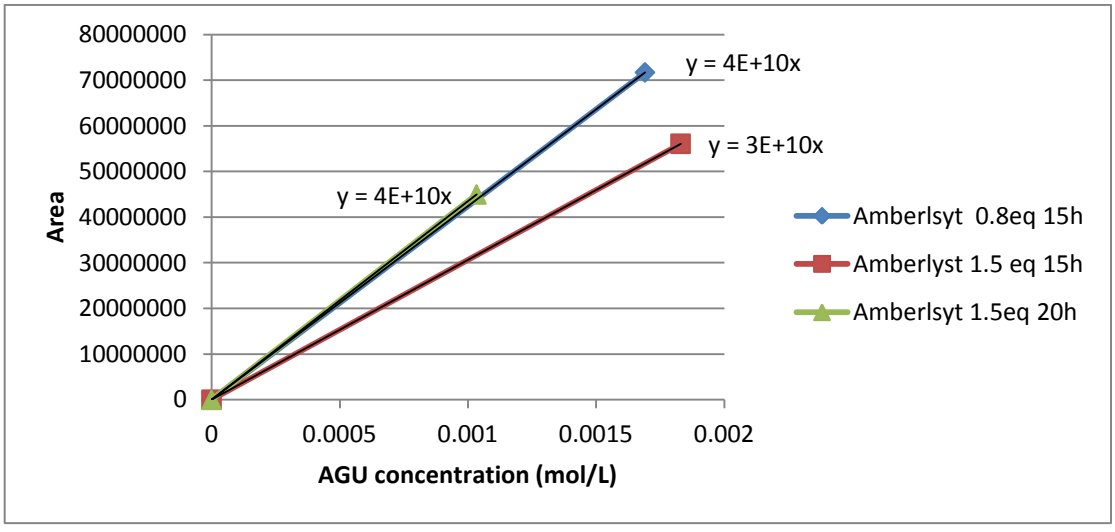


Figure 3.24 Water soluble products calibration constructed with samples considered 100% soluble after the solubilization step.

Thanks to the HPLC–ELSD analysis was calculated that in the samples with 100% WSP about 0.20% cellobiose solution result from the milling process and this correspond to

inject a sample in the ESI-MS whit a concentration of 20 µg/ml. Due that the linear calibration range for cellobiose in the ESI-MS is from 0 µg/ml to 50 µg/ml, the contribution of cellobiose in the total ESI-MS area could be determined quite accurately with the equation reported in Figure 3.21. In the same way (with the equation in Figure 3.19) the glucose contribution area of the 1.1min peak could be calculated, thus allowing the calculation of the reminder contribution area of the Glc_{3-5} oligosaccharides. This permits a roughly comparison between the divers milled samples on the Glc_{3-5} oligomers presence that in other ways wouldn't be possible. For instance, the first sample (Amberlyst 0.8eq 15h) shows a total ESI-MS area of the 1.1min peak of 71708805 (average area value) and concentrations of glucose and cellobiose in the injected sample equal to 15.6 µg/ml and 20.5 µg/ml respectively. With the ESI-MS calibration line for glucose and cellobiose it is been calculated that those concentration values lead in area values of the 1.1min peak equal to 965686 and 59519915 respectively. Thus the Glc_{3-5} oligomers contribution area is easily calculated by subtraction resulting 11223204 as illustrated in Table 3.4. Also with this instance it is clear that the glucose contribution for the 1.1min peak is almost negligible compared to the which one of cellobiose and Glc_{3-5} oligomers.

Total ESI-MS area	71708805	-
Glc_1 contribution Area	965686	-
Glc_2 contribution Area	59519915	=
Glc_{3-5} contribution Area	11223204	

Table 3.4 Determination of the Glc_{3-5} oligomers contribution area of the 1.1min peak total area.

Furthermore the total of WSP resulting from the reaction can be considered as the conversion of the overall milling process and thus allowing the selectivity determination for glucose and cellobiose. The Glc_{3-5} oligomers yield can be easer determinated from the overall conversion value substrating the glucose and cellobiose yield, considering that the glucosan-levoglucosan chains are included in this latter class of compunds and some other lower sugar fragments are negligible, as the the mass-spectrums suggest.

For example for the same sample considered:

Glucose Yield = 6.5%

Cellobiose Yield = 8.3%

Glc_{3-5} oligomers Yield = 85.1%

In this way the calibration line for glucose and cellobiose are no more essential to determine the WSP amount and the Glc_{3-5} oligomers yield, but must be anyway constructed to be sure that the 1.1min peak is proportional to the sugar amount injected.

In particular the glucose calibration was fundamental to understand that the glucose amount that didn't undergo in any reactions inside the column is constant also varying the concentration injected.

3.2.6 Validation of the developed method

In order to understand if the WSP analysis method constructed is reliable or not a milled sample was analyzed and the amount of soluble products checked by visual analysis.

SAMPLE: Cellulose + Amberlyst 15 0.2eq milled 20h at 500rpm.

The surnatant of the solubilisation step was injected in the HPLC for the glucose and cellobiose determination and diluted 100 times for the further three injections in the ESI-MS instrument.

Areas of 1.1min retention peak resulted from the ESI-MS detector: 66919456; 93400016 and 82466160.

Also with this sample emerge that the area determination of a single sample in different injections fluctuate in a wide range showing that is not a precise technique.

Average area value = 80928544.

With that average value area and thanks to the previously 100% WSP samples calibrations (Figure 3.28) was calculated the AGUs concentration in the sample injected. Once transformed that concentration in number of AGUs moles, considering the dilution factor, and afterward converting it in mass of AGUs solubilized, it's possible determine the percentage of WSP in the sample selected. For that sample is been calculate a WSP value of 77.8%. This could be considered a possible value if compared with the insolubilized sample remained in the centrifuge tube as illustrated in Figure 3.29.

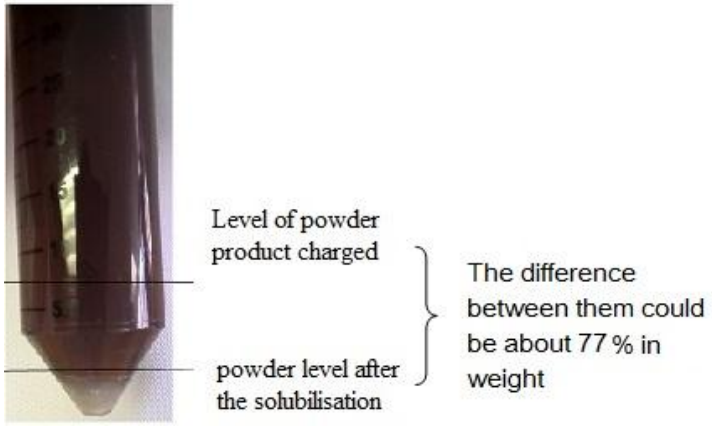


Figure 3.29 Visual analysis of the water soluble products performed comparing the insoluble polysaccharides remained at the bottom of the centrifuge tube to the overall solid charged in the mill.

3.3 STUDY OF THE REACTION USING THE PLANETARY BALL MILL

To understand and compare the performances of the solid acids listed in Table 3.5 it was considered as a conversion value of the depolymerization process the total amount of soluble products arisen after the solubilization step, determined with the ESI-MS analytical method previously described. Indeed, after the milling, the powder products was discharged from the bowl and dissolute in water at as reported in the solubilization step paragraph. Thanks to the ESI-MS analysis conducted on the aqueous solution was concluded that are totally soluble only oligomers until five AGUs. Obviously, following this protocol, all the sugar oligomers produced by the grinding treatment insoluble in water, because too long, cannot be determined. In this way not all the depolymerization extend will be accurately quantified but an underrated error will surely occur. Keeping in mind the purpose of this work, compare the catalytic performances of various solid acids toward the cellulose depolymerization, could be approved that this underrated error arise for each catalytic material studied and thus, the comparison realized confronting only the WSP yield could be considered reliable. Moreover, to be exploited for further green industrial transformations, the saccharide products should be preferably soluble in water in order to restrict the utilizations of organic solvents, making truthful the definition of conversion earlier announced. On a processing level, use of a solid catalyst over a homogeneous analogue offers several practical level, particularly in terms of catalyst recycling and process intensification. Based on the previously proposed mechanism of depolymerization, it is evident that strong Bronsted acidic catalysts are required to break the glycosidic bonds responsible for the strength of the cellulose polymer. Accordingly, a variety of Bronsted acid catalysts were selected including amberlyst 15, para-toluen-sulfonic acid and moreover some Lewis acid species, such as kaolinite and $AlPO_4$. In this way it is possible to compare catalysts that own different acid strengths and different physical features, in order to understand the properties that lead to the best depolymerization rate. The today state of the art of the cellulose depolymerization assisted by mechanical forces, suggest acidulate cellulose as one of the catalytic systems that offers the best activity toward this reaction. Hence acid impregnated cellulose was chosen as benchmark against which compare the performances of the solid acids selected in this study. Obviously, in order to have a correct comparison, acidulate cellulose, amberlyst 15 and p-TSA were used in such amount to have the same number of acid sites. Due to it is impossible to calculate the catalytic sites of kaolinite an equal amount

of cellulose and that clay was charged in the milling resulting in a weight ratio of 1:1. This value was chosen because in literature it is reported that with this ratio a reaction performed at 800rpm gives 80% of WSP in three milling hours[2]. For the same reason when $AlPO_4$ was used as catalyst it was charged in a weight ratio equal to 1:1. Thus, to compare the catalysts performances, the amount of the water soluble products obtained with different solid acids is plotted in function of the milling time and, since glucose is the preferable wanted product for the following industrial valorization and cellobiose its dimer, that could be easily split under hydrolysis, interesting conclusions on the catalyst performances could be effected by comparison of their yields. Moreover, thanks to the ESI-MS analysis, it is possible to determine the oligosaccharides distribution arisen from each milling process, and thus have a roughly idea of the selectivity features of the catalysts tested.

SOLID ACID CATALYSTS TESTED
HCl Acidulate Cellulose (benchmark)
Amberlyst 15 anhydrous form
para-toluen sulfonic acid
Kaolinite
$AlPO_4$

Table 3.5 List of the solid acid under investigation.

First of all pure cellulose in total absence of any catalyst was milled for twenty hours to verify if just the mechanical forces exerted in neutral environment are able to induce some depolymerization effects, but not soluble products were detected both with HPLC-ELSD and ESI-MS analysis. This confirms also the total inert feature of the grinding media, suggesting that acid sites are essential to shorten down the saccharide chains. Thus the depolymerization tests were performed introducing in the planetary ball mill the cellulosic substrate with one catalyst per time, obtaining the following WSP conversion and sugars yields at different milling times.

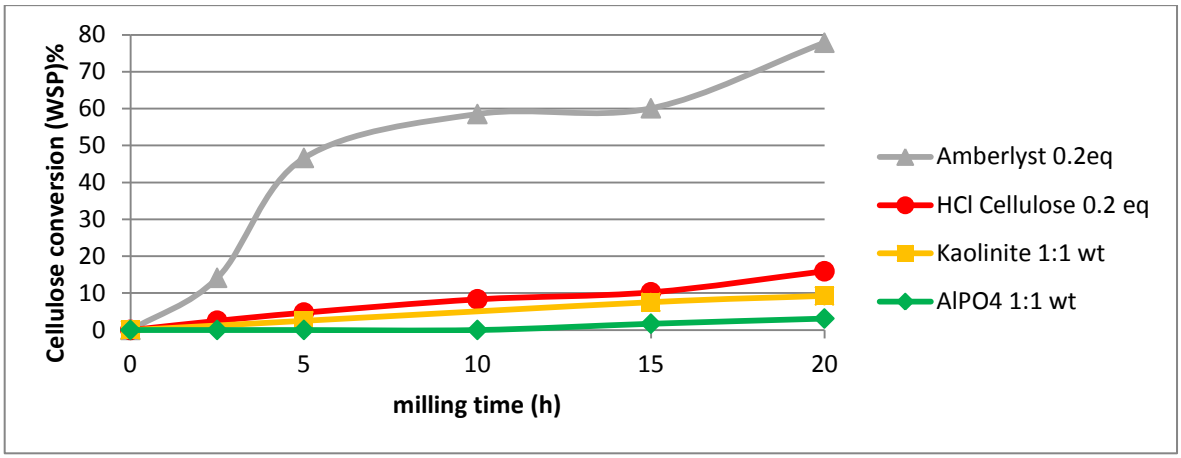


Figure 3.30 Water soluble products amount obtained from the milling of cellulose with the solid acids tested in function of the grinding time. Each reaction was performed in planetary ball mill at 500rpm.

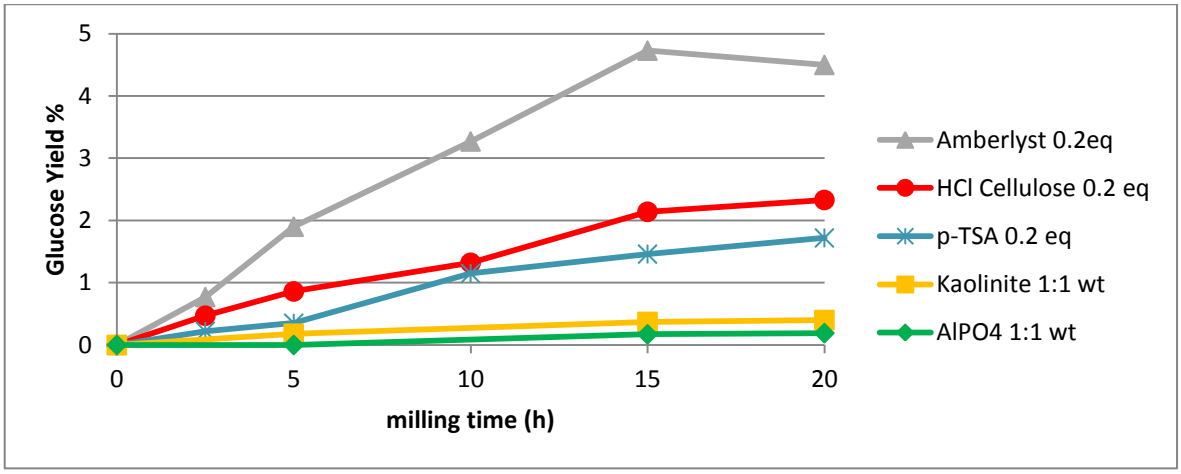


Figure 3.31 Glucose yielded from the milling of cellulose with the solid acids tested in function of the grinding time. Each reaction was performed in planetary ball mill at 500rpm.

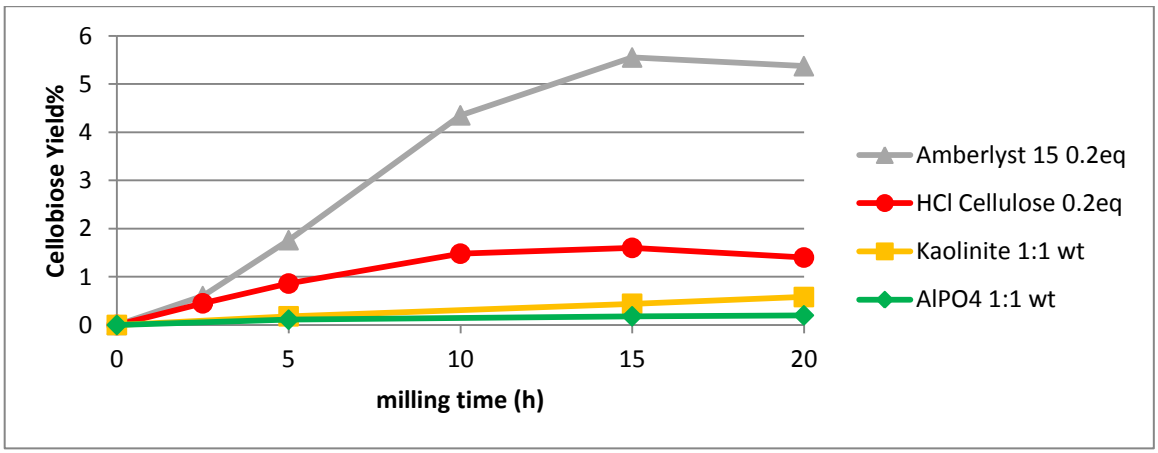


Figure 3.32 Cellobiose yielded from the milling of cellulose with the solid acids tested in function of the grinding time. Each reaction was performed in planetary ball mill at 500rpm.

Prior to studying any solid catalysts, the system optimized in this work was first used to benchmark the performance of acidulate cellulose, the state of the art homogeneous catalyst for cellulose depolymerisation as reported by Rinaldi et al[1]. As can be seen from Figure 3.30, over the course of the reaction, approximately 20 % of WSP were detected, following milling for 20 h at 500 rpm. When compared to the literature, it is clear that the system optimized here produces far fewer WSP than the previous Rinaldi system. Since it has been reported that the yield of WSP increases exponentially with grinding speed, we hypothesize that our lower results are due to the lower maximum grinding speed of the Retsch PM100 model. Despite obtaining lower rates of depolymerisation, the result still provides a useful benchmark against which the solid catalysts tested in this work can be compared. As can be seen in Figure 3.30, the various catalysts displayed various levels of activity. Kaolinite shows slightly less activity than acidulate cellulose due that it produces 9% of WSP if grinded 20 hours. $AlPO_4$ yields in only 3% of WSP in 20 hours of milling, not resulting a good catalyst toward the depolymerization reaction. Despite the low yield produced by this last solid, is still an important result because means that it own some activity features. Indeed must be remembered that the milling of pure cellulose for twenty hours doesn't conduce in any product. On the other hand amberlyst 15 is able to heavy depolimerize the cellulosic substrate, revealing itself the best catalytic system among those ones studied. Indeed when it was milled for 20 hours with microcrystalline cellulose it yielded 78% of WSP. In this way the acid polymeric resin shows a remarkable activity toward the reaction studied. In particular using the same number of acid equivalents, amberlyst was able to produce in only 2.5 hours more than three times of the conversion that arose with acidulate cellulose in 20 hours of grinding. Unfortunately the WSP amount when p-TSA was tested cannot be determined due that under the MS peak used for this determination include other unknown species that induce an overrating error, making not acceptable the conversion value obtained for a correct comparison. Observing Figure 3.31 could be affirmed that the catalyst activity series arisen toward the glucose production perfectly follow the ones obtained in term of WSP conversion. Ambelryst 15 was the solid acid more efficient to convert cellulose in glucose. HCl impregnated cellulose was active to produce glucose but significant differences arise if compared to amberlyst 15. In particular, with 20 hours of grinding, the polymeric resin has yielded 4.5% of glucose whereas acidulate cellulose only 2.5%, almost the double value. Thanks to the HPLC-ELSD method used to quantify glucose, it was possible to determine the p-TSA activity

toward the sugar production. Thus 1,7% of glucose was yielded in 20 milling hours, revealing p-TSA slightly less performing than the impregnated cellulose. Because the activity trend resulted in terms of glucose yield is quite similar to that one of WSP could be expected that p-TSA is placed between impregnated cellulose and kaolinite also in terms of conversion. A visual analysis, comparing the amount of unconverted cellulose, remained at the bottom of the centrifuge tube, to the total solid quantity inserted in the milling system, could confirm this last point because the level of the powder doesn't show a significant decrease meaning that the WSP yield could be almost 15%, an intermediate conversion value between the ones obtained with HCl cellulose and kaolinite in 20 hours of milling time. Indeed also toward the glucose production kaolinite has shown a really low activity, 0.5% with the longest grinding time, slightly higher than $AlPO_4$ but lower than acidulate cellulose, perfectly agreeing the catalytic activity trend arisen toward the WSP. It is important indicate the low glucose yield produced by $AlPO_4$, 0.19% because confirm the catalytic behavior of this substance, since the just milling treatment of cellulose isn't able to generate any sugar product. Studying the cellobiose yield against the milling time, Figure 3.32, can be deducted the same activity trend arisen toward glucose and WSP yields. Unfortunately also in this case, the p-TSA trend cannot be traced because in the chromatogram resulted from the HPLC-ELSD analysis cellobiose and p-TSA peaks are overlapped and so the disaccharide concentration cannot be determined.

A further comparison of the catalytic performances of the solid acids tested could be performed thanks to the spectrums resulted from the ESI-MS analysis. Indeed the peaks intensity of the mass-spectrum, at every m/z value, are proportional to the ions amount produced and so direct correlated with the analytes injected. Hence, thanks to this technique, it is possible to understand the distribution of the sugar oligosaccharides produced by the depolymerization reaction occurred in the milling device and thus, have a roughly idea on the selectivity features of the solid acids tested. For every catalyst was chosen to report only the spectrums of the most significant samples, useful to deduce some information and to make performances comparison.

- HCl impregnated cellulose

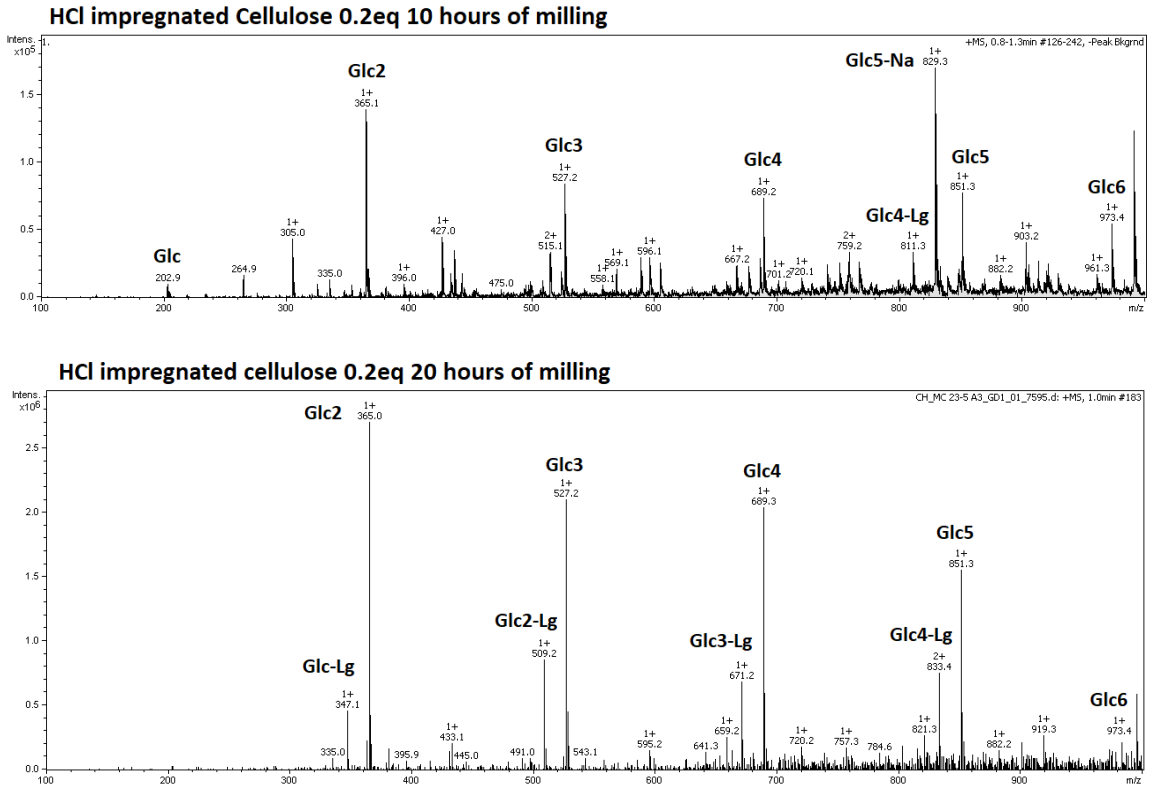


Figure 3.33 Mass-spectrums of sample obtained from the milling of acidulated cellulose in different milling times.

Despite the complex products distribution resulted from the acidulate cellulose milling, shown in Figure 3.33, it is clear that the major products of the depolymerization reaction are the sugar oligomers constituted by only anhydrous glucosan units. Indeed comparing the ion intensities could be concluded that the copolymer, including one levoglucosan units, are always present in lower quantities than their correspondents omopolymers, constituted only by glucosyl units. This means that if some dehydration reactions occur inside the mill they are not so frequent as the hydrolysis process, and obviously occur randomly. Furthermore were detected lot of unknown species, more evident at shorter milling time due to the lower production of sugar oligomers. Indeed for milling time minor than ten hours is not worth to plot the mass-spectrums because the polysaccharides yields are so low that it is almost impossible to distinguish their peaks from other compounds. These unknown species could be attributed to some unselective fragmentations of the cellulosic chains and this suggests the not perfect selective behavior of the acidulate system toward the glycosidic bond cleavage. Nonetheless the main and highest peaks that were identified were referred to some polysaccharides. This represents the first important observation that could be performed on the products

distribution. Indeed this mean that if the milling process produces some fragments of the sugar oligomers structure, it occurs in a minor extend than the depolymerization process itself. In other words the acid milling process is more selective toward the breaking of the glycosidic bonds than the fragmentation of other kind of linkages in the saccharide structure. This could be explained considering the presence of the solid acids inside the mill that drive the system toward the hydrolysis of the glycosidic bonds rather than other kind of random breakages. Indeed must be considered that acid presence is mandatory for the production of some soluble oligomers, due that just mechanical forces are unable to fragment so far the cellulose substrate. Despite the fragmentation process, that could lead in some not complete sugar oligomers, it is important to recognize that species lighter than glucose weren't detected. This means that the fragmentation process, that probably occurs, splits oligomers only in structures heavier than glucose and, more importantly, that the milling process combined with the acid conditions is unable to decompose the sugar structure of glucose. If the milling time of acidulate cellulose is extended it conducts in yields enhancement of the longer oligomers and must be noted that also the ion intensities of the unknown species increase, but in lower amount compared to the oligomers. This could be explained considering that once the protons on the cellulosic frame have broken its structure, though in unclear species, have produced tinier particles and molecules that result more free to move. Thus, thanks to the milling motion, the acidulate particles can approach others, becoming more selective toward the glycosidic linkages.

- Amberlyst 15

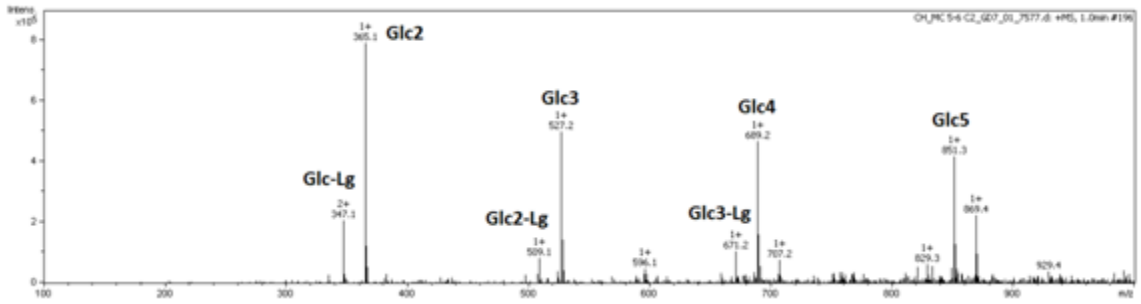


Figure 3.34 Mass-spectrums of a sample obtained from the milling of cellulose and 0.2 equivalents of amberlyst 15 in 10 hours of milling time.

For amberlyst 15 was chosen to report the spectrum of the sample milled 10 hours, showed in Figure 3.34, because in the intermediate milling time are present sugars

oligomers in a suitable relative ratio to make good a comparison with the others catalytic species tested. Indeed can be foretold that increasing the milling time the longer saccharides reach the same peak intensity of cellobiose due to the depolymerization mechanism characteristic of the cellulosic substrate. Nonetheless this topic is deeply discussed in the following "Study of the depolymerization mechanism" paragraph. Due that the only few unknown peaks detected have really low intensities, compared to the main products, can be affirmed that amberlyst 15 own more selective behavior toward the depolymerization reaction than acidulate cellulose, preferring the cleavage of the glycosidic bonds rather than the approaching to other kind of linkages. This could reside in the physical difference of the two catalytic system. In HCl impregnated cellulose, the acid sites are represented by protons dispersed on the cellulose chains and thus are really close to the substrate chemical bonds. Indeed for the tiny dimension of the hydrogen cations could be imagined that they are proper accommodated on the carbon or oxygen atoms of the carbohydrate or strictly enclosed inside the cellulose network. Moreover, for the randomly consequence of the impregnation process, they could be placed everywhere on the cellulosic chains not preferring a special location where settle themselves. Thus when the milling process begin, thanks to the mechanical energies induced, this protons break down the closest bonds available, without interest the chemical nature of them because aren't able to move freely due that are absorbed on the saccharide chains. On the other hand, in the amberlyst resin the acid sites are obviously larger than the protons dimension because supported on a cumbersome polymer matrix. Thus at the grinding begin they aren't closed on the cellulosic substrate because enclosed in a different physical frame. Indeed in this kind of catalysis the mechanical forces are not mandatory only to input the required energies, but also to approach the two solid reactants, what was not necessary for the acidulate cellulose system. Moreover as explained in the "reaction mechanism" chapter this mechanical forces are able to activate the closed cellulose structure in a more open shape where the glycosidic bonds are more exposed at the external reaction environment. In this way the amberlyst acid sites, having to approach the cellulosic chains prefer to move toward the bonds more accessible like the glycosidic ones. Indeed, with amberyst 15, the yield of unknown species starts to increase during the last milling hours where the solid resin is already been heavy grinded and thus, it results in a finer molecules that can also approach the ring bonds of the sugar units. For fairness must be considered that during the last whiles of the grinding also the cellulose lattice is been chopped and so more bonds are exposed for unwanted chemical reactions.

Furthermore, comparing the acid strengths, the resin's sites are weaker than the naked protons and so if they appear on a different kind of chemical bonds is more difficult that they could split them. This is confirmed by the fact that if larger amount than 0.2 equivalents of HCl are enclosed on the cellulose easily decompose the carbohydrate through unselective reactions whereas the amount of amberlyst can be increased until 1.5 equivalents showing anyway a selective behavior.

- Para-toluen-sulfonic acid

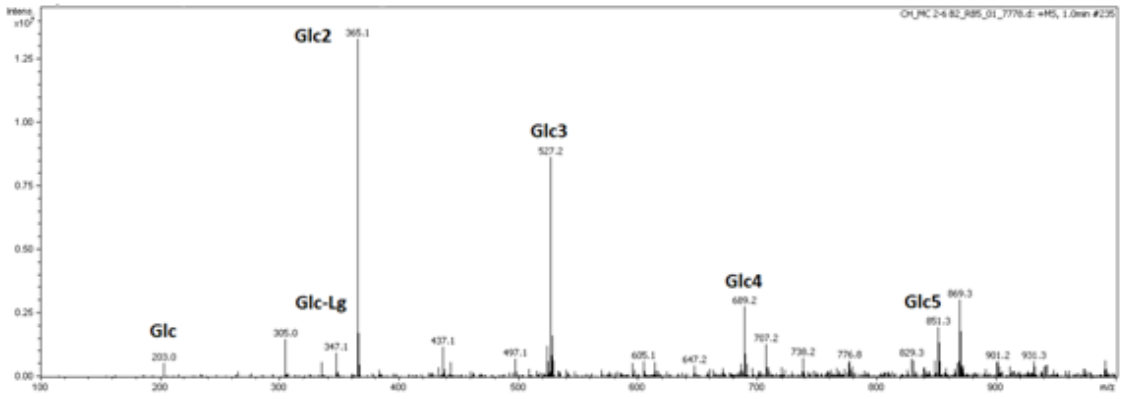


Figure 3.35 Mass-spectrums of a sample obtained from the milling of cellulose and 0.2 equivalents of para-toluen-solfonic acid in 20 hours of milling time.

The spectrum represented in Figure 3.35 suggests that when p-TSA was used as catalyst the yield of the soluble sugars produced decrease with the length increasing of their chains, confirming also for this catalyst the preferred formation of shorter saccharides. Despite this the weaker intensities of the Glc_4 and Glc_5 peaks could be due to the lower overall conversion obtained with this solid acid. Probably if the milling times would extended higher amount of soluble products were obtained and the Glc_4 and Glc_5 oligomer yield increases reaching the values of the shorter saccharides as it occurs with the use of amberlyst. Unfortunately, for the same reason, increasing yields of the longer oligosaccharides in function of the milling time couldn't be verified due that all the spectrums show roughly the same products distribution. Only lower intensities of the cellobiose and Glc_3 peaks were detected. Hence was reported only the spectrum of the longest milling time where greater amount of products can be observed.

- Kaolinite

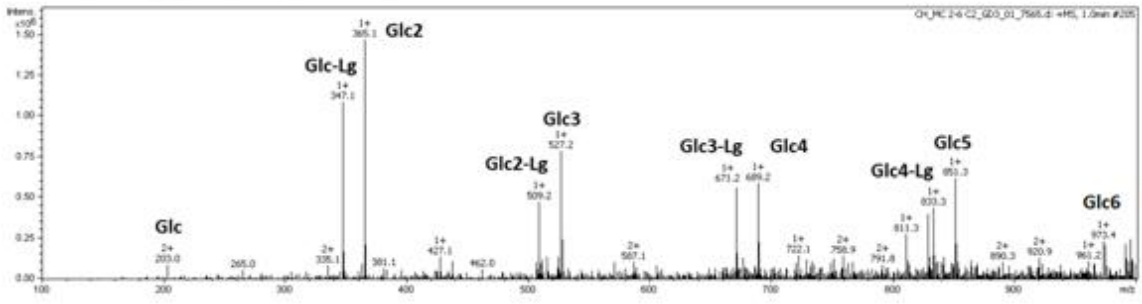


Figure 3.36 Mass-spectrums of a sample obtained from the milling of cellulose and kaolinite in weight ratio of 1:1 in 20 hours of milling time.

When kaolinite was used as catalyst the production of oligomers is so poor that a only the spectrum of the sample milled twenty hours results useful for some conclusions, and thus is reported in Figure 3.36. With this solid catalyst the cellobiose yield seems higher than the ones of the longer oligosaccharides probably because, due to the poor activity of the clay, longer milling times are required for a homogeneous products distribution like the one resulted from the reaction with amberlyst 15. A interestingly feature of this catalyst is that it appears the more likely to induce dehydration reactions and thus produce more levoglucosan copolymers than every other solid tested, though the copolymers yield is never higher than the omopolymers ones. This is an important observation because means that the dehydration reaction of the glucose units is not a intrinsic behavior of the milling process but depend on the acid solid introduced. Moreover it is worth to mention that in all the spectrums obtained were detected only copolymers with no more than one levoglucosan unit. Probably this is due to a statistic reason due that, in every circumstance studied, the glucose oligomers amount is always higher than the copolymer already formed.

- Aluminum phosphate

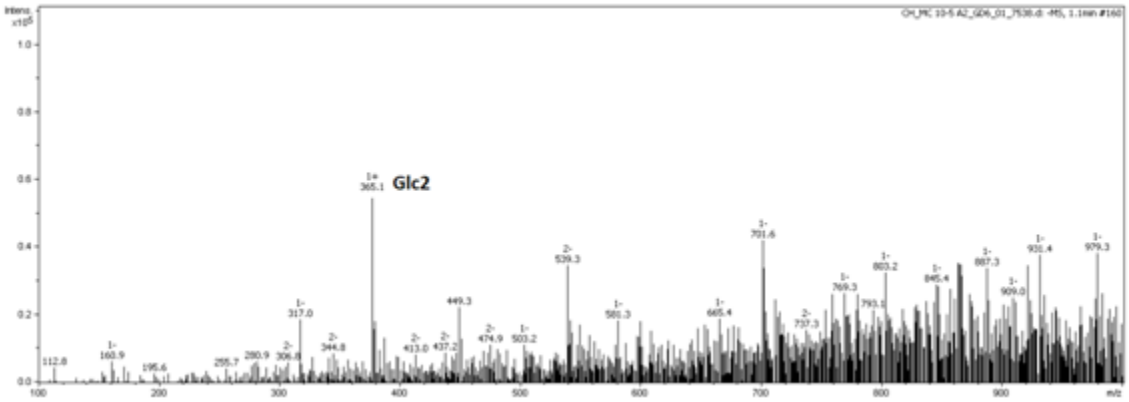


Figure 3.37 Mass-spectrums of a sample obtained from the milling of cellulose and $AlPO_4$ in weight ratio of 1:1 in 20 hours of milling time.

As a confirm to the low activity that $AlPO_4$ shows toward the depolimerization reaction, when it is milled with cellulose for 20 hours the only one saccharide that could be identified is cellobiose. In this way the mass-spectrum illustrated in Figure 3.37 confirms the presence of this dimer as yet found with the HPLC-ELSD analysis. All the others peaks at major m/z values are attributed to unknown species or to instrumental background noise due that their intensities are really low.

3.3.1 Effect of the catalyst content on the depolymerization

Despite the strong activity shown by amberlyst 15 total conversions in WSP weren't still obtained. Hence the catalytic amount of the resin was increased until 0.8 equivalents to understand if, enhancing the number of acid sites present inside the milling system, higher depolymerization rates can be achieved. Thanks to this reagents ratio 100% of WSP were obtained in 15 milling hours against the 60% of conversion yielded with 0.2 equivalents. Acknowledging the beneficial effect due to the acid sites increment, major quantities of amberlyst 15 were introduced in the bowl until achieving the value of 1.5 equivalents. This greater amount of acid resin have enhanced again the depolymerization rate, especially during the intermediate milling times, but in a lower contribution compared to when the acid sites were increased from 0.2 to 0.8 equivalents.

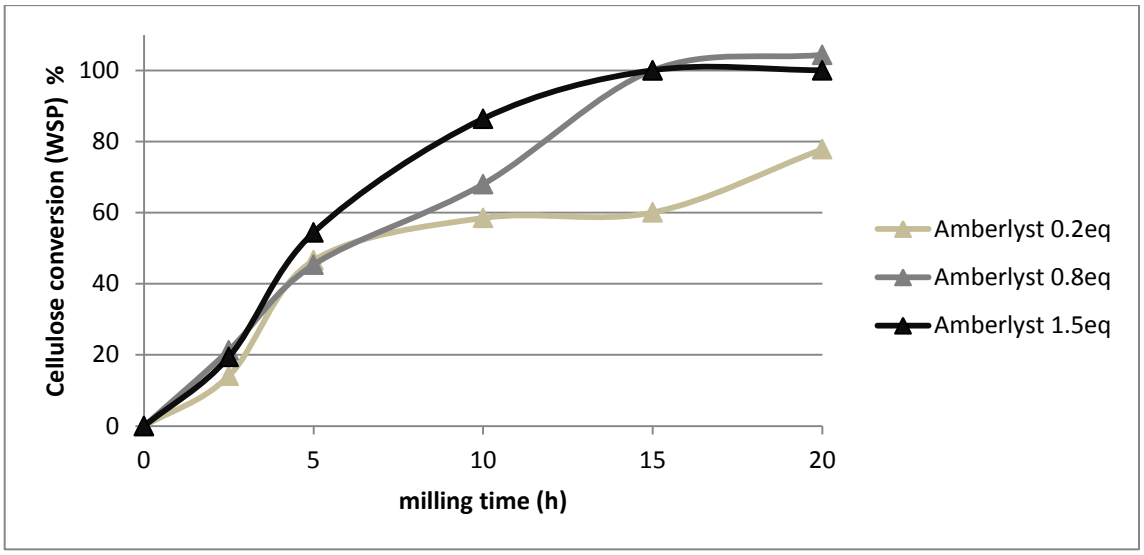


Figure 3.38 Water soluble products amount obtained from the milling of cellulose with different equivalents of amberlyst 15 in function of the grinding time. Each reaction was performed in the planetary ball mill at 500rpm.

In Figure 3.38 it is clear that the WSP conversion is greater when higher quantities of amberlyst 15 are introduced in the system, as theoretically expected because the number of acid sites increase. Unfortunately this phenomena cannot be confirmed increasing the acid equivalents of the acidulate cellulose because greater amounts than 0.2 of acid equivalents conduce in unselective side reactions that don't permit to obtain reliable conversion values. The begnign effect of the acid sites improvement on the studied reaction can be also demonstrated in term of glucose and cellobiose yield as reported in Figeue 3.39 and Figure 3.40.

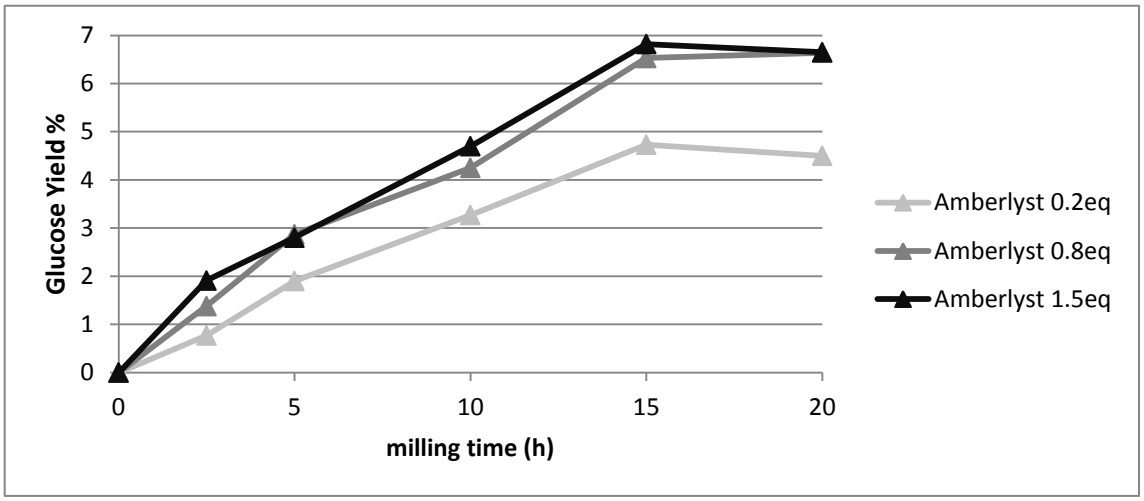


Figure 3.40 Glucose yielded from the milling of cellulose with different equivalents of amberlyst 15 in function of the grinding time. Each reaction was performed in the planetary ball mill at 500rpm.

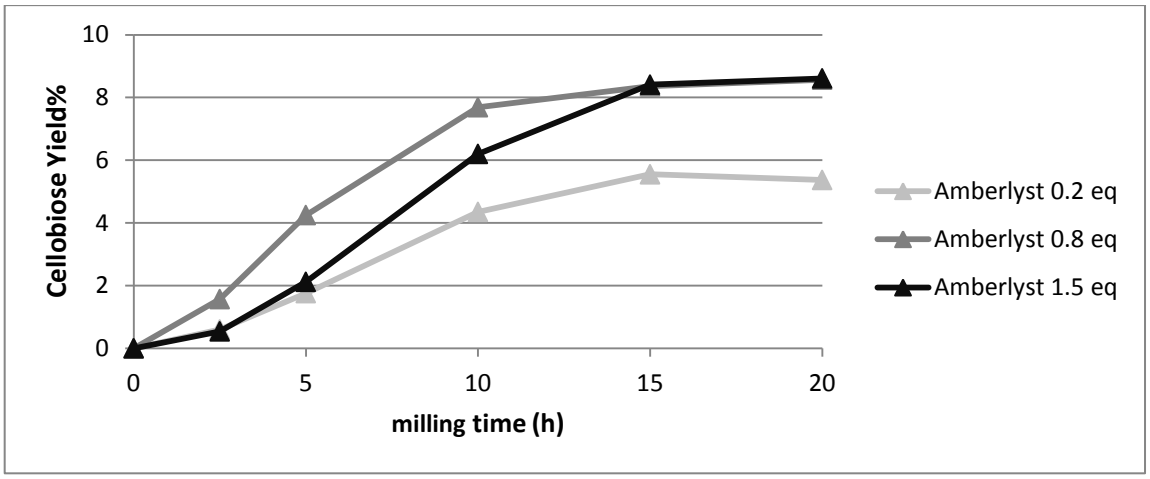


Figure 3.40 Cellobiose yielded from the milling of cellulose with different equivalents of amberlyst 15 in function of the grinding time. Each reaction was performed in the planetary ball mill at 500rpm.

As happen for WSP trends, yields of both sugars, glucose and cellobiose, remarkably increase when higher amount than 0.2 equivalents of amberlyst 15 are utilized. It is worth to mention the unusual higher cellobiose yield that was reached, during the intermediate milling times, when middle equivalents of amberlyst 15 were utilized. Indeed in these circumstances if 1.5 equivalents of acid resin were used lower cellobiose yields were produced compared to when almost the half of the catalyst was charged in the mill. Probably with higher acid conditions the cellobiose yield drops because it is converted in its hydrolysis product glucose; though the increase in glucose yield arisen when 0.8 and 1.5 equivalents of amberlyst were introduced is lower to the decrease that is shown in the cellobiose yield.

Aware of the massive depolymerization rate enhancement, occurred when the acid sites number inside the milling device was increased, it was considered worth to deeper study this phenomena. With this purpose the yielded WSP amounts were plotted in function of the acid equivalents of amberlyst 15 for each milling time as reported in Figure 3.41

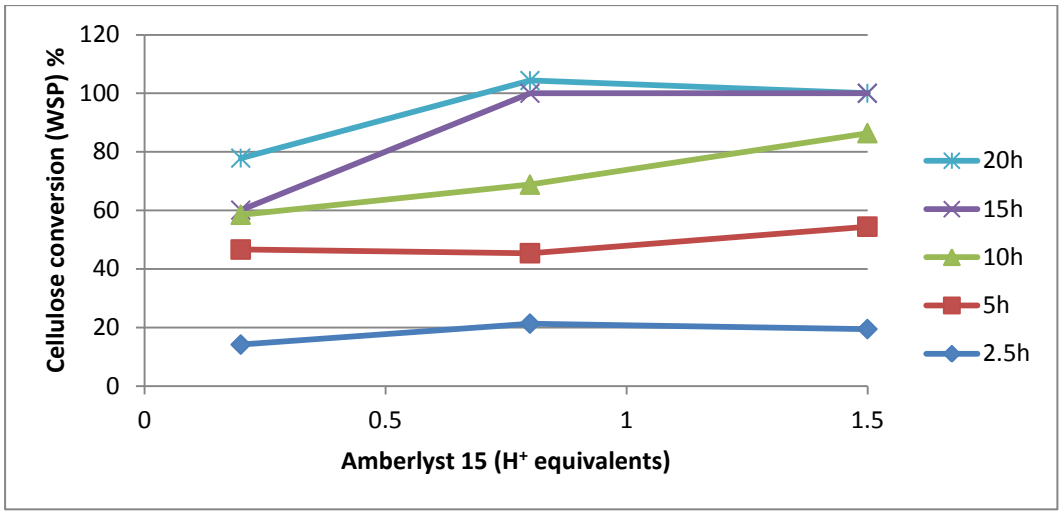


Figure 3.41 Water soluble products amounts plotted in function of the acid equivalents of amberlyst 15 for each milling time. Each reaction was performed in the planetary ball mill at 500rpm.

As already mentioned the cellulose depolymerization improvement with the acid sites increase is evident. Especially, the gain in WSP formation is larger when the acid content is enhanced from 0.2 to 0.8 equivalents rather than when the acidity is extended to 1.5equivalents. This means that the depolymerization extent cannot be more improved when an optimum of acid condition is reached. Furthermore this conversion increment in function of the acid condition is more apparent with higher milling times. Indeed as can be seen from Figure 3.41 the slope of the WSP trend as function of the environment acidity becomes steeper at higher milling times until when value of 100% of conversion is reached. This could mean that during the first reaction whiles the main effects responsible of the soluble oligomers formation are the mechanical forces exerted on the cellulose substrate whereas while the depolymerization proceed the average length of the cellulose chains decrease, becoming in this way more likely to undergo to the hydrolysis from the acid sites. Indeed shorter are the oligomers and easer can interact with the solid resin permit a better approach between the reactive sites of the two reagents. Moreover could be expected that the soluble products obtained during the first reaction whiles are only the external fragment of the whole cellulose chains because the inner bonds are enclosed by the remainder polymeric structure. Whereas at higher milling time more glicosidic bonds are ready to be hydrolyzed and in this way are more likely to the environment acidity. Hence could be supposed that at the first milling hours the depolymerization mainly occurs due to the mechanic forces, whereas at higher grinding times the bonds breaking is more due to the acidic hydrolysis. Since plotting the WSP amount in function of the acid equivalents, for each milling time, has been demonstrated

useful to undarstand different topics of the studied reaction, the same study was performed for the glucose and cellobiose yield, observing the Figures 3.42 and 3.43.

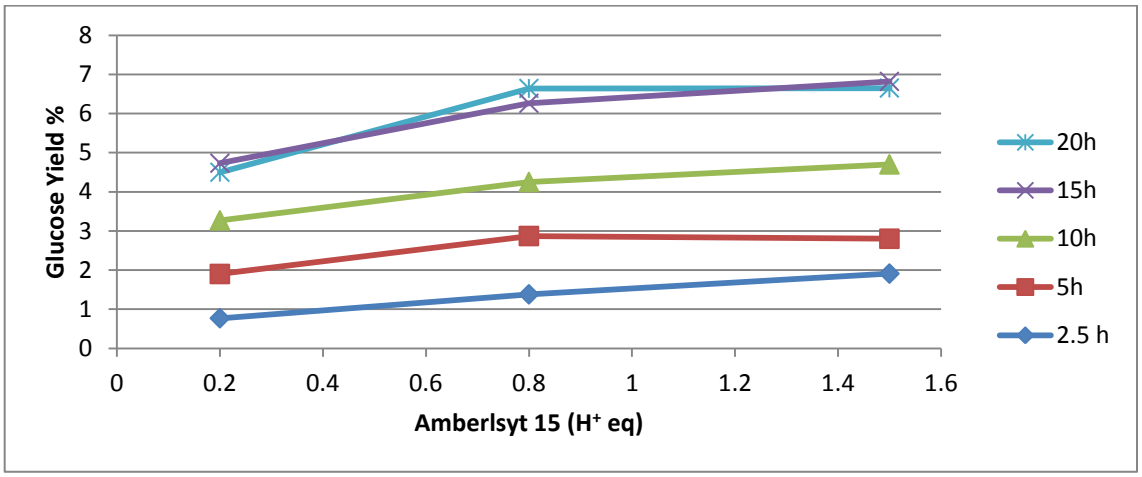


Figure 3.42 Glucose yielded plotted in function of the acid equivalents of amberlyst 15 for each milling time. Each reaction was performed in the planetary ball mill at 500rpm.

From the trends illustrated in Figure 3.42 it is clear that with higher number of equivalents of amberlyst acid sites the glucose yield increases following almost a linear trend, moreover with the same slope conversely to the WSP formation. It is worth to mention the exception observed for the last five hours of milling when a 100% of WSP were obtained, where the glucose yield doesn't increase if the acidity is enhanced from 0.8 to 1.5 equivalents.

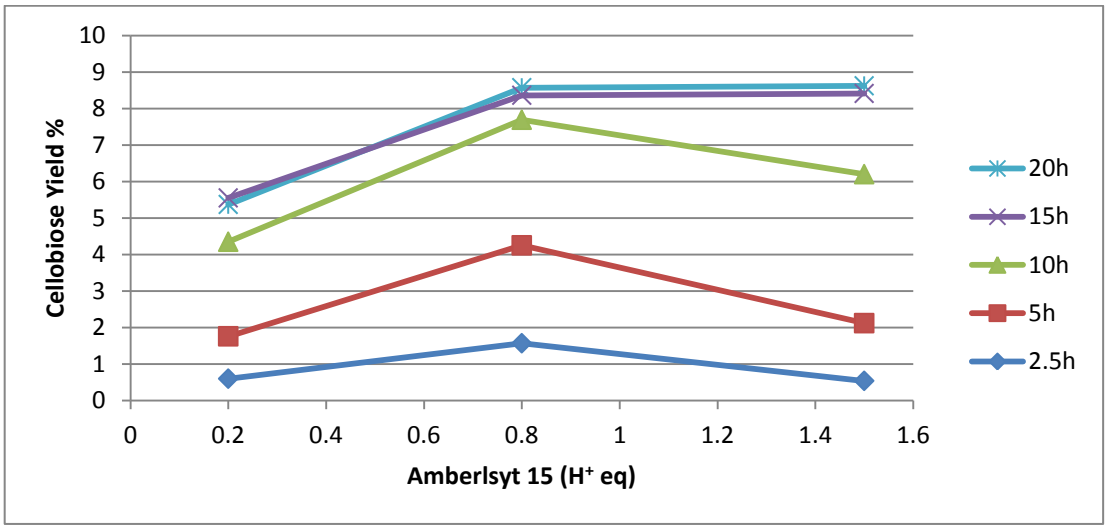


Figure 3.43 Cellobiose yielded plotted in function of the acid equivalents of amberlyst 15 for each milling time. Each reaction was performed in the planetary ball mill at 500rpm.

On the other hand cellobiose yield reaches a maximum value for intermediate acidity conditions but is remarkable that, for each milling time, the rate how the cellobiose yield increases or decreases as a function of the equivalents number is almost constant. Both

for glucose and cellobiose, their yield trends in function of the acid equivalent number at 15 and 20 hours could be perfectly overlapped, if the experimental error is considered. This means that the increment of yield due to the more acid environment is not a function of the milling time. If the milling time is correlated with the average length of the sugar chains, because it can be supposed that more the cellulosic substrate is grinded and more undergo in bonds breakages, the following and resulting conclusion is that the improving of the sugars yields with the enhancing of the acid sites is not dependent on the length of the saccharide polymers that suffer the depolymerization reaction. This is a further confirm that the first AGUs that undergo to the hydrolysis reactions, toward the glucose or cellobiose formation, are the more external of the sugar macromolecule and so, all the inner sugar units, don't suffer the increasing of the environment acidity. It is worth to remember that also with HCl impregnated cellulose with lower amount than 0.2 of acid equivalent both glucose and cellobiose yield decrease. Thus also with different catalytic system if the number of acid sites are lowered the performances worsen as a confirm that they are extremely important for the depolymerization development. It is important to remember that when higher amounts than 0.2 of acid equivalents were introduced as acidulate cellulose, glucose and cellobiose yields decrease due to the occurring of some side reactions and so, depending on the type of the catalytic system, an optimum of the environment acidity could be present and must be determined in order to have the better depolymerization rate.

Thanks to the ESI-MS analysis could be also studied the products distribution depending on the number of acid sites insert in the milling device, or rather if different amounts of acid sites influence the length of the oligomers produced by the depolymerization reaction. Thus it was chosen to plot in Figure 3.44 the mass-spectrums of samples grinded 10 hours where intermediate yields of glucosan oligomers were present in order to have a better comparison.

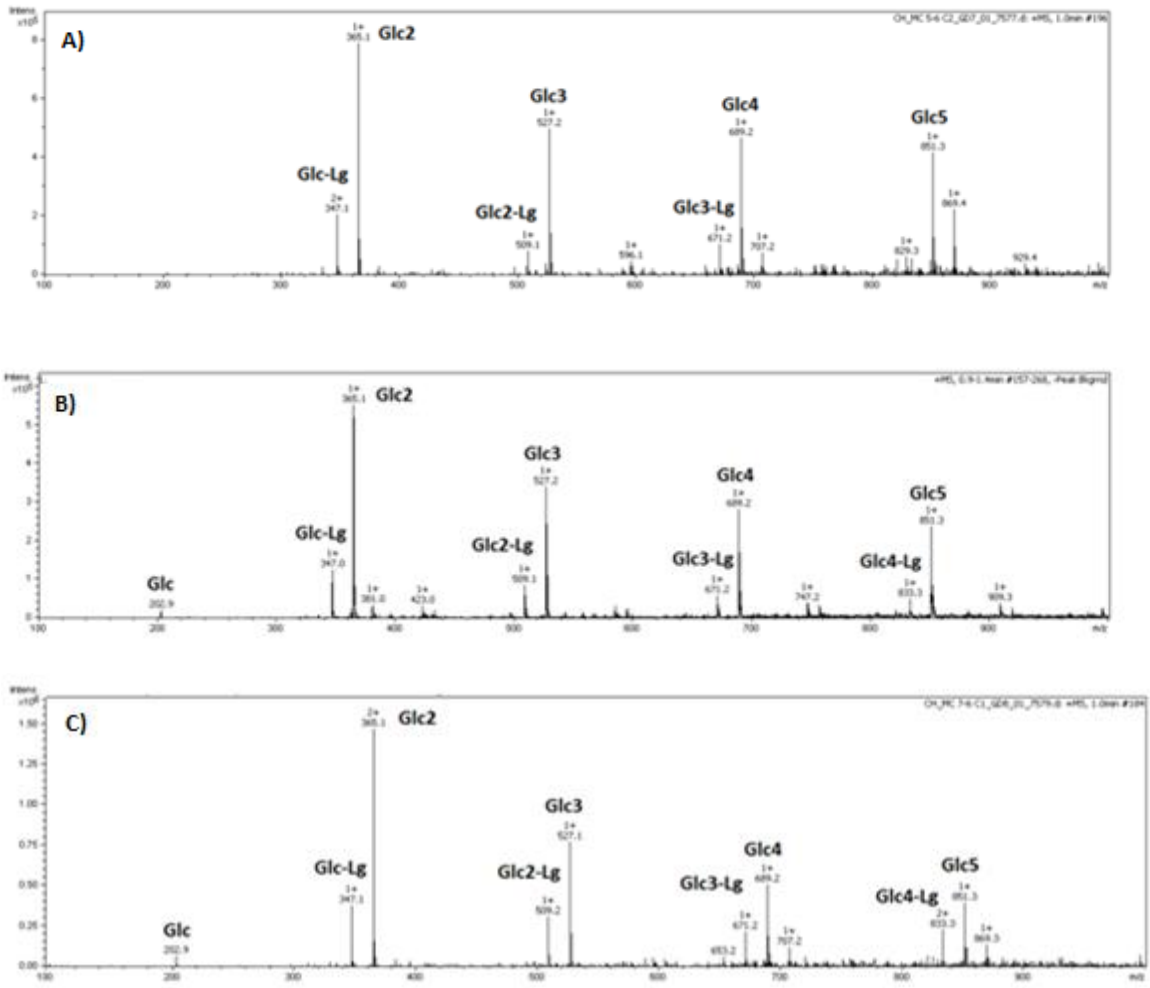


Figure 3.44 Mass-spectrums of samples obtained milling cellulose and increasing amounts of amberlyst 15. **A)** 0.2 equivalents. **B)** 0.8 equivalents. **C)** 1.5 equivalents. Each reaction was performed in the planetary ball mill at 500rpm for 10 hours.

Observing the previously spectrums it emerges that the environment acidity doesn't affect the distribution of the oligosaccharides produced due that the height of the peaks related to a particular oligosaccharide doesn't change varying the number of acid sites utilized. This phenomena, that the products distribution is not dependent on the number of acid sites, is a further confirm that the major obstacle that prevent the $Glc_{3\text{-}5}$ oligomers formation during the first whiles of reaction is a physical reason, like the cellulose structure, and not chemical, like the environmental acidic strength. Thus once the inner bonds are exposed to the acid sites of the solid catalyst, they show the same chemical features of the more external ones, due that are chemically identical. Also the ion intensity ratio between the glucosan omopolymers to the levoglucosan copolymers appears constant enhancing the system acidy. This explain that the dehydration process that occurs on some chains inside the mill is not due to the acid sites as supposed.

3.3.2 Study on the depolymerization mechanism

Analyzing the WSP conversion development in function of the milling time, in Figures 3.30 and 3.38, it can be understood the depolymerization mechanism, or rather, which sugar fragments are knock off from the cellulose structure to originate soluble oligosaccharides. With all the solid acids studied, the amount of WSP is proportional to the milling time as obviously expected. In particular all the conversions produced with different catalysts seems to have the same parabolic trend if plotted against the milling time. The not perfectly parabolic increase arisen when ambelryst 15 was used, especially with 0.8 or 0.2 equivalents, must be referred more to some analytical errors rather than to a trend deviation of the conversion values. These parabolic trends suggest that faster depolymerization rates occur at the first reaction whiles whereas conversion increments are more hard to be reached during the last grinding hours. For instance, in Figure 3.38, when 1.5 equivalents of amberlyst 15 were introduced during the first five hours of milling, an increasing of 55% of conversion was obtained, whereas during the last five hours, starting to count that when 100% of WSP was reached, only a 15% of conversion increasing was showed. This trend mustn't reputed commonplace because it means that saccharide chains shorter than five units were produced as the grinding begins, and due that the conversion at that time was not complete, can be assumed that those soluble oligomers were detached from the more external side of the whole cellulosic chains. Indeed if these first soluble chains were enclosed in the inner part of the cellulosic polymer all the rest of the sugar fragments were crushed down in the same way resulting in a total conversion of water soluble products. Furthermore no activation time is observed before the water soluble products formation, confirming again that the first oligosaccharides produced are the more external cellulose fragments, otherwise no initial conversion increment was observed while the cellulose chains were broken down in middle length oligomers and only in subsequently moments, fragmented in smaller soluble oligosaccharides. In this way can be deducted that the depolymerization process doesn't occur homogeneously, but cellulose is casually broken down in different parts of its chains while some smaller oligomers are detached from the external pendants. The rapid increment of depolymerization in the first whiles of the grinding and its gradually decrease until an asymptotic value could be explained considering the hardness and dimension of the cellulose particles. Indeed as the grinding begins the cellulose particles are harder and more rigid due to the whole original length of the polymeric chains and thus are more likely to the mechanical forces exerted on them. On the other hand during

the intermediate or last milling times the chains are yet partially broken down and so are more flexible, more softer and thus less likely to absorb mechanical energies to be transformed in soluble products. This explain why to reach the last conversion points more grinding hours are required. Moreover must be considered that the WSP are softer and stickier than the starting cellulose, thus when their formation occurs, besides of settling themselves on the bowl internal wall, mingle themselves with the reagents and buffer the milling forces leading in a longer required times to complete the reaction. As a confirm of this latter point the impregnated cellulose and kaolinite trends in Figure 3.30 are strictly linear, also at longest milling times, because they produce few amounts of soluble products that don't influence at all the grinding effect.

Comparing the trends of the glucose yield, in Figure 3.40, and the total soluble products conversions, in Figure 3.39 could be also deducted further information on the depolymerization mechanism. When ambelryst 15 was used glucose yield reached an asymptotic value at the same grinding times when also the water soluble products yield become to have a constant value. This probably means that if some depolymerization degree is reached and the substrate is milled again the products distribution doesn't change. For instance when 0.8 and 1.5 equivalents of Amberlyst 15 were used, a total conversion in WSP was obtained in 15 hours of milling and if the grinding process is extended the yield in glucose doesn't increase meaning that the oligomeric chains already formed don't break down again in the sugar monomers but probably are split in some longer oligosaccharides. This can be confirmed by the glucose and WSP yield ratio that remains constant in the last hours of the milling process when 100% of conversion is reached. In Figure 3.42 it is more clear that when 100% of water soluble products are achieved, the yield of glucose doesn't enhance if the milling time is extended, considering that with 0.2 equivalents of amberlyst a full conversion doesn't occur also with 15 or 20 hours of milling. Instead, during the last five grinding hours investigated, only if the amount of acid resin is increased at 0.8 equivalents a 100% value of conversion is reached and the glucose yield remain constant. Also for cellobiose, Figure 3.43, when 100% of WSP are obtained no increment of its yield is registered extending the milling time, probably confirming that its selectivity doesn't change while the depolymerization process occurs. Indeed cellobiose yield neither increases or decreases continuing to grinding the powder when cellulose is full converted in soluble oligosaccharides. Unfortunately, plotting the glucose and cellobiose selectivity as

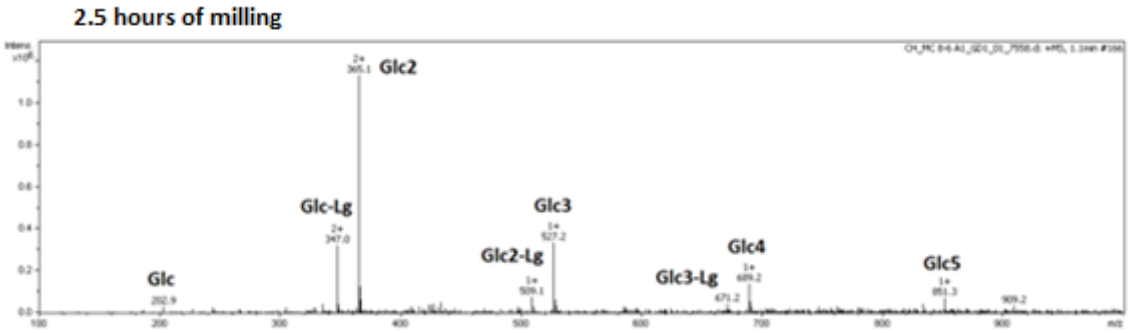
function of the milling time no specific trend is obtained probably due to the high difference of magnitude order between the sugar yield and the relative WSP value that originate fluctuating data. Nonetheless, only considering the values obtained when 100% of WSP are achieved, glucose and cellobiose selectivity are remarkably constant as the Table 3.6 represents.

	Milling time	WSP	Yield %		Selectivity %	
	(h)	%	Glucose	Cellobiose	Glucose	Cellobiose
Amberlyst 15 0.8 eq	15	100	6.5	8.4	6.5	8.4
	20	100	6.6	8.6	6.6	8.6
Amberlyst 15 1.5 eq	15	100	6.8	8.4	6.8	8.4
	20	100	6.7	8.6	6.7	8.6

Table 3.6 Glucose and Cellobiose yields and selectivities of samples 100% water soluble.

This means that probably once the overall amount of cellulose is fragmented in a five units oligomers or shorter, the milling process doesn't affect the products already formed, probably because the saccharide chains are yet too short to undergo to further linkage breaking. Indeed is well-known from the mechanochemistry specialist that if the dimensions of the grinding substrate are to tiny it tempts to aggregate in small agglomerations that avoid the relative huge milling balls.

Other information on the depolymeryzation mechanism can be obtained thanks to the ESI-MS instrument, observing the distribution of the oligomers produced with different times of milling. For this purpose are chosen spectrums of samples milled with 1.5 equivalents of amberlyst 15 because they are the ones that exhibit the stronger depolimerization rate and so their products can better be detected and thus compared.



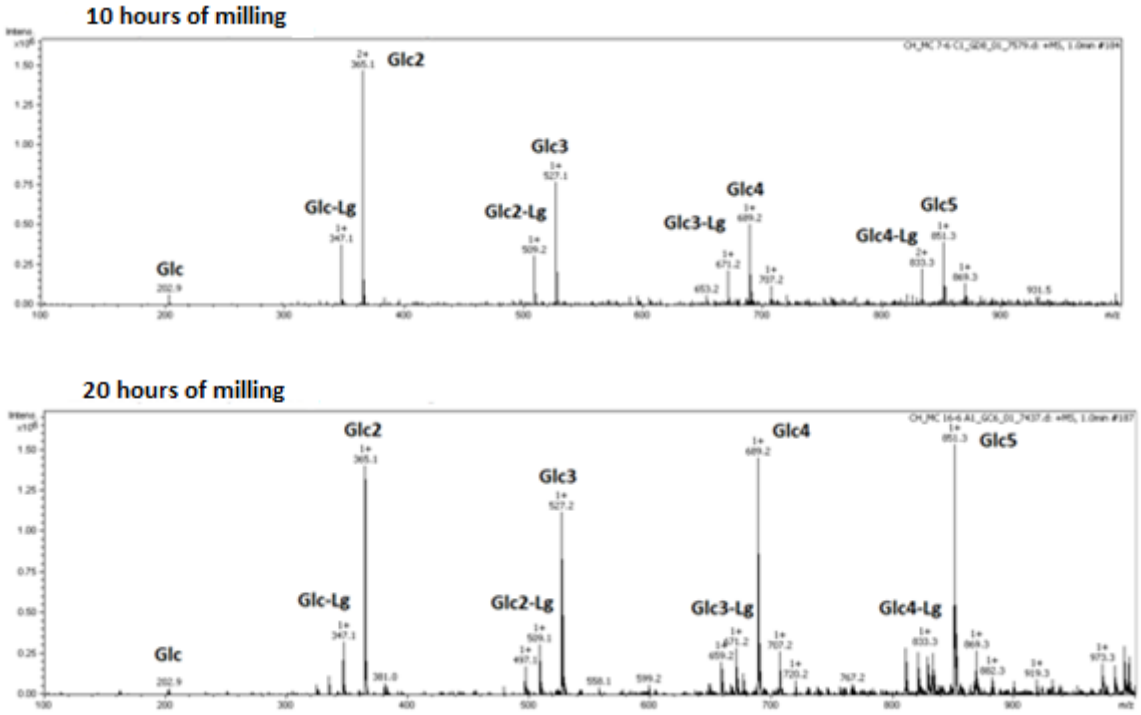


Figure 3.45 Mass-spectrums of samples obtained milling cellulose and 1.5 equivalents of amberlyst 15 for different time of grinding. Each reaction was performed in the planetary ball mill at 500rpm.

Comparing the spectrums in Figure 3.45 it is clear that increasing the milling time the production of bigger oligomers enhance and also the amount of unknown fragments become higher. It is evident that cellobiose starts to forming with the first whiles of the reaction whereas longer chains, for example those constituted by four and five sugar units, are predominantly detached during the longer reaction hours. This is in agree with the theory that more exposed bonds of the cellulosic chains are more likely to undergo to the hydrolysis reactions than the inner ones, as already concluded. Indeed could be expected that the fourth or fifth glycosidic linkages at the beginning of the reaction are enclosed and protected by the cellulose structure itself, whereas the first or second bonds are already set to be depolymerized. With the grinding progress the microcrystalline cellulose lattice is cracked in smaller subunits where also the inner glycosidic links are more exposed to the acid sites of the catalyst and so longer oligomers could be formed. Indeed is remarkable that by comparison of the polysaccharide peaks, with twenty hours of milling all the oligomers seems to reach the same value of intensity. The increasing, and not the decreasing, of the $Glc_{3\text{-}5}$ oligosaccharide intensities, leads in the further conclusion that extension of milling time doesn't induce in a breaking or hydrolysis of the soluble oligosaccharides yet formed but is more likely to crush the bigger cellulose chains. This is a confirm of the theoretical behavior that longer chains more suffer the

mechanical forces due to their harder and more rigid structure. Unfortunately comparison on glucose yields cannot be performed due that is well known that this compound undergoes in some reaction inside the LC column used for the previously separation. On the other hand, the high intensity of the longer polysaccharides detected at longer milling hours suggest the these compounds don't undergo in any reactions inside the column, agreeing with the behavior shown by cellobiose. Others confirms of the depolymerization mechanism described arise if the cellobiose and glucose yield ratio is calculated for each milling time and for each catalyst tested. Indeed considering one solid catalyst and comparing for each milling time its cellobiose-glucose ratio production, appear that the most of the times cellobiose is produced in higher amounts than glucose especially during the last milling hours. Indeed the only few times when the glucose yield is higher than the one of cellobiose are during the first five milling hours. This is in agree with the hypothesis that when the grinding begins the first oligomers detached are the fragments more external of the whole cellulosic chain and in this way the first glycosidic bond is more likely to be broken rather than the second. Whereas, increasing the milling time the cellulosic polymer is already heavy fragmented in shorter chains, though not still enough light to be soluble, but can be better crushed by the grinding balls and the second glycosidic linkage is more accessible by solid acid sites, producing higher amount of cellobiose. Despite the behavior here described the cellobiose-glucose ratio doesn't show a linear increment with the milling time confirming that at longer grinding hours the depolimerization mechanism proceed randomly but producing anyway more cellobiose than glucose. Nonetheless can be concluded that impregnated cellulose, is more likely to produce glucose whereas higher yields in cellobiose are favorite if ambelryst 15, kaolinite or $AlPO_4$ were used. No significant trends result plotting the cellobiose-glucose ratio in function of the resin acid equivalents meaning that the acidity doesn't lead in a preferred production of one of the two sugars. This conclusion is in agree with the fact that the chemistry of the first and second glycosidic bond is exactly the same and thus no one of them should be preferred by the acid sites for the hydrolysis reaction.

3.3.3 Study of the effect of the acid sites distribution on a inert support

Thanks to the milling results obtained when amberlyst 15 and p-TSA were used as catalytic system it is possible to draw conclusions on how the distribution of acid sites on a inert support could influence the depolymerization rate. Indeed the functional group responsible of the acid features in these two solid catalysts, is the same toluene-sulfonic group, thus could be expected that the acid force owned by the two system should be almost equal if introduced in the same equivalents number. Conversely the polymeric resin shows an activity, in term of glucose production, more than four times greater than the organic acid. Also in terms of WSP, determined by visual analysis, ambelryst 15 is remarkably more active than p-TSA due that in 15 milling hours totally depolimerize cellulose whereas with the organic acid all the cellulose charged remains on the bottom of the centrifuge tube. Hence can be understood that not only the amount of acid sites with their intensity introduced in the system is the reason of the catalyst activity but more physical features must be considered. The main physical difference between amberlyst 15 and p-TSA is that on the polymeric resin the acid sites are distributed among a cross-linked network of carbonaceous chains while the organic acid could be considered as a pure toluen-sulfonic groups in contact with the cellulosic substrate. Thus can be deducted that one further parameter important for the solid catalysis is the distribution of the acid site on the solid acid. The benign distribution effect appears also when kaolinite and $AlPO_4$ activities are compared. Kaolinite is a clay where the Lewis acid sites are represented by the aluminum atoms that are coordinate in a octahedral structures supported by tetrahedral silicon structures, whereas in $AlPO_4$ each aluminum atom is just coordinated with a phosphate counteranion forming a framework structure. Moreover, due that kaolinite and $AlPO_4$ were both inserted in weight ratio with cellulose equal to 1:1, the aluminum acid sites are in a greater amount in the presence of the salts rather than when the clay was used due to the presence, in this latter case, also of silicon structures. This means that the distribution of the acid sites is a parameter more important for the depolymerization reaction than the acid sites quantity itself. Furthermore exactly the distribution of the acid sites on an inert support, like in the case of amberlyst 15 the styrenic polymer, has permitted to increase the amount of acid equivalents inside the milling device, making possible, the previously study of the dependence of the depolymerization rate on the acid sites number. Indeed must be remembered that when greater amounts than 0.2 of acid equivalents were present on the cellulosic material

produce an heavy decomposition of the substrate that leads in a side production of carbonaceous compounds. This result shows again the beneficial behavior of the acid sites when they are distributed on a support as in the polymeric resin. Indeed in this special conformation also if the number of acid sites are increased don't induce in any decomposition process because they are diluted in a matrix such, in this case, the styrenic chains. In this way also if they are present in higher quantities the distribution and dilution effect prevent the possibility to overcome the energy barrier toward the decomposition compounds, stopping instead the reaction at the depolymerization products. Whereas with acidulated cellulose the acid sites are so closed to the substrate linkages that, if are present in larger amounts, result in too high concentration that produce undesired side products.

3.4 STUDY OF THE RACTION USING THE MIXER BALL MILL

Several experiments were conducted also in the mixer ball mill in order to compare the performances of the two different devices. Indeed, as already described in the mechanochemistry chapter, the two mills differ not only on the power that gives kinetic energy to the grinding balls but also how the milling bowls are swung to generate this energies. In particular the vial of the planetary ball mill is rotated around a fixed axis whereas the capsules of the mixer ball mill are swung back and forth.

3.4.1 The grinding of acidulate cellulose inside the mixer ball mill

Due that as catalytic benchmark for the solid acids performances comparison was chosen acidulate cellulose, the first depolymerization test was performed with this kind of catalytic system. With HCl impregnated cellulose with ten hours of milling no soluble products could be detected, after that a slightly amount of soluble products start to forming. Unfortunately, as soon as the sugar production begins, an heavy layer of cellulosic cake starts to attaches at the bottom of the capsules that it shows impossible to be removed. Sometimes some aliquots of that product were managed to be detached but due that the total amount of the power charged cannot be obtained, it is reveal futile because a total conversion cannot be calculated. Indeed the most of the times the sample portion that could be removed was the one unconverted, producing a fake result if analyzed. As a confirm, the couple of times when this sample aliquot was injected, no sugar products were detected. Lot of efforts were made to recuperate that product

converted also with the help of cold and hot water but without any successful attempt. Moreover wet grinding, adding in each capsules three milliliters of water, were conducted thinking that aqueous system is able to prevent the sticking of the products, but in this conditions, after the milling, no sugar was detected. This is a clear evidence that water acts as a buffer component for the mechanical forces exerted on the powder reagents, preventing the depolymerization reaction.

3.4.2 The catalytic activity of amberlyst 15 inside the mixer ball mill

Aware of the poor activity shown by acidulate cellulose in this kind of device and that, in the planetary ball mill, amberlyst 15 was the only catalyst able to produce higher depolymerization rate than the comparison benchmark, this resin was be the succeeding solid acid tested in the mixer ball mill. Fortunately when amberlyst 15 was used the whole powder content charged in the capsules could be removed. Probably the acid solid introduced acts as solid diluent among the cellulose particles and the sugar products formed, preventing in this way their agglomeration in one single sticky layer. In this way the powder products could be discharged and solubilized with the classical solubilization step described.

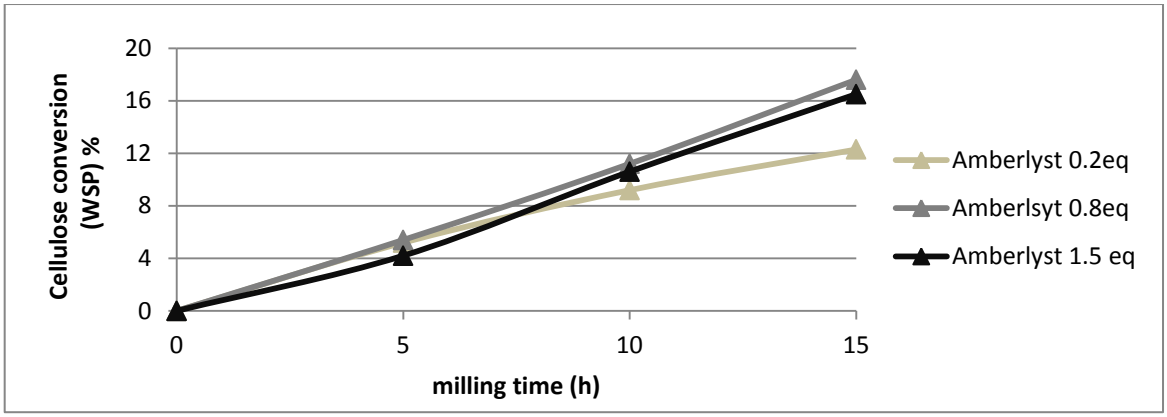


Figure 3.46 Water soluble products amount obtained from the milling of cellulose with different equivalents of amberlyst 15 in function of the grinding time. Each reaction was performed in a mixer ball mill at 30Hz

Despite the low accuracy of the ESI-MS method used for the WSP determination, a linear increment of the reaction conversion, as a function of the milling time, could be detected, as illustrated in Figure 3.46. This is the first good observation because means that also with this device the grinding balls are continuously producing lighter

oligosaccharides and thus that the depolymerization process advances. Moreover the conversion linear increment shows that isn't yet reached an asymptotic value of production, suggesting that if the milling time was extended most likely deeper depolymerization could be obtained.

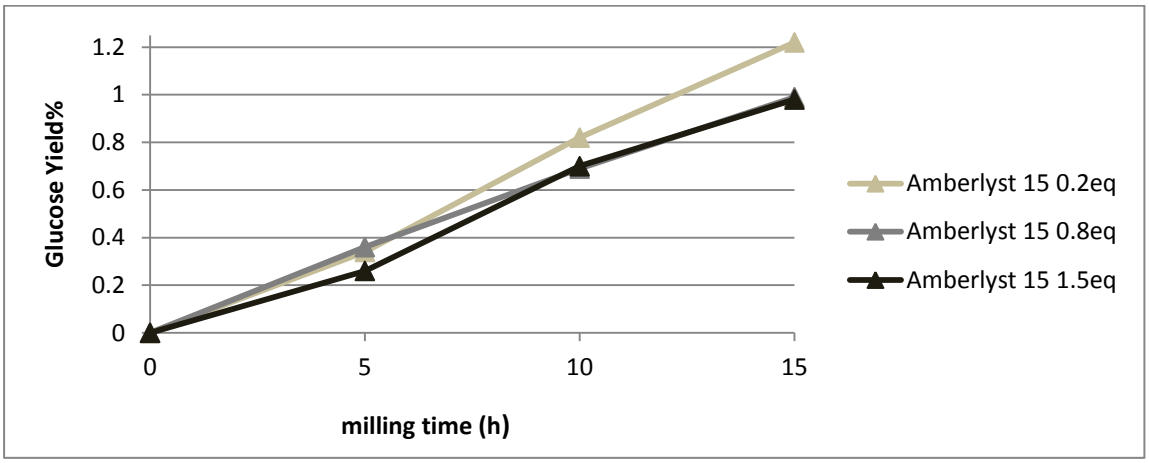


Figure 3.47 Glucose yielded from the milling of cellulose with different equivalents of amberlyst 15 in function of the grinding time. Each reaction was performed in a mixer ball mill at 30Hz

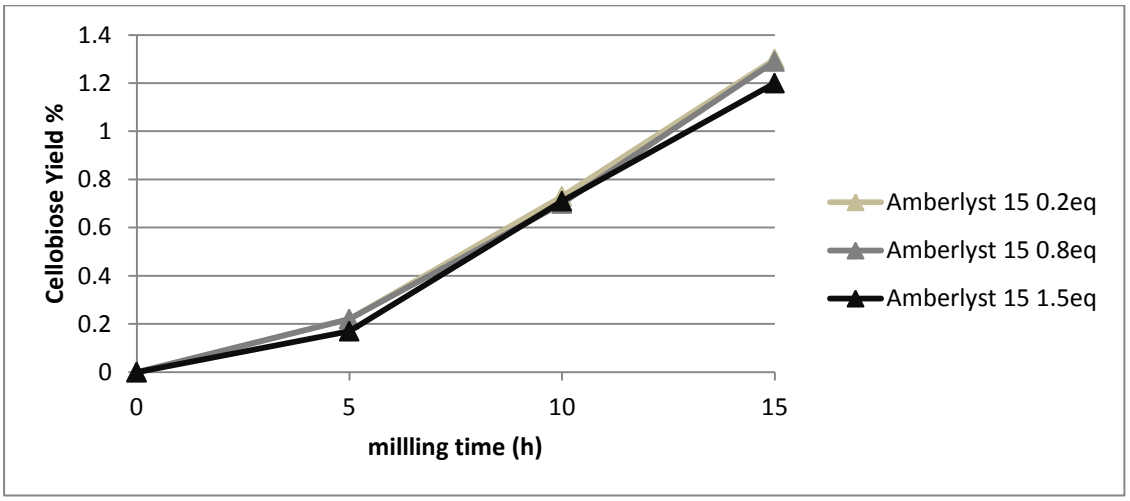


Figure 3.48 Cellobiose yielded from the milling of cellulose with different equivalents of amberlyst 15 in function of the grinding time. Each reaction was performed in a mixer ball mill at 30Hz

Unlike the trends arisen with the planetary ball mill different amounts of catalyst don't involve great increments of the depolymerization rate. Indeed all the three trends could be almost overlapped if the experimental error is considered. This different behavior could be explained assuming that the mixer ball mill has a weaker milling power than planetary ball mill, as could be confirmed comparing the depolymerization rates occurred in the two devices at the same milling time. Thus the weak mechanical forces exerted by

the mixer ball mill are unable not only to depolymerize cellulose but neither to deconstruct the polymer matrix of amberlyst 15. In this way not the overall acid equivalents inserted in the grinding vial result available for approaching the cellulose glycosidic bonds because some of the acid sites are still enclosed inside the resin structure. Thus also if greater amount of catalyst are charged in the mill, the real increment of the acid equivalents is low because the acid sites available for the reaction are the only ones present on the surface of the resin beads. As noticed on the WSP analysis, both glucose and cellobiose yield enhance with the milling time advancing, Figure 3.47 and 3.48. Must be observed that also with this device no activation time is required meaning that, as soon as the grinding begins, glucose and cellulose start to forming. This still confirms the hypothesis that shorter oligomers start to detach with the first milling whiles and no crushing of the whole cellulose chains in shorter oligosaccharides is required before the formation of the shortest sugars. Hence is confirmed that despite the different strength of the milling process the cellulose depolymerization proceed in the same way, suggesting that it is a own behavior of the nature of this reaction and not of the device where it is performed. Interestingly, considering also the experimental error, no advantages arise if greater amount of acid resin are used, both on the glucose both on the cellobiose yield. Nonetheless looking at the glucose production it seems that with higher amount of acid equivalents, the performances worsen. Probably this could be explained considering that the mechanical forces exerted in this system aren't so strong, thus the extra amount of catalyst inserted acts as a solid diluent buffering the grinding effect. On the other hand the cellobiose yield trend is remarkably the same if different amounts of acid resin were introduced. It seems also that the average increment of yield in function of time is the same for the two sugars, meaning that in this case no abetment in terms of sugar productions or bonds breaking is present.

As for study performed in the planetary ball mill important information on the depolymerization reaction and on the grinding treatment could be obtained comparing the mass-spectrums of samples milled with increasing amount of catalyst, reported in Figure 3.49.

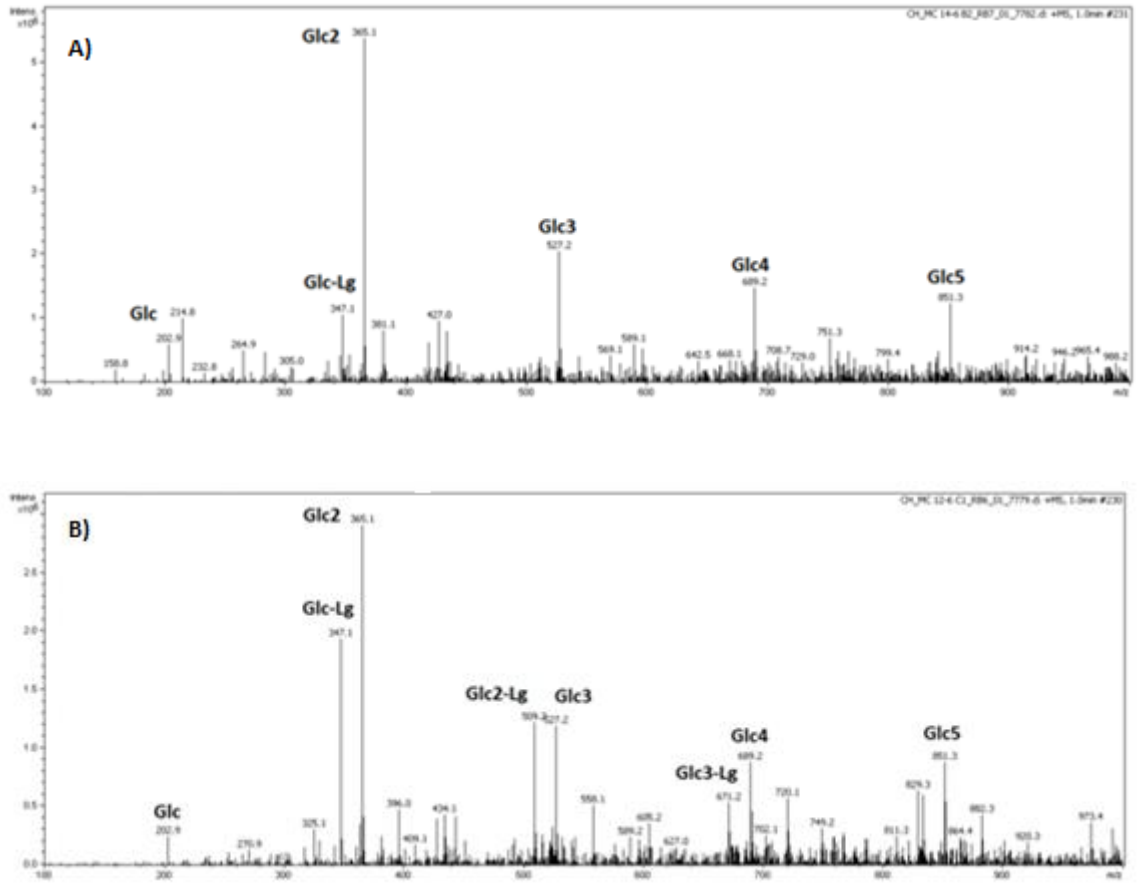


Figure 3.49 Mass-spectrums of samples obtained milling cellulose and increasing amount of amberlyst 15. **A)** 0.2 equivalents. **B)** 1.5 equivalents. Each reaction was performed in the mixer ball mill at 500rpm for 15 hours.

When the depolymerization process was performed in the mixing ball mill, good resolution of the mass-spectrum useful for the product distribution analysis, were obtained only when samples were milled for 15 hours. Indeed, probably for the low amount of the products obtained, in the spectrums of samples milled five and ten hours could be identified only cellobiose and Glc_3 peaks, though with really weak intensities. For this reason it is considered worth to report only the spectrums of samples milled the longest times. Nonetheless this trend still explains the easier depolymerization in shorter saccharides rather in longer sugar chains, confirming also the same deductions drawn examining the glucose and cellobiose yield trends, obtained with the HPLC-ELSD analysis. These results are in agree with the depolymerization mechanism also observed

in the planetary ball mill. Thanks to the mass-spectrums could be examined the production of the longer soluble saccharides, that instead with the HPLC-ELSD analysis cannot be performed. Hence, looking all the oligomers intensities peaks obtained with the longest milling times, the relative products distribution seems remaining constant varying the amounts of catalyst used, agreeing with the sugar yield trends already discussed. Is worth to mention that milling the reactants with this kind of device induce a greater production of fragmented species than in the planetary ball mill, as the major number of unidentified peaks reports. Interestingly, increasing the environment acidity larger amount of levoglucosan copolymer were obtained. For instance with 1.5 equivalents of amberlyst Glc-Lg and Glc_2-Lg intensities peaks are remarkably high, whereas with 0.2 equivalent almost no levoglucosan units appear. Moreover this higher formation of dehydration products wasn't be observed in any reactions conducted in the planetary ball mill. Hence could be affirmed that in this kind of milling the dehydration reaction is influenced by the acid condition of the system.

3.4.3 Other catalyst tested

All the other catalysts tested in the planetary ball mill, such p-TSA, kaolinite and $AlPO_4$ were used to perform solid-solid reaction also in this milling device, but unfortunately, no sugar products were detected also at the highest milling frequencies and longest times, most likely for their less catalytic activity.

3.4.4 Comparison of the milling devices performances

Thanks to the catalytic activity shown by ambelryst 15 in both of the mechanical devices, it is possible to compare their performances in terms of WSP and sugar yield originated. For instance when amberlyst 15 was used as catalyst in the planetary ball mill, 20% of conversion in WSP were arisen in only 2.5 milling hours, whereas with the mixer ball bill this value of conversion isn't reach also with 15 milling hours. However, for clarity, is following reported in Table 3.7 the sugars yields and WSP conversion obtained with the two devices in order to have a easier comparison. Due that with mixer model the depolymerization rate isn't influenced by the quantity of catalyst used, it was chosen to report only the values obtained with 0.2 equivalents of amberlyst 15.

Milling time (h)	WSP %		Glucose Yield %		Cellobiose Yield %	
	Planetary	Mixer	Planetary	Mixer	Planetary	Mixer
5	47	5	1.9	0.3	1.8	0.2
10	59	9	3.3	0.8	4.4	0.7
15	60	12	4.7	1.2	5.6	1.3

Table 3.7 Comparison of the planetary ball mill and mixer ball mill in term of WSP, Glucose and Cellobiose yield.

It is clear that planetary ball mill is the more performing device in every term of comparison, both in WSP production and sugar yields. Indeed must be also remembered that when the less active solid acids were used in the mixer ball mill, such as p-TSA, kaolinite or $AlPO_4$ no one oligosaccharides were produced, most likely for its lower powerful grinding and thus, a catalytic comparison of that species couldn't be performed.

CONCLUSIONS

During the thesis period were conducted several activities with the purpose to develop a reliable experimental methodology that permit to depolymerize cellulose trough the utilization of several solid catalysts. In order to achieve the greater sustainable features of the process the whole work was conducted in absence of any organic solvents neither water. Hence in order to overcome the physical hurdles that arise in this singular solid-solid environment, such as the interaction between the reactants active sites, all the tests were performed in milling devices which mechanical forces, exerted on the starting materials, permit the chemical transformations toward the desired products, thus originating the so called mechanochemistry approach.

Since in nature and on the chemical market are present plenty of different kinds of cellulose and lignocellulosic materials the first study conducted was select the best cellulosic starting material to depolymerize. The selection was focused mainly on microcrystalline and α-cellulose for their homogeneous and secure chemical composition. According to the today state of art, the catalytic system that shows the best activities toward the studied reaction is cellulose impregnated with a electrolytic acid. Thus both kinds of cellulose were impregnated with HCl and grinded the same time in a planetary ball mill. After the milling microcrystalline cellulose has resulted the best starting material to study because has conducted to the more regular products distribution and to the milled mixture easier to handle. Thus the study on α-cellulose was neglected.

With the purpose to understand the performances of the various solid catalysts used, acidulate cellulose was also set as a benchmark to make a comparison. Indeed, as reported in literature, it is able to convert cellulose in 100% of products water soluble if grinded in a planetary ball mill. Since the acid species is a mandatory requirement for the good occurring of the depolymerization reaction, because it was verified that the only mechanical forces are unable to yield in some soluble oligosaccharides, the acid impregnation treatment with which is obtained the acidulate cellulose was previously studied and optimized in order to have the same acid strength on the cellulosic substrate after every impregnation step. Thanks to this study was discovered that acid equivalent number resulted on microcrystalline cellulose depend on:

- The amount of the electrolytic acid on which the cellulosic substrate is treated;
- The duration of which cellulose and the concentrated acid remain in contact.

Moreover it was established that the optimum acid load on the cellulosic substrate correspond to 0.2 equivalents since grater acid conditions lead, after the milling, to unselective reactions that produce carbonaceous undesired products, whereas lower acid amounts yield in less sugar quantities.

Afterward were tuned up the milling parameters in order to achieve the highest depolymerization rate but with the mind of doesn't degrade the starting materials both the compounds produced, since they are really likely to undergo in degradation reactions if the system reaches too high temperatures. Thus it was chosen to perform the milling at the highest powers and speeds that the mechanical devices were able to achieve but introducing grinding breaks to prevent overheating of the reaction system. In this way temperatures higher than 40°C and materials degradations were avoided. Since the moisture content of the selected cellulose was a sufficient water amount to hydrolyze all the glycosidic bonds present in its structure, no extra water quantities were added in the reaction environment due that liquids presence could buffer the reagents impact inside the milling vial. For the same reason and to increase the green approach of the whole developed process neither organic solvents were added in this reaction.

In this way the product resulting from the milling is a powder mixture, therefore was developed a recovery process to obtain compounds in a suitable form for the successive qualitative and quantitative analysis. Thus was conceived a water solubilization step where all the soluble products yielded from the milling were dissolved in a aqueous solution and afterward separated from the solid catalyst and the unconverted cellulose trough a centrifugation process. To verify if the developed recovery procedure is able to hydrolyze some oligosaccharides already produced, thus falsifying the products distribution obtained from the grinding, a cellobiose standard solution was treated in the same conditions of the solubilization step and analyzed with an HPLC-ELSD instrument. No one glucose molecule was detected with this analysis, suggesting that no hydrolysis reaction was occurred, confirming that this methodology is suitable for the purpose prefixed.

Unfortunately today doesn't exist a reliable analytical method to exactly quantify the depolymerization rate of cellulose, due that, for its recalcitrance features, it is insoluble in most of the solvents used by the chemical industries, first of all water. Thus it was chosen to consider a conversion value of the solid-solid reaction the total amount of water soluble products generated from the milling process. Thanks to the ESI-MS analysis was determined that only oligosaccharides until five AGUs are totally soluble in water and thus, though longer oligomers are formed from the depolymerization reaction, cannot be determined neither quantified. Hence with this conversion definition of course an underrating error of the depolymerization rate occurs. This is not important because it could be expected that this determination error arise in equal extend for each milling test. Moreover must be remembered that one of the purposes of this work is to compare catalytic performances of several solid acids, not quantify the depolymerization rate occurred, thus the water soluble products amount originated from the grinding could be considered a suitable value to perform that comparison.

A gravimetric analysis, weighting the total solid material insert in the mill and the successive unreacted and insoluble fraction remained at the bottom of the centrifuge tube, couldn't be exploited for the WSP quantification because it requires a drying process in oven that burn the unconverted compounds, probably due to the presence of some acid residue. Moreover, after the milling, some solid acids show soluble features. Hence this analytical technique implies too many errors that don't permit to obtain a suitable conversion values for the catalysts comparison. Therefore an analytical method to quantify the WSP yield was developed thanks to samples identified as 100% water soluble by visual analysis after the solubilization step. This samples were injected in the ESI-MS instrument that return a chromatogram where all the oligosaccharides produced by the milling process were eluted together in the same retention peak. Nonetheless that peak was integrated for each sample injected to have its area value. Knowing the total amount of cellulose previously insert in the mill, and thus the total amount of AGUs present in the aqueous solution injected, was constructed a calibration line as AGUs concentration against its relative area. Thanks to this calibration was possible to determine the overall WSP amount and thus, the cellulose conversion of every milling reaction with which perform the catalyst performances comparison. Moreover thanks to the HPLC-ELSD analysis also glucose and cellobiose yields could be easily determined, whereas longer oligosaccharides cannot be quantified because pure material of that

compounds couldn't be found on the chemical market, preventing their calibration line construction with standard solutions.

Prior to studying any solid catalysts, the methodology optimized in this work was first used to benchmark the performance of acidulate cellulose in the mechanical devices utilized. Therefore impregnated cellulose was grinded in the planetary ball mill at 500rpm at different milling times. Unfortunately the WSP yielded were far lower if compared to the ones obtained in literature. Since it has been reported that the yield of WSP increases exponentially with grinding speed, we have hypothesized that our lower results were due to the lower maximum grinding speed of the Retsch PM100 model. Despite obtaining lower rates of depolymerization, the result has still provided a useful benchmark against which the solid catalysts tested in this work could be compared. Hence for each depolymerization reaction performed, one solid acid and microcrystalline cellulose were charged in the planetary ball mill in proper quantities to have a constant equivalents ratio. Every catalytic test was repeated with different milling times, from 2.5 to 20 hours, in order to study the depolymerization extent as function of the reaction time. Amberlyst 15 has resulted the catalytic system more performing, yielding in 79% of WSP in 20 hours of milling, showing itself four time more active than acidulate cellulose, since this has produced approximately 20% of WSP in the same milling conditions. On the other hand kaolinite and $AlPO_4$ didn't managed to heavy depolymerize cellulose. They have converted respectively only the 9% and 3% of the overall starting material. p-TSA activity was not determined due that the MS peak used for its conversion quantification include other unknown species that induce in an overrating error, making not acceptable the obtained conversion value for a correct comparison. Observing the glucose amount yielded in each reactivity test, emerge the same performances trend arisen in term of WSP. Indeed amberlyst 15 was able to convert the cellulosic substrate in 4.5% of glucose, whereas the grinding of acidulate cellulose has produced only 2.5% of the sugar monomer. Kaolinite and $AlPO_4$ still showed poor activities due that they have yielded in 0.5% and 0.2% of glucose. Thanks to different analytical method used to quantify the sugar monomers, p-TSA activity was determined, ranking itself between acidulate cellulose and kaolinite, producing 1.7% of glucose. For every catalytic test conducted the cellobiose amount yielded was always higher than glucose but still in a comparable value. Accordingly, the catalytic ranking emerged in term of cellobiose yield matches the ones based on the glucose yield.

Moreover thanks to the ESI-MS analysis was possible to observe the oligosaccharides distribution arisen by every depolymerization reaction and thus, have a roughly idea on the selectivity features of the catalyst tested. Acidulate cellulose was still used as a benchmark and was deduced that has as a selective reactivity toward the cleavage of the glycosidic bonds rather than to other chemical linkages of the sugar structure. Indeed the highest mass peaks detected were attributed to oligosaccharides species, especially composed by two until five AGUs. A greater amount of copolymer composed by one levoglucosan unit were also detected but nonetheless in lower quantity than the pure omopolymers. Furthermore were emerged small unrecognizable peaks, thus attributed to unknown species probably formed by some fragmentations of the oligosaccharides inside the milling devices. Nonetheless these undesired products were detected in very tiny quantities compared to the main oligosaccharides produced and thus confirming again the selectivity of the depolymerization reaction toward the breaking of the β-1,4-glycosidic bond. Amberlyst 15 was resulted, besides the more active solid acid, the more selective catalyst toward the oligosaccharides formation, due that the production of unknown compounds were the lowest emerged. Moreover it has yielded in less quantities of levoglucosan copolymer than acidulate cellulose. p-TSA was still a selective species but more predisposed to produce greater amount of cellobiose than longer oligosaccharides. The use of kaolinite has yielded the higher production of levoglucosan copolymers, which mass peaks almost reached the which ones of the relative omopolymers. Despite this the clay didn't conducted in a larger formation of unknown sugar fragments. $AlPO_4$ has revealed itself the less selective catalyst, due that the only product recognizable was cellobiose but whit a very weak peak intensity.

Since a total depolyrimerization extent wasn't still achieved, the amberlyst 15 to cellulose amount ratio was increased until reaching 0.8 equivalents of acid resin. This conditions had led to obtain 100% of WSP in 15 hours of milling at 500rpm in the planetary ball mill, enhancing also the yield in glucose and cellobiose. Acknowledging the benefit gained with greater amounts of catalyst, the quantities of amberlyst 15 were still increased at 1.5 equivalents of acid sites, improving again the depolymerization rate. Indeed in this acid conditions 100% of WSP were achieved in less time and higher sugar yields were obtained. The overall performances of the tested catalysts, also if used in various quantities, are summarized in Table 4.1 in term of cellulose conversion (WSP), glucose and cellobiose yield.

Solid Catalysts	Yield (%)		
	WSP	Glucose	Cellobiose
Acidulate cellulose 0.2 eq	10.2	2.1	1.6
Amberyst 15 0.2 eq	60.0	4.7	5.5
Amberyst 15 0.8 eq	100	6.5	8.3
Amberyst 15 1.5 eq	100	6.8	8.4
p-TSA	unknown	1.4	unknown
Kaolinite	7.5	0.3	0.4
AlPO4	1.7	0.1	0.2

Table 4.5 Cellulose conversion and glucose, cellobiose yields obtained using the solid catalysts tested with 15 milling hours at 500rpm in the planetary ball mill.

Plotting conversion and yields values as function of the acid equivalents number of amberlyst 15, whit which the depolymerization reactions were performed, a straight dependence was resulted with a steeper gradient during the longer milling times. In other words the depolymerization rate most depend on the acidity environment in the last reaction whiles when some oligosaccharides are yet formed and the cellulose structure yet decomposed. Studying this behavior brought to the deduction that, at the beginning of the reaction, the main hurdle to overcome to produce oligosaccharides is the cellulose crystalline morphology, but once it is deconstructed, thanks to the mechanical forces, the depolymerization process mainly proceed through hydrolysis reactions supported by the acid species. For fairness must be reported that cellobiose yields had shown a maximum value at intermediate acid conditions, probably because, if too larger quantity of catalyst are present it undergoes in hydrolysis reactions producing glucose. Unfortunately the dependence of the reaction performances against the amount of acid sites couldn't be deeper studied on acidulate cellulose because greater amounts than 0.2 acid equivalents on that substrate conduce in unselective side reactions, that don't allow to obtain reliable conversion values. Nonetheless milling acidulate cellulose with lower amount than 0.2 acid equivalents has yielded in less glucose and cellobiose productions, confirming the trend. Moreover to deepen this phenomena it were analyzed mass-spectrums of cellulose samples milled with different amount of amberlyst 15 at intermediate grinding times. In particularly it emerged that the oligosaccharides distribution remain constant varying the acid strength of the reaction environment. This is a further confirm that the major obstacle that prevent the $Glc_{3\text{-}5}$ oligosaccharides formation during the first reaction whiles is a physical reason, like the cellulose structure, and not chemical. Thus, once the

cellulose structure is crushed, the inner bonds of the polymer are exposed to the acid sites of the catalyst, showing the same chemical features of the external ones, due that are chemically identical.

Analyzing the oligosaccharides production as function of the milling time it was understood that the cellulose depolymerization begin with the detaching of the terminal pendants of the whole cellulosic polymer. In a second while, once the mechanical forces have deconstructed the cellulose crystalline morphology, also the inner glycosidic bonds are exposed to the catalyst acid sites, and thus also longer oligosaccharides are produced. This behavior was confirmed thanks to the ESI-MS analysis observing products distribution of samples resulted from different milling times. Furthermore it was observed that once a great yield of water soluble products is already formed, conversions improving are more harder to achieve, thus confirming that the oligosaccharides produced buffer the mechanical forces exerted on the reaction materials slowing the depolymrization process. In the few samples in which cellulose was totally converted in water soluble products was found that if the milling is prolonged glucose and cellobiose yields didn't increase, neither decrease. Since were classified as total soluble only oligosaccharides shorter than six AGUs, it was concluded that once these compounds are formed don't further depolymerize with milling extension, otherwise glucose or cellobiose yields improvement must be registered. This trend confirms the theoretical behavior that under some sizes the particles inside the milling vial tent to dodge the grinding balls and so, don't undergo in further depolymerization reactions.

Compare the results of reactions catalyzed by ambelryst 15 and p-TSA was useful to understand the reason of the remarkably greater activity of the acid resin than the other solid acids studied. Indeed the functional group responsible of the acid features of these two catalyst is the same toluen-sulfonic group, hence could be expected that the acid strength of the two materials is almost equal. Thus the main difference of these two catalytic systems is that on amberlsyt 15 the acid sites are distributed on an inert support, such as the polystyrene matrix, whereas in p-TSA no dispersion contribution is present because is a proper organic molecule of that acid group. Since amberlyst 15 was resulted remarkable more active than p-TSA, it was concluded that the dispersion of the acid sites is an important catalyst features for the occurring of the depolymerization reaction, confirmed also by the performances comparison between kaolinite and $AlPO_4$. Indeed kaolinite and $AlPO_4$ posses roughly the same aluminum Lewis acid sites, but in the clay,

thanks to the presence also of silicon structures, they are more distributed. Moreover studying the glucose yields provided with 0.2eq and 1.5eq of ambelryst 15 and 0.2eq of p-TSA, was proved that the dispersion effect is a more performing features than the increment of the catalyst quantity. Furthermore this distribution factor was considered the reason of the higher selectivities provided by ambelryst 15 than the other catalysts, indeed the lower concentration of its acid functions prevents the overcoming of the energy barrier that conduces to undesired side reactions, that instead HCl on impregnated cellulose is able to perform.

Having gained all these information about the catalysts activities and selectivities and the cellulose depolymerization mechanism, reactivity tests were performed also in a mixer ball mill in order to understand its performances. In this device amberlyst 15 was the only solid tested that has produced some glucose, cellobiose or water soluble products yields but in really tiny amounts if compared to the ones obtained in the planetary ball mill. As occurred in the planetary ball mill, increments of reaction times have conducted in depolymerization rate improvements but, in this case, following a perfectly linear trend, probably because high yield of WSP weren't produced and thus, no buffering effect of the mechanical forces occurs during the last milling hours. Furthermore also in this mechanical device the major product detected during the first reaction whiles was cellobiose, whereas extending the milling time also longer oligosaccharides were produced, suggesting that the depolymirazion mechanism previously described is a specific features of the cellulosic substrate and don't depend by the grinding equipments. Contrarily, in this device, greater amount of catalyst didn't enhance the depolymerization rate, probably because the weaker mechanical forces exerted are unable not only to depolymerize cellulose but neither to deconstruct the polymer matrix of amberlyst 15. In this way not the overall acid equivalents inserted in the grinding vial result available for approaching the cellulose glycosidic bonds because some of the acid sites are still enclosed inside the resin structure. Thus also if greater amount of catalyst are charged in the mill, the real increment of the acid equivalents is low because the acid sites available for the reaction are the only ones present on the surface of the resin beads.
It must be reported that in this mill all the others catalysts used didn't show any activity behavior.

Thanks to the catalytic features shown by amberlsyt 15 in both mechanical devices was possible to compare their performances toward the studied reaction. Planetary ball mill demonstrated itself the best equipment in which perform the cellulose depolymerization due that in five hours of milling it produces 47% of WSP when 0.2 equivalents of ambelryst 15 were used as catalytic system, against the 5% of conversion arisen in the mixer ball mill. Also in term of glucose and cellobiose the mixer device is less performing than the planetary and this behavior could be attributed to the different power of milling that the two devices exhibit. As a confirm of the weaker milling performances of the mixer ball mill, must be remembered that this model is not able to exploit the activities of p-TSA, kaolinite and $AlPO_4$ that instead were shown in the planetary ball mill.

As a general conclusion of this work could be affirmed that the mechanochemistry approach could be exploited to valorize cellulose with the experimental methodology here developed, because assists its transformation in useful chemical platform molecules. Indeed quantitative conversions in water soluble products were obtained in 15 hours of milling and 15.3% of overall yield of glucose and cellobiose was achieved with the best milling conditions. Nonetheless deeper studies must be conducted to improve the entire process productivity and selectivity. For example it was found that the depolymerization rate decrease when approximately 50% of cellulose conversion is obtained, that correspond almost at five milling hours, due that the oligosaccharides produced buffer the mechanical forces exerted by the grinding items. Therefore a technical strategy to improve the overall process productivity could be stop the grinding when that conversion value is reached, separate the soluble products from the insoluble starting material trough a water extraction and reprocess the unconverted cellulose. In this way is avoided to conduct the reaction at the slowest reaction rate, thus saving almost ten hours per milling, resulting in a overall increment of the process productivity. The alternatively route developed in this work strongly differs from the today common processes exploited to convert biomass in useful saccharides since it prevents the use of toxic organic solvents or concentrated acids, offering an overall clearer chemistry approach. Moreover the preventing of corrosive chemicals permits to simplify the operational equipments employed thus reducing the capital cost invested for their provision. Nonetheless was proved that the acid catalyst presence is a mandatory requirement since the only

mechanical forces are unable to depolymerize cellulose. In particularly, among the catalysts studied, amberlyst 15 is the solid acid that provides the higher activity and selectivity. This is a commercial compound that could easily purchased yet today on the chemical marked, thus preventing synthesis optimization processes that would increase its cost. Moreover it is plenty less expensive than α-amilase, the enzymatic species today exploited in the biobased route. Unfortunately this acid resin doesn't perfectly match all the advantages that could show a heterogeneous catalyst due that, after the milling, it results totally soluble as the reaction products. Hence if the sugar aqueous solution want to be exploited in further industrial transformations must be purified trough some separation operations, for example an ion exchange process able to restrain the acid resin fragments. Alternatively this aqueous solutions could be used as such if the saccharide content want to be converted, trough acid catalysis, in some consecutive derivates. In both cases the recovery of the acid solid cannot be performed, thus preventing its recycling for further depolymerization reactions. This is an undesired aspect for the sustainability features of the whole process because it increases the amount of catalyst used that furthermore results as a waste at the end of reaction. Nonetheless, if amberlyst 15 is used as catalyst in a mechanical device, the few unwanted compounds which result among the reaction products are mainly constituted by carbonaceous fragments coming from its deconstruction and thus result less toxic or dangerous than the concentrate alkaline or acid species present in the end products of the industrial processes today utilized. Thus, also if a refusal or contaminant is still produced, it is easier and safer to handle and dispose. Furthermore the distribution of the acid sites on the ambelryst 15 polymer matrix permits its utilization in lower amount if compared to the other solid acids studied, still reaching the same conversion values, thus restricting both the overall amount of the starting materials utilized as the quantity of matter that must be disposed at the end of the reactions. All these factors, besides improving the intrinsic safety of the process, minimize the separation or neutralization apparatuses required after the reaction, thus saving chemical auxiliaries, energy and capital costs invested in these equipments. Hence at the end of this work could be considered worth to deeper study this new route to obtain valuable platform molecules from biomass because could provide both economical advantages as human and environment safety benefits compared to the industrial processes today exploited for this purpose.

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CHAPTER 3- RESULTS AND DISCUSSION

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