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Optimization of levulinic acid production from lignocellulosic biomass: experiments and simulation

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Abstract

Levulinic acid has gained much interest from the scientific community because of its wide application as a green intermediate to produce fuels and chemicals from renewable biomass sources. This thesis aims to optimize levulinic acid production from corn stover, finding out the best green-operating conditions to increase its yield. The effectiveness and the advantages in pre-treating the biomass before its acid hydrolysis is also discussed. To this goal, experiments were carried out, investigating the effect of reaction time and acid concentration on the yield of levulinic acid. Afterwards, the effect of a hydrothermal pretreatment of the biomass to break the lignin structure and possibly increase hydrolysis effectiveness was investigated. A comparison between the results found and those reported in literature was done to understand better the advantages brought by the proposed pretreatment. Finally, a simplified simulation and a preliminary economic assessment of the entire process was developed to analyze the feasibility of a hypothetic industrial process.

Riassunto

Con il costante incremento della popolazione mondiale, si ricercano alternative sempre meno impattanti per soddisfare i bisogni delle società, limitando quanto più possibile il consumo delle materiali fossili, principale causa dei cambiamenti climatici e dell'effetto serra. Ciononostante, queste materie prime rimangono la risorsa maggiormente utilizzata per la produzione di energia e prodotti di uso comune. Per limitare le dannose conseguenze derivanti dall'uso spregiudicato di queste risorse, è decisivo ricercare fonti di energia alternative, capaci di rigenerarsi in tempi brevi e il cui utilizzo risulti meno impattante sia per l'uomo che per l'ambiente. Tra queste ricordiamo l'energia solare, quella eolica, il calore geotermico, le maree e le biomasse. Negli ultimi anni, le biomasse hanno assunto un ruolo sempre più importante all'interno dell'industria chimica a tal punto da promuoverne una sua conversione verso una dimensione green e bio. Nello specifico, questo lavoro di ricerca indaga il processo di produzione dell'acido levulinico, derivante dall'idrolisi acida dei residui non edibili del mais.

Nel 2004, il dipartimento di Energia degli Stati Uniti ha individuato 14 composti aventi le potenzialità di essere i nuovi "green building block", alternativi a quelli della tradizionale raffineria, che possono essere combinati per ottenere prodotti più complessi tra cui polimeri, resine, combustibili, fino ai beni legati all'industria alimentare. Per le proprietà chimico-fisiche e per il suo elevato range di applicabilità, l'acido levulinico è considerato un componente fondamentale, e questa ricerca analizza i vantaggi e le criticità dei processi chimici coinvolti nella sua produzione, nell'ottica di introdurre dei miglioramenti per ottimizzarne la resa.

Comunemente, l'acido levulinico è prodotto dall'idrolisi acida della cellulosa; tuttavia, a causa della complessa struttura della biomassa formata da cellulosa, emicellulosa e lignina, in percentuali variabili a seconda della tipologia e dell'area geografica in cui cresce, ogni componente reagisce, ottenendo composti diversi. Inoltre la lignina e l'emicellulosa creano un effetto barriera verso la cellulosa, limitandone l'idrolisi e riducendo la resa di acido levulinico.

Per ridurre questo fenomeno, si introduce un pretrattamento iniziale della biomassa con lo scopo di modificarne la struttura, limitando l'effetto barriera. Confrontando le rese ottenute dalla biomassa tal quale e pretrattata, viene discussa l'effettiva utilità del pretrattamento.

Sperimentalmente, il pretrattamento iniziale è condotto in condizioni semi-batch utilizzando un reattore contenente la biomassa e riscaldato per mezzo di una fornace elettrica, mentre una soluzione basica viene costantemente pompata al suo interno. Il pretrattamento è realizzato a 190°C e 34 atm. In seguito la biomassa pretrattata è sottoposta ad idrolisi utilizzando un reattore batch in cui viene inserita anche la soluzione acida. Il reattore viene riscaldato per mezzo di bagno di sabbia. La reazione è condotta a 190°C e 34 atm per garantire condizioni subcritiche all'interno del reattore. Diversi esperimenti vengono eseguiti variando il tempo di reazione e la concentrazione di acido per comprenderne meglio gli effetti sulla resa finale. I prodotti ottenuti dal pretrattamento sono analizzati misurando il pH, il carbonio organico contenuto nel liquido di idrolisi e si è definita la composizione elementare della biomassa pretrattata. Il liquido e il residuo solido ottenuto dall'idrolisi vengono analizzati con la cromatografica ad alta pressione e l'analisi elementare. Definite la concentrazione di acido levulinico e dei principali sottoprodotti si confrontano i risultati con quelli riportati in letteratura. Da questo confronto si evince che il pretrattamento iniziale della biomassa, non solo permette di raddoppiare la resa finale di acido levulinico, rispetto a quella ottenuta dalla biomassa tal quale, ma permette anche di ridurre il tempo di reazione a 5 min. Inoltre dal liquido di idrolisi generato durante il pretrattamento è possibile estrarre un composto di valore, l'acido lattico, che trova largo impiego nell'industria alimentare.

Il lavoro di ricerca si conclude con una valutazione delle potenzialità economiche di un ipotetico processo industriale, tenendo conto di tutti i processi chimico-fisici per la produzione di acido levulinico. Questa analisi prende in considerazione solo i costi legati al consumo di materie prime ed energia dei processi sopra esposti, a cui si aggiungono la separazione liquido-solido, la neutralizzazione e la distillazione, per ottenere acido levulinico al 98% in massa. I costi legati alla colonna di distillazione sono calcolati sulla base dei costi energetici ottenuti dalla simulazione con Aspen+.

Da una prima indagine si evince che l'impianto è capace di generare un profitto pari a 9730.67 €/h, è tuttavia importante ricordare che un'analisi più dettagliata, prendendo in considerazione anche il costo operativo e i diversi fattori economici, è fondamentale per stimare il reale profitto generato dall'impianto.

Introduction

The world is highly dependent on the utilization of fossil resources (e.g., petroleum, natural gas and coal) to fulfil its energy needs. Furthermore, a wide range of modern products like polymers, resins, textile etc. is derived from fossil resources. However, the reduction of fossil sources exploitation and their effects have been the centre of several discussions among the scientific community. The effects of human activity are reflected in the ice melting and more extreme climate events. The only way to reduce these events is a transition from a fossil-based economy to renewable alternatives, such as solar, eolic and biomass energy. This work highlights the possible applications of biomass as a green alternative to produce chemicals, which are commonly derived from the traditional refinery. More specifically, the focus of this thesis is on levulinic acid production from lignocellulosic biomass.

Levulinic acid is a chemical compound that belongs to the list of the most promising bio-based platform molecules found by the US Energy Department in 2004. It is investigated because of its wide application as green intermediate to produce fuels, solvents, polymers, resins etc. The reaction for levulinic acid production is based on the acid hydrolysis of cellulose, which is one of the major structural components of biomass. The goal of this thesis is to find out a possible green-way to optimize the yield of levulinic acid, analyzing the advantages of processing biomass through a hydrothermal pretreatment, before its hydrolysis.

Chapter 1 contextualizes the biomass presenting its common usage, and discussing the advantages and the drawbacks of its wide applications that are forcing towards a conversion of the traditional way to produce chemicals and fuels. The methods and the instrumentations used to carry out the experiments and to analyze the products are presented in chapter 2. Chapter 3 reports a comparison between experimental results and those reported in the literature to better understand the advantages brought by the proposed pretreatment. Finally, in Chapter 4 a preliminary economic assessment, based on material and energy balances of a hypothetic industrial process for levulinic acid production from biomass is presented, quantifying the thermal and electrical energy costs, as well as feedstock supply and the final revenues.

This master thesis, aiming at investigating the mechanism to produce levulinic acid, was carried out during a 6 months internship in the Biomass Research Laboratory (BRL) of Old Dominion University, in Virginia (USA). I would like to thank Dott. Sandeep Kumar, for his

advice on overcoming the difficulties and patience to supervise me for the entire project. I would also like to thank Anuj Thakkar for being such a good lab mate and an amazing friend wherewith spending a great time.

Chapter 1

Literature review

At present, our society is highly dependent on finite fossil fuels such as petroleum, coal and natural gas, primarily used to satisfy the needs of energy, fuels, organic chemicals, and polymers. The increasing energy demand, gradual depletion of fossil fuels, and hence the rise of crude oils price are foremost motivations for the exploration of renewable resources for sustainable production of electricity, heat, fuels, organic chemicals, and polymers (1). In this chapter, the use of biomass as a potentially renewable resource will be discussed, understanding its main composition to know its chemical applications better. A specific focus is dedicated to one specific component, i.e. levulinic acid, to understand how it can be produced from biomass and which are its primary uses in our society.

1.1 Overview of biomass

The term "biomass" commonly refers to a renewable organic substance produced by plants through photosynthesis process. As well known, during photosynthesis, plants combine carbon dioxide from air and water from the soil to form carbohydrates that are biochemical building blocks of biomass. Solar energy is accumulated in chemical bonds of carbohydrates, and other molecules included in biomass.

Biomass can be defined as any organic matter that is available on a renewable or recurring basis, including dedicated energy crops and trees, agricultural food and feed crop residues, aquatic plants, wood and wood residue, animal waste materials (2). It can be wet or dry. The moisture content depends on biomass type and condition of its storage. Dry biomass mainly has a low moisture content (about 30 wt%). Wood, straw, or other sun-dried wastes are examples of dry biomass. Other types of biomass can be classified as wet, for instance, algae suspensions, sugar solutions and sewage sludge. Biomass of all the Earth is concentrated in a thin surface layer called biosphere. Although it can be considered just a tiny fraction of the total mass of the Earth, for the human being, it represents a big storage of energy that can be

used as food or in fuel production. The yearly amount of biomass is estimated around 146 billion metric tons, mostly from uncontrolled plant growth Baskar, Baskar (1).
Even if, due to the specific origin and formation conditions, the composition of biomass is highly variable, which can make it challenging to use it in some biochemical applications, there are some advantages of using this material in fuel production, such as:

- Renewable energy source;
- CO_2 natural conversion and climate change benefits;
- Conservation of fossil fuels;
- High concentrations of combustible volatile matter, such as CH_4, C_2H_2, CO, H_2, which ensure the highly reactive nature of the fuel;
- High concentrations of extractive, which consist of various organic and inorganic components extracted individually by different polar and non-polar solvents, namely water, ethanol, benzene and toluene. Their higher concentrations are strong indicators for potential production of biodiesel, bioethanol and other biofuels and biochemicals.
- Biodegradable resource with significant reactivity and low initial ignition and combustion temperatures during conversion.

However, there are also some disadvantages related to the use of biomass as fuel, such as:

- High values of moisture and oxygen in biomass that leads to some problems during biofuel pretreatment and in the biomass conversion reducing the combustion temperature and its efficiency.
- Competition with edible biomass (food, feed), fiber and biomaterial productions;
- Damage of natural ecosystems (water, soil, land-use change, deforestation, biodiversity);
- Insecurity of feedstock supply;
- Low energy density (bulk density and calorific value);
- Low pH and ash-fusion temperatures (3).

1.1.1 Structure of biomass

As previously mentioned, although biomass can be of different types, it is mainly plant-derived, and the knowledge of its chemical structure is essential to develop an energy-

efficient biorefinery process. In general, the chemistry of biomass is quite complex due to the significant amount of chemical compounds that are involved; carbohydrates, lignin, proteins, and fats are the most common chemical compounds present in the biomass, together with a lower extent of several other chemicals, such as vitamins and dyes. Based on chemical nature, biomass can be classified into different categories, such as (2):

- Triglycerides feedstock (vegetable oil, animal fats, waste cooking oil, and microalgae oils);
- Sugar and starchy feedstock (sugar beet, sugar cane);
- Lignocellulosic feedstock (wood, straw, grasses).

The latter is the object of this dissertation.

Generally, lignocellulosic biomass is primarily composed of cellulose (40-50%), hemicellulose (25-35%) and lignin (15-20%), as reported in figure 1.1.

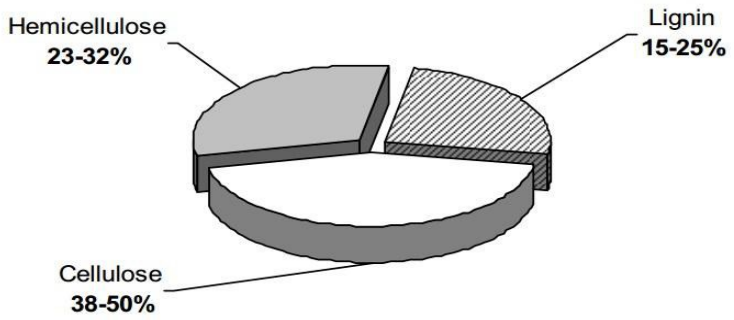


Figure 1.1: *Composition of lignocellulosic biomass ([2])*

There are also traces of pectin, protein, extractives (non-structural sugar, nitrogenous material, and waxes) and ash. However, the compositions of lignocellulosic biomass vary significantly depending on type and geographical origin.

Cellulose is a high molecular weight (10^6 kg/kmol or more) linear polymer of β-glucose (5000-10000 units) linked together by β-1,4 glycosidic bonds. Its complex structure is reported in figure 1.2.

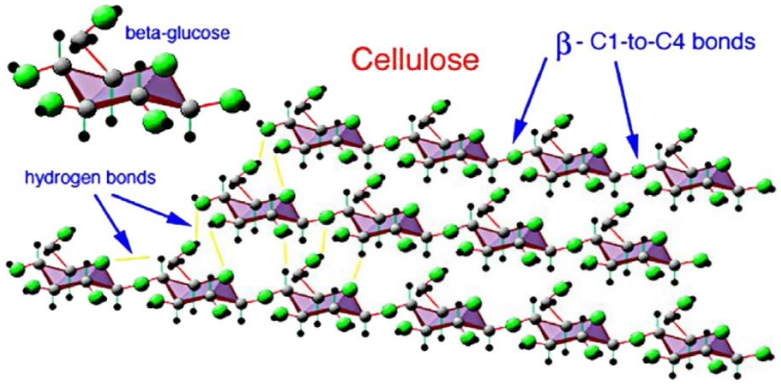


Figure 1.2: *Cellulose from intra-molecular condensation of β-glucose at the 1,4-position*

The polymer chain of cellulose is bundled together by hydrogen and van der Waal bonds leading to high strength and high resistance to biological attack. Cellulose is highly crystalline, with only a small fraction being amorphous. The crystalline property of cellulose makes it completely insoluble in aqueous solutions. This property also leads to high resistance to hydrolysis that reduces the conversion efficiency of this polymer to monomers during biorefining processes.

Hemicellulose is an amorphous and branched polymer made of five carbon (xylose and arabinose) and six-carbon (galactose, glucose, and mannose) sugar together with uronic acid substituents (e.g. 4-o-methylglucuronic, D-glucuronic and D-galacturonic acids). Its structure is reported in figure 1.3.

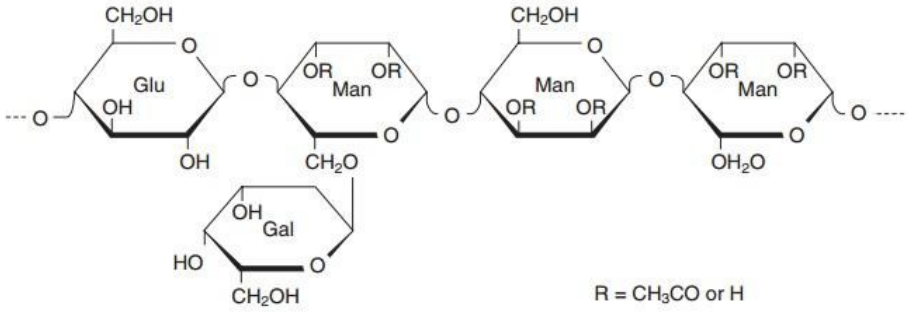


Figure 1.3: *Chemical structure of hemicellulose ([2])*

Hemicellulose is either a homopolymer or a heteropolymer with short branches. The monosaccharides are linked together by β-1,4 glycosidic bonds and sometimes β-1,3 glycosidic bonds. The number of repeating monosaccharides is only ~150 in hemicelluloses. The most abundant building block of hemicellulose in hardwood and agricultural plants (like grasses and straw) is xylan. It is a polymer of xylose linked at 1 and 4 positions. While in

softwoods, the abundant hemicelluloses building block is glucomannan. It is a straight-chain polymer of D-mannose and D-glucose linked by β-1,4 glucosidic bonds with a small amount of branching. The hemicellulose bridges lignin and cellulose fibers, leading to a rigid network of cellulose-hemicellulose-lignin. Being amorphous, hemicellulose is highly soluble in water, so its hydrolysis to monomer sugar is relatively easy compared to cellulose.

Lignin is the most abundant high-molecular weight aromatic polymer (6×10^5-15×10^6 kg/kmol). It is an amorphous and three-dimensional polymer composed of three different methoxylated phenylpropane units (coniferyl alcohol, sinapyl alcohol, and coumaryl alcohol), as shown in figure 1.4, that are bonded together by different kind of linkages. The overall lignin structure is shown in figure 1.5.

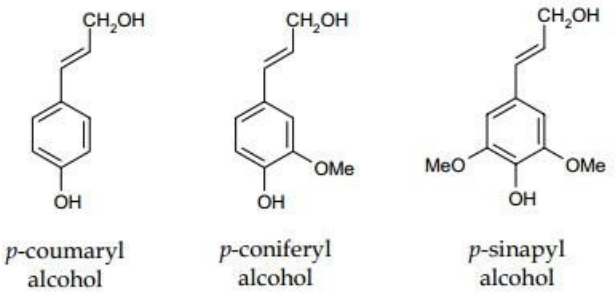


Figure 1.4: *Polymers of lignin ([2])*

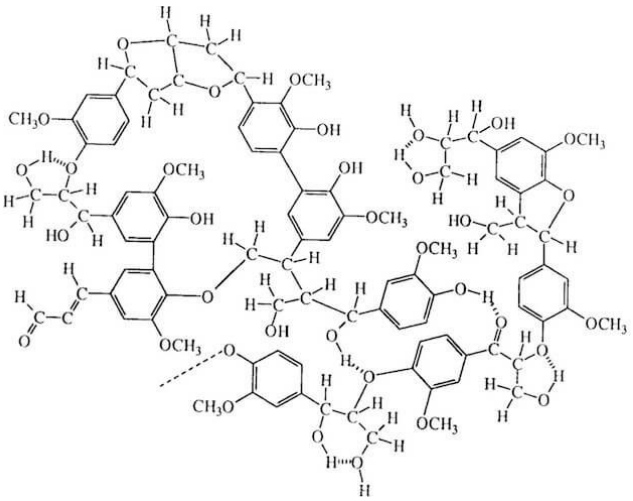


Figure 1.5: *Lignin structure*

The distribution of these phenylpropane building blocks in lignin depends on types of biomass. The plants cell walls are primarily composed of lignin that provides a structural

support, resistance against microbial attack, and a hydrophobic vascular system for the transportation of water and solutes (2).

1.2 Biorefinery

The concept of biorefinery was originated in the late 1990s as a result of the scarcity of fossil fuels and increasing trends of use of biomass as a renewable feedstock for the production of non-food products. The American National Renewable Energy Laboratory (NREL) defined biorefinery as a facility that integrates biomass conversion process and equipment to produce fuels, power and chemicals from biomass. The biorefinery was classified into three types, phase I, II and III, based on conversion technologies to produce various bio-products.

1.2.1 Type of biorefineries

The phase I biorefinery, called the "whole crop biorefinery", has fixed processing capability. It uses cereals, such as, wheat, triticale as well as maize, to produce a fixed amount of ethanol, other products for feed, and carbon dioxide. The first step is the mechanical separation into corn and straw, which are obtained almost in the same amount. The straw represents an Lignocellulosic-Feedstock (LCF) and may further be processed in an LCF-Biorefinery regime. On the one side, there is the possibility of separation into cellulose, hemicellulose, lignin, and their further conversion within separate product lines, which are shown in the LFC-Biorefinery in figure 1.6. Furthermore, straw is the starting material for the production of syngas via pyrolysis technologies. Syngas is the primary material for the synthesis of fuels and methanol. Corn may either be converted into starch or directly used after grinding to the meal. According to further processing, the meal can be treated and finished by extrusion into binder, adhesive, and filler, while starch can undergo multiple treatments.

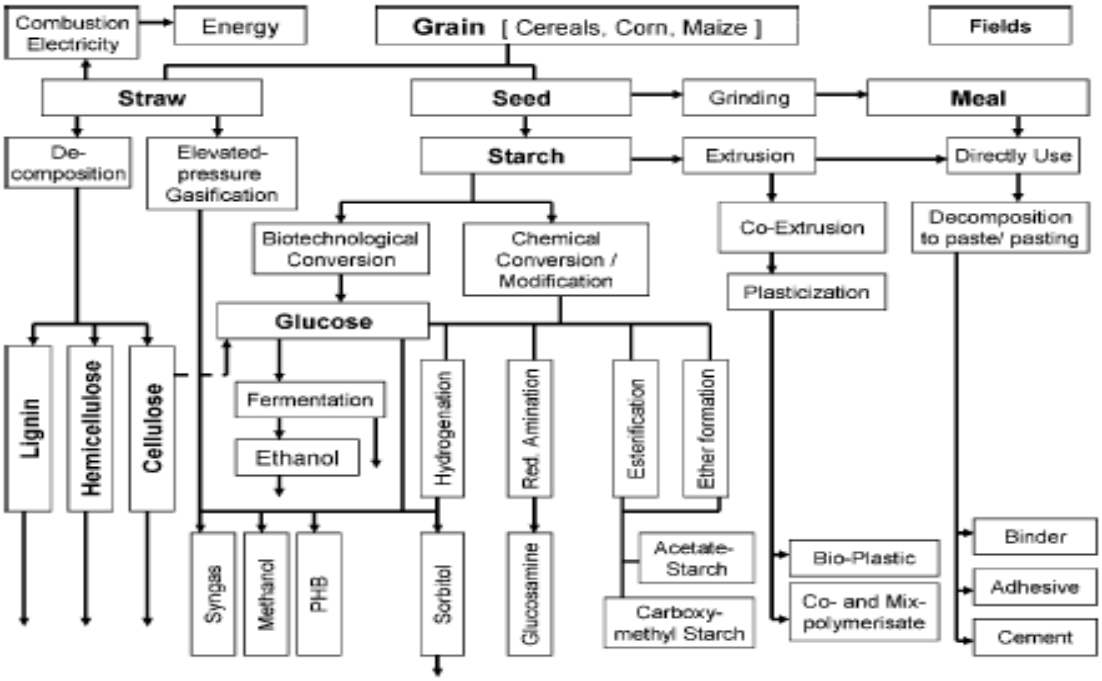


Figure 1.6: *Type I biorefinery ([6])*

Phase II biorefinery, called "Green Biorefinery", uses nature-wet biomass such as green grass, lucerne, clover, or immature cereal to get an assembly of products such as starch, high fructose corn syrup, ethanol and corn oil depending on their demands and price. The careful wet fractionation technology is used, as first step (primary refinery), to isolate the content-substances in their natural form. Thus, the green crops goods (or humid organic waste goods) are separated into a fiber-rich press cake (PC) and a nutrient-rich green juice. Besides cellulose and starch, the press cake contains valuable dyes and pigments, crude drugs, and other organics. The green juice contains proteins, free amino acids, organics acids, dyes, enzymes, hormones, other organic substances and minerals, and the main focus is directed to products such as lactic acid and corresponding derivatives, amino acid, ethanol, and proteins. The press cake can be used for the production of green feed pellets as raw material for the production of chemicals, such as levulinic acid, as well as for conversion to syngas and hydrocarbons (synthetic biofuels). All the residues of substantial conversion are suitable for the production of biogas combined with the generation of heat and electricity, as reported in figure 1.7.

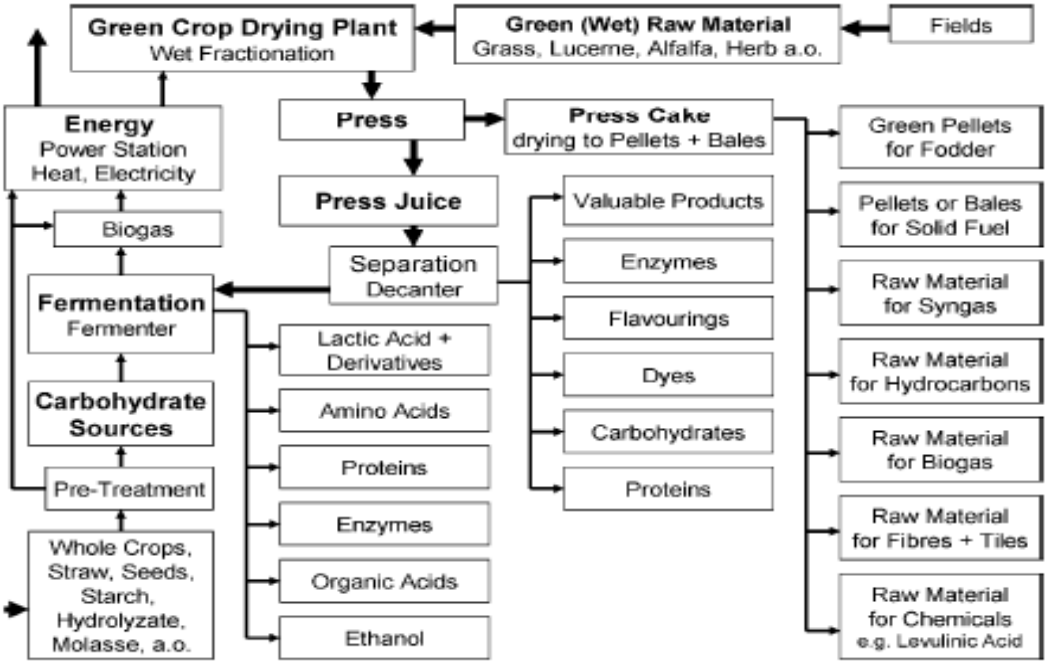


Figure 1.7: *Type II biorefinery (6)*

The phase III biorefinery, i.e. the "Lignocellulose Feedstock biorefinery ", uses a mixture of nature-dry raw materials, such as cellulose-containing biomass and wastes to produce a multitude of products using a combination of technologies. As already said, lignocellulosic materials are composed of three primary chemical fractions, namely hemicellulose/polyose (a sugar-polymer of pentoses predominantly) cellulose (a glucose-polymer) and lignin (a polymer of phenols). General chemical reactions of LCF-Biorefinery are reported in equations 1.1,1.2,1.3 and 1.4 (4) :

$$\text{Lignocellulose} + H_2O \rightarrow \text{Lignin} + \text{Hemicellulose} + \text{Cellulose} \quad (1.1)$$

$$\text{Hemicelulose} + H_2O \rightarrow \text{Xylose} \quad (1.2)$$

$$\text{Xylose} + \text{acid catalyst} \rightarrow \text{Furfural} + 3H_2O \quad (1.3)$$

$$\text{Cellulose} + H_2O \rightarrow \text{Glucose} \quad (1.4)$$

The main bio-based products are today obtained from the conversion of biomass into primary products like starch, oil, and cellulose. Other already commercially available bio-based

products include adhesives, cleaning compounds, detergents, lubrificants, solvent, packaging materials, paints and coating, plastic filler, polymers (5); a general overview about potential products of a LCF-Biorefinery is shown in figure 1.8.

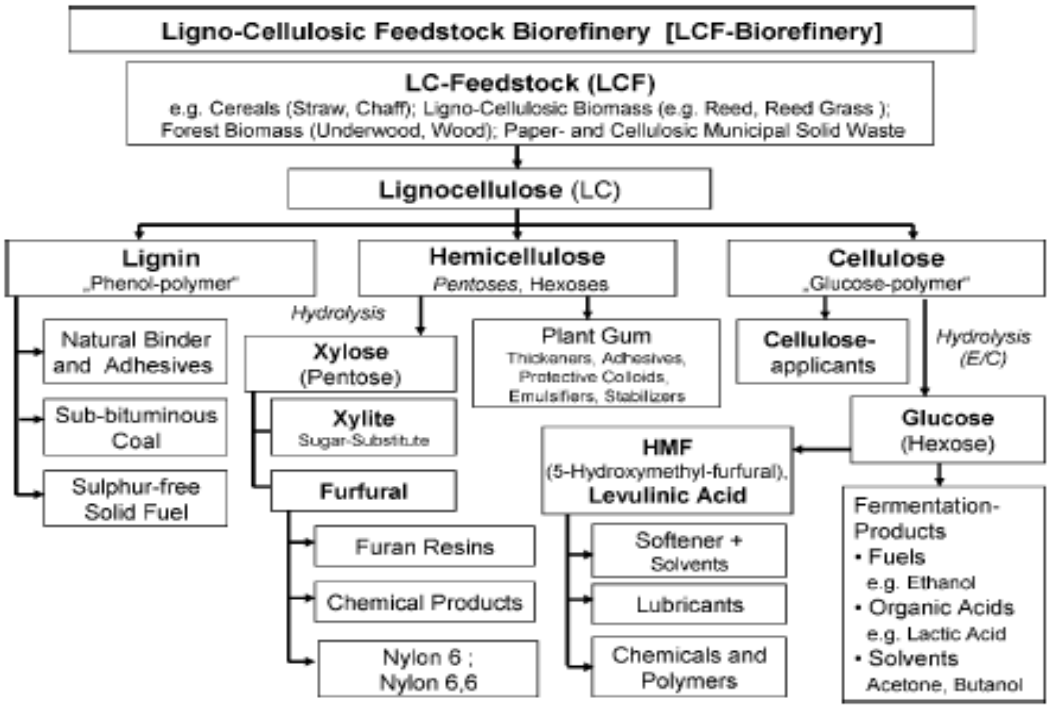


Figure 1.8: *Type III biorefinery ([6])*

As already said, the biorefinery industry aims to produce bulk chemicals from biomass according to a selection of simple platforms that differ from those currently used in the petrochemical industry. Due to the chemical complexity of biomass, there is some limit in the choice of which platform chemicals can be produced, since different processing strategies of the same material can lead to different break-down products. From a theoretical print of view, all the oil refinery platform chemicals can be derived from biomass, but the lower process' yields and the higher costs, are against their low market price (6). As well, the future biorefineries are expected to be based on a limited number of platforms, from which all the other chemicals can be derived. Indeed, the carbohydrate fraction of biomass feedstock (i.e. cellulose and hemicellulose in lignocellulosic biomass) is expected to play the most significant role as a renewable carbon source for biochemical products. Indeed, biomass polysaccharides can be effectively hydrolyzed to monosaccharides (e.g., glucose, fructose and xylose) which can then be converted, via fermentation or chemical synthesis, to an array of

bio Platform Molecules (bPM – building block chemicals with potential use in the production of numerous value-added chemicals), analogous to the petro-platform molecules of the current oil refinery. In 2004, the US Department of Energy compiled a list of the most promising bPMs, which is reported in figure 1.9 (5).

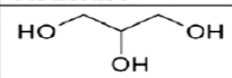
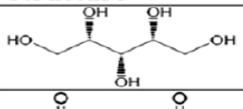
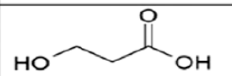
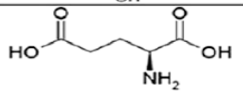
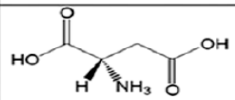
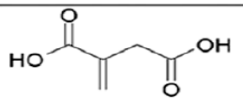
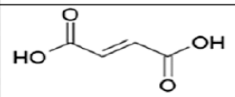
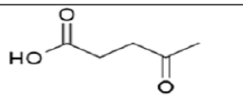
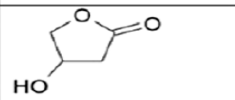
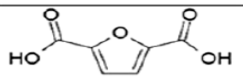
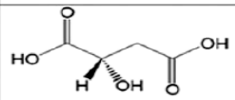
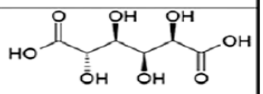
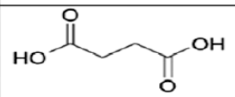
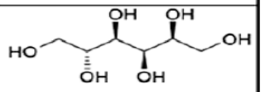
Bio-PM	Structure	Bio-PM	Structure
glycerol		**(S, R, R)-xylitol**	
3-hydroxy propionic acid		**L-glutamic acid**	
L-aspartic acid		**itaconic acid**	
fumaric acid		**levulinic acid**	
3-hydroxy butyrolactone		**2,5-furan-di-carboxylic acid**	
L-malic acid		**glucaric acid**	
succinic acid		**sorbitol**	

Figure 1.9: *List of the most promising bio Platform Molecules (bPMs) ([5])*

1.2.2 Biomass conversion process

As mentioned before, biomass can be converted into several chemical products that can be used to derive other commodities and bulk chemicals or for energy generation; conversion of biomass to energy is undertaken using different process technologies, such as thermochemical, mechanical, biochemical, and chemical routes.

1.2.2.1 Thermochemical processes

The burning of biomass in air, i.e. combustion, is used over a wide range of output to convert the chemical energy stored in biomass into heat, mechanical power, or electricity, using different process equipment. It contributes to over 97% of bioenergy production in the world.

It is possible to burn any type of biomass, but in practice, combustion is feasible only for biomass with moisture content ≤ 50%, unless the biomass is pre-dried. Three many stages occur during biomass combustion: drying, pyrolysis and reduction, and combustion of volatile gases and solid char. Combustion of biomass produces hot gases at a temperature around 800-1000 °C, and it contributes more than 70% of the overall heat generation. The combustion of biomass on a large scale is still considered to be a complicated process with a technical challenge associated with biomass fuel characteristic and type of combustor (7, 8).

Pyrolysis is another thermal technology based on thermal decomposition of biomass that takes place in the absence of oxygen to get solid charcoal, liquid (bio-oil or bio-crude), and gases ate high temperatures. There are three stages for a typical pyrolysis process: the first stage, pre-pyrolysis, occurs between 120 and 200°C with a slight observed weight loss when some internal rearrangements take places, such as bond break, the appearance of free radicals and formation of carbonyl group, with a corresponding release of a small amount of water, carbon monoxide and carbon dioxide. The second stage is the main pyrolysis process, during which solid decomposition occurs, accompanied by a significant weight loss from the initially fed biomass. The final stage is the continuous char devolatilization, caused by the further cleavage of C-H and C-O bonds. This technology can be used to produce predominantly bio-oil by means of flash pyrolysis, which occurs with a high heating rate (as high as hundreds of °C/min) and short residence time, enabling the conversion of biomass to bio-crude with efficiencies of up to 80%. This product can be used in the engine and turbine, and its use as feedstock for refineries is also being considered (7, 8).

Finally, biomass gasification is a process that converts carbonaceous biomass into combustible gases (H_2, CO, CO_2), with specific heating values, in the presence of partial oxygen (O_2) supply or suitable oxidants such as steam and CO_2. When air or oxygen is employed, gasification is similar to combustion, but it is considered a partial combustion process. There are some differences between the two processes; in general, combustion focuses on heat generation, whereas the purpose of gasification is to create valuable gaseous products that can be used directly for combustion or as a feedstock (syngas) in the production of chemicals. Also, gasification is considered to be more environmentally friendly because of the lower emission of toxic gases into the atmosphere and the more versatile usage of solid byproducts. Gasification can be viewed as a particular form of pyrolysis, taking place at a higher temperature to achieve higher gas yields. Biomass gasification offers several

advantages, such as reduced CO2 emission, compact equipment requirement with a relatively small footprint, accurate combustion control, and high thermal efficiency. The production of syngas from biomass allows the production of methanol and hydrogen, each of which may have a future as fuel for transportation (7, 8).

Torrefaction is another thermal pretreatment technology that can be described as slow pyrolysis carried out in a range of temperatures between 200-300°C in an inert and reduced environment at shorter residence time. Torrefaction serves to pretreat the biomass to infer superior fuel properties in comparison with its parent biomass and with respect to its further application processes such as pyrolysis, gasification, or combustion. Common biomass reactions during torrefaction include devolatilization, depolymerization, and carbonization of hemicellulose, lignin, and cellulose. This process leads through a significant alteration of the physical and chemical composition of biomass, making it perform better for cofiring and gasification purposes. It usually deals with the production of a brown to a black uniform solid product, as well as condensable (water, organics, and lipids) and noncondensable gases (CO_2, CO, and H_2). The purpose of torrefaction is to provide a solid product that is appropriately standardized and has specific properties that meet the requirements for its end-use. The property by which torrefaction improves upon the original biomass feedstock include :

- Increased heating value;
- Increased product homogeneity in terms of physical (particle size distribution) and chemical properties;
- Improved grindability: as the torrefied material is more brittle, it requires less energy to grind it down to the required particle size (9, 10).

Carbonization is a slow pyrolysis process in which biomass is converted into a highly carbonaceous, charcoal-like material. The characteristic feature by which carbonization differs from other dry thermochemical conversion techniques is that the heating time is significantly longer than the pyrolysis reaction time. Typically carbonization consists of heating the biomass in an oxygen-limited environment, and reaction conditions are tailored to maximize the production of “char”, which is a generic term used to describe any solid material obtained through pyrolysis. Among the main products, it’s important to remember the charcoal and biochar. The term “charcoal” is used to describe the char produced by carbonization that is by purpose made as a fuel or as a reducing agent in metallurgical applications; instead, “biochar” is a name given to any type of biomass pyrolysis char.

1.2.2.2 Biochemical processes

Unlike thermochemical processes, biochemical processes occur at lower temperatures and have lower reaction rates. Fermentation is one of the common types of biochemical processes. It uses microorganisms and/or enzymes to convert a fermentable substrate into recoverable products (usually alcohol or organic acid). Ethanol is currently the most required fermentation product; glucose is the most common substrate, while pentose (sugar from hemicellulose), glycerol, and other hydrocarbons required the development of customized fermentation organisms to enable their conversion to ethanol.

Anaerobic digestion, instead, involves the bacterial breakdown of biodegradable organic material in the absence of oxygen over the temperature range from about 30-65 °C. The principal product is biogas (a gas mixture made of methane, CO_2, and other impurities)(5).

1.2.2.3 Mechanical Processes

These processes aim to perform a size reduction or a separation of feedstock components. Usually, this is applied first in order to prepare biomass within a specific size range according to the requirements. Biomass size reduction is a mechanical treatment that refers to either cutting or commuting processes that significantly change the particle size, shape, and bulk density of biomass.

The separation process involves the division of substrate into its components, while with extraction methods, valuable compounds are extracted and concentrated; lignocellulosic pre-treatment belongs to this category (5).

1.2.2.4 Chemical processes

These processes introduce a change in the chemical structure of the molecule by reacting with other substances. The most common chemical processes are hydrolysis and transesterification. The first one uses acids, alkalis, or enzymes to depolymerize polysaccharides and protein into their component sugar (e.g. glucose from cellulose) or derivate chemicals (e.g. levulinic acid from glucose). Transesterification is a process by which vegetable oil can be converted to methyl or ethyl esters of fatty acids, also called biodiesel (5).

1.3 Pretreatment of biomass

Pretreatment processes aim to increase accessible surface area, to decrystallize cellulose, and to remove hemicellulose and lignin, to make biomass more accessible for enzymatic hydrolysis. Many pretreatment strategies have been developed to enhance the reactivity of cellulose and to increase the yield of fermentable sugar. Typical goals of pretreatment include:

- ❖ Production of highly digestible solid that enhance sugar yield during enzyme hydrolysis;
- ❖ Avoiding the degradation of sugar;
- ❖ Minimizing the formation of inhibitors for subsequent steps;
- ❖ Recovery of lignin for conversion into valuable co-products.

Pretreatment technology can be classified into: physical, chemical, physicochemical, and biological.

1.3.1 Physical pretreatment

Physical pretreatment belongs to mechanical processes. As already mentioned, it involves the breakdown of biomass size and crystallinity by milling or grinding. The reduction of particle size leads to an increase of available specific surface and a reduction of the degree of polymerization (DP). These increase the total hydrolysis yield of the lignocellulose in most cases by 5-25%, and also reduce the technical digestion time (11, 12).

1.3.2 Chemical pretreatment

1.3.2.1 Alkaline

Alkaline pretreatment involves the use of bases, such as sodium, potassium, calcium, and ammonium hydroxide, for the pretreatment of lignocellulosic biomass. The use of alkali causes the degradation of ester and glycosidic side chains resulting in a structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose, and partial solvation of hemicellulose. Operating conditions are usually less severe than other pretreatments, and it

can be performed at ambient conditions, but longer pretreatment time is required. Another important aspect of this treatment is the change of the cellulose structure to a form that is denser and thermodynamically more stable than the native cellulose (11, 12).

1.3.2.2 Wet oxidation

Wet oxidation is the process of treating material with water and air or oxygen at a temperature above 120°C and pressure ranging from 16 to 32 atm. It was found that the initial reaction during wet oxidation is the formation of acids. The acids are formed by the solubilization of the acidic hemicellulose components, mainly xylan, by de-esterification of the acetate groups on the hemicellulose and by oxidation. As the acid concentration increases and the pH drops, hydrolytic reactions become favorable. More and more of the hemicelluloses are broken down into lower- molecular weight fragments that dissolve in the water. This reaction affects not only the hemicelluloses, but also the cellulose and lignin fractions; one important effect on cellulose in that its rate of acid hydrolysis is substantially increased after wet oxidation. On the other side, the amount of lignin removed after pretreatment ranges from 50% to 70% depending on the type of biomass pretreated and the operating conditions.

This technique can be combined with other pretreatment methods to increase the yield of sugar further; combining wet oxidation with alkaline pretreatment has been shown to reduce the formation of by-products, thereby decreasing inhibition (11, 12), Gary D. McGlnnls (13).

1.3.2.3 Acid pretreatment

Pretreatment of lignocellulose with acids at ambient temperature is done to enhance the anaerobic digestibility. The objective is to break the rigid structure of the lignocellulosic material to obtain its single components, that react following a different reaction pathway, as reported in figure 1.10, and to solubilize hemicelluloses, by this making cellulose more accessible.

The pretreatment can be done with dilute or concentrate acids. The most commonly used acid is dilute sulphuric acid (H_2SO_4). The primary reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose; solubilized hemicelluloses can be subjected to hydrolytic reactions producing monomers, furfural, hydroxymethylfurfural, and other (volatile) products in acidic environments. During acid pretreatment, solubilized lignin quickly condensates and precipitates. The solubilization of hemicellulose and the precipitation of solubilized lignin are more pronounced during strong acid pretreatment compared to the dilute one (11, 12).

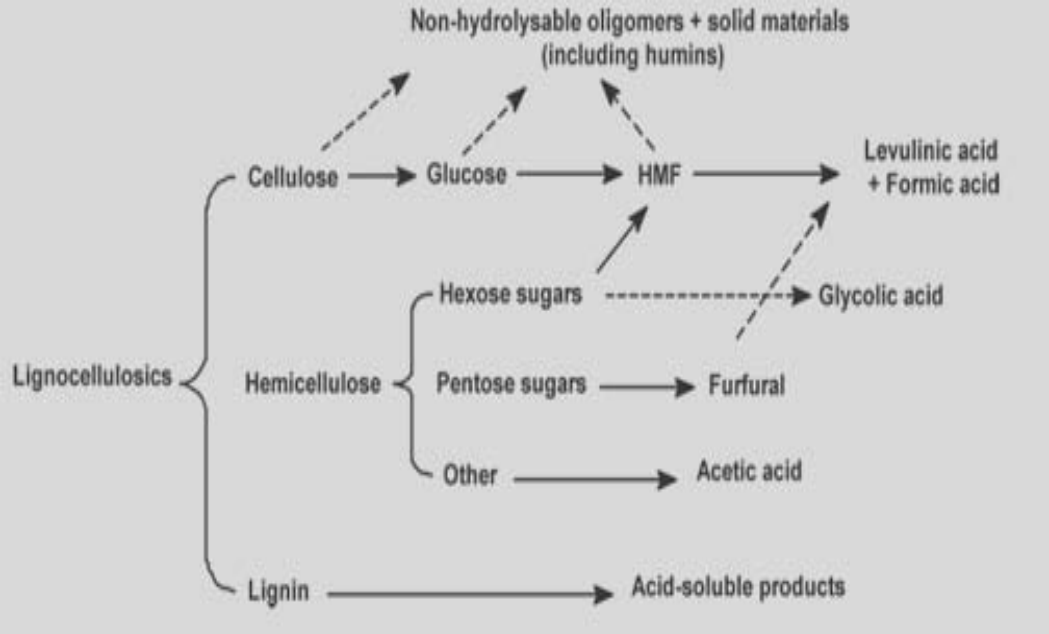


Figure 1.10: *The reaction products of cellulose, hemicellulose, and lignin (14)*

1.3.3 Physicochemical Pretreatments

During this pretreatment, the lignocellulosic biomass is heated. If the temperature increases above 150–180 °C, a small amount of lignocellulosic biomass, firstly the hemicelluloses and shortly after the lignin, starts to solubilize. During thermal processes, a part of the hemicellulose is hydrolyzed and forms acids (11, 12). Among the pretreatments, we recall the stream-explosion, the liquid hot water, and the supercritical fluids pretreatment.

1.3.3.1 Steam-Explosion

Steam-explosion pretreatment is one of the most commonly used pretreatment options, as it uses both chemical and physical techniques to break the structure of the lignocellulosic material. This hydrothermal pretreatment method subjects the material to high pressures and temperatures for a short duration of time, after which the system is rapidly depressurized, disrupting the structure of the fibrils. The disruption of the fibrils increases the accessibility of the cellulose to the enzymes during hydrolysis. Particle size is a major contributing factor to the effectiveness of the process, and it has been seen that relatively large particle sizes have

been able to yield maximum sugar concentrations. Three products are derived from steam explosion pretreatment :

- A solid containing less recalcitrant cellulose plus lignin;
- A liquid containing solubilized hemicellulose and some degradation products of lignin and pentoses, e.g., furan derivatives and phenolic compounds;
- Water vapor containing volatile compounds that are produced during pretreatment, e.g., 60-70% of the produced furfural (11, 15).

1.3.3.2 Liquid hot water (LHW) pretreatment (autohydrolysis)

This pretreatment is performed at elevated temperatures in the range of 150-230°C and high pressure to maintain water in liquid form. Indeed, autohydrolysis is the hydrolysis reaction of hemicellulose in aqueous medium. In this process, the extensive hydrolysis of hemicellulose to oligomers and monomers occurs, while lignin content changes are moderate, and the glucan losses are negligible. During the pretreatment, hemicaetal linkages are cleaved, and thus acetyl groups are released in the form of acetic acid. Water also acts as an acid at elevated temperatures. The presence of acetic acid, along with the acidic nature of water, in the main driver for the production of solubilized monomers and oligomers from hemicellulose. As the previous treatment, also LHW increases the accessible surface area of the biomass by hemicellulose and lignin removal. Enhanced accessibility of enzymes to biomass increases the enzymatic digestibility of lignocellulose (15).

1.3.3.3 Supercritical Fluid (SCF) pretreatment

A supercritical fluid is a material which can be either liquid or gas, used in a state above the critical temperature and critical pressure where gases and liquids can coexist. It shows unique properties that are different from those of either gases or liquids under standard conditions; it possesses a liquid-like density and exhibits gas-like transport properties of diffusivity and viscosity. Thus, SCF can penetrate the crystalline structure of lignocellulosic biomass, overcoming the mass transfer limitations encountered in other pretreatments. An advantage of this process is the lower temperature used, which aids in the stability of the sugar and prevents degradation observed in other pretreatments.

Supercritical carbon dioxide (CO_2), with a critical temperature (Tc) of 31°C and critical pressure (Pc) of 7.4 MPa, has excellent potential for biomass pretreatment even if it can be combined with steam to the best overall yield of sugar (11).

1.4 Hydroxymethylfurfural and Levulinic Acid

Originally, glucose and fructose were used as raw materials for the synthesis of HMF and LA. The earliest paper published on HMF production was in 1875, in which acid catalyst was employed for the conversion of sugar. HMF derived from biomass, despite not receiving much attention until the 1980s (16).

HMF is the product of the acid-catalyzed dehydration of cellulose biomass at mild temperatures. It is a platform molecule that can be obtained not only from fructose but also (more recently) from glucose via isomerization to fructose, as well as directly from cellulose. It is used as an intermediate for the production of pharmaceuticals, fine chemicals, and furan-based polymers, which have recently been proposed as a biorenewable replacement for PET plastic (17). Furthermore, it is advantageous not only as intermediate for the production of the biofuel dimethylfuran (DMF) but also for essential molecules such as levulinic acid, 2,5-furandicarboxylic acid (FDA), 2,5-diformylfuran (DFF), dihydroxymethylfuran and 5-hydroxy-4-keto-2-pentenoic acid, as reported in figure 1.11. In addition, Table 1.1 shows the main properties of HMF.

Table 1.1: Physical properties of HMF

Property	Value
Molecular mass [g/mol]	126.11
Melting point [°C]	28-35
Boiling point [°C]	114-116
Density [g/cm^3]	1.20

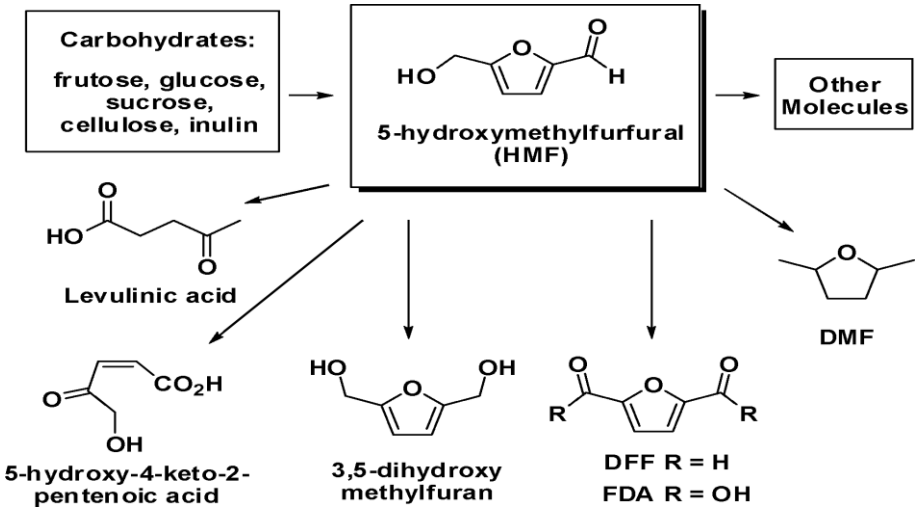


Figure 1.11: *Application of HMF*

The preparation of HMF mainly starts from lignocellulosic biomass. The conversion of lignocellulose into HMF generally includes three steps :

1. Hydrolysis of cellulose into glucose;
2. Isomerization of glucose into fructose;
3. Dehydration of fructose into HMF and by-products such as humins, 2-hydroxy acetyl, and 5-methylfurfural.

The conversion of C6 sugar into HMF was investigated in many reports, and the mechanism of acid-catalyzed production process has been carefully studied to explore the possible pathways.

Fructose is initially produced from glucose through mutarotation and isomerization. To explain the dehydration process of fructose into HMF, there are two types of mechanisms proposed by researchers and reported in figure 1.12:

1. A cyclic route through the fructofuranosyl intermediate;
2. An acyclic route through the 1,2-enediol intermediate.

The cyclic route begins with cyclic fructofuranose. A ternary carbenium cation is produced from the dehydration of the hemiacetal at C2. Then HMF is formed through two consecutive β-dehydrations.

In the acyclic pathway, fructose was dehydrated into 1,2-enediol (an intermediate of the reaction), which is assumed as the rate-limiting step. HMF is produced via two conservative β-dehydration and a ring closure reaction (18).

It has been widely accepted that the reaction pathway for levulinic acid and HMF production is quite similar, and HMF is a crucial intermediate for levulinic acid formation. By rehydration of HMF with two molecules of water, levulinic acid, together with formic acid as by-product, are produced (17, 19)

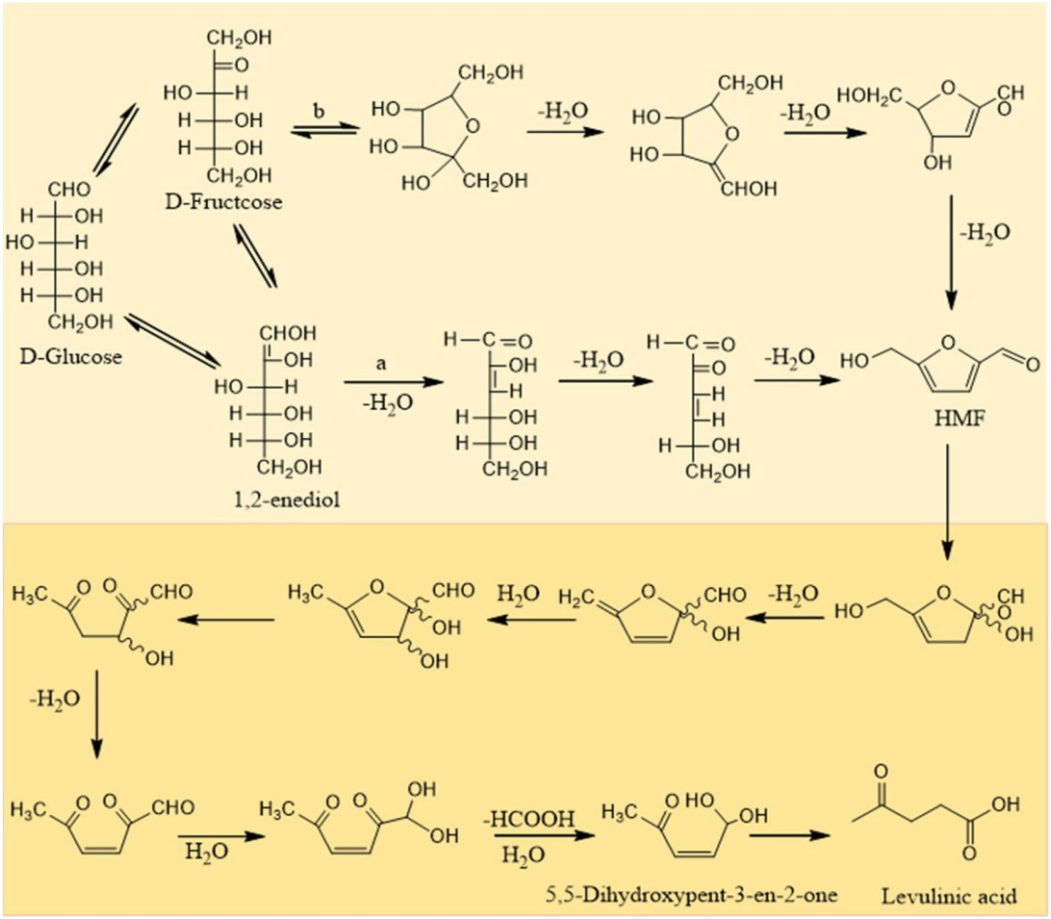


Figure 1.12: *Reaction pathway for levulinic acid production ([16])*

1.4.1 Levulinic acid

Levulinic acid ($C_5H_8O_3$), also know as leavulinic acid, 4-oxopentanoic acid, β-acetylpropionic acid, and γ-ketovaleric acid, is a water-soluble, organic compound with a

ketone (= O) and carboxylic group (-COOH), as reported in figure 1.13, which give it a wide range of functionality and reactivity. It is also soluble in alcohol and ether. The reactivity of levulinic acid makes it an ideal intermediate for the production of useful derivatives.

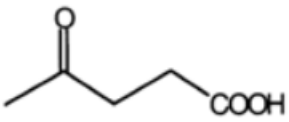


Figure 1.13: *Molecule of levulinic acid ([18])*

Table 1.2 reports some physical and chemical properties related to levulinic acid.

Table 1.2: Properties of levulinic acid

Properties	Values
Melting point	37 °C
Boiling point (101.325 kPa)	245-246 °C
Specific density (20°C)	1.14
Refractive index (25°C)	1.4396
Flashpoint	138 °C
Surface tension (25°C)	39.7dyne cm-1
Heat of vaporisation (150°C)	0.59 kJ mol-1
Heat of fusion	79.8 J mol-1

The increased relevance of levulinic acid is in part due to its inherent capability to serve as a biobased chemical intermediate to produce fuels via conventional petrochemical technology (19). However, it also has potential applications such as textile dye, antifreeze, animal feed, solvent food, pharmaceutical compounds, and even cosmetics, as briefly shown in figure 1.14.

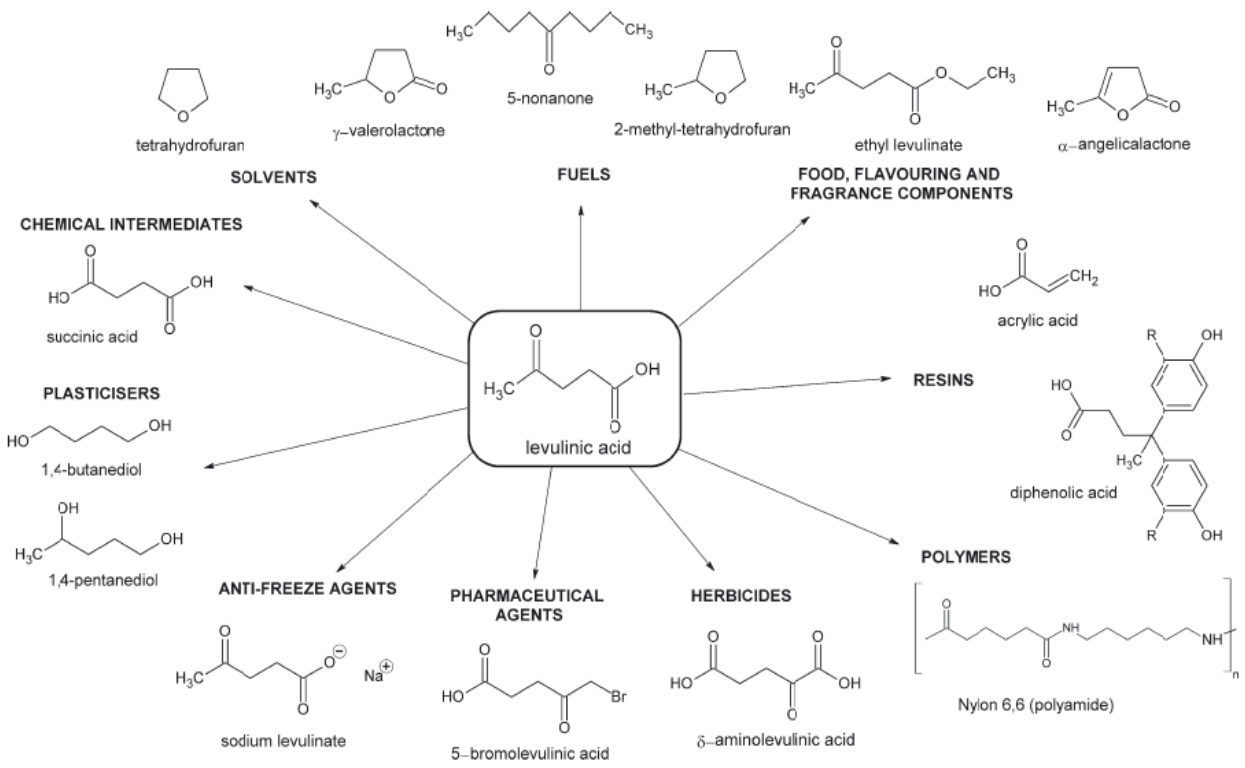


Figure 1.14: *Possible application of levulinic acid ([19])*

Other chemical applications include its use as a chiral reagent, polyhydroxyalkanoates, lubrificant, adsorbent, and in polymer industry. Moreover, LA can be chemically converted to 2-methyl-tetrahydrofuran and various levulinate esters that are used as gasoline and biodiesel additives, respectively; this is the most important application of levulinic acid and its derivatives (20).

More in detail, to expand the market for levulinic acid, researchers have mainly focused their attention on three products, which are methyltetrahydrofuran (MTHF), δ-aminolevulinic acid (DALA), and diphenolic acid (DPA).

MTHF is mainly used as a transportation fuel extender. Because it is miscible with gasoline, up to 60% by volume without adverse engine performance, and hydrophobic, MTHF could be blended at the refinery and transported by pipeline. A direct conversation of levulinic acid to MTHF is reported to occur in only low yield; however, several indirect LA processing routes that lead to MTHF are reported in figure 1.15.

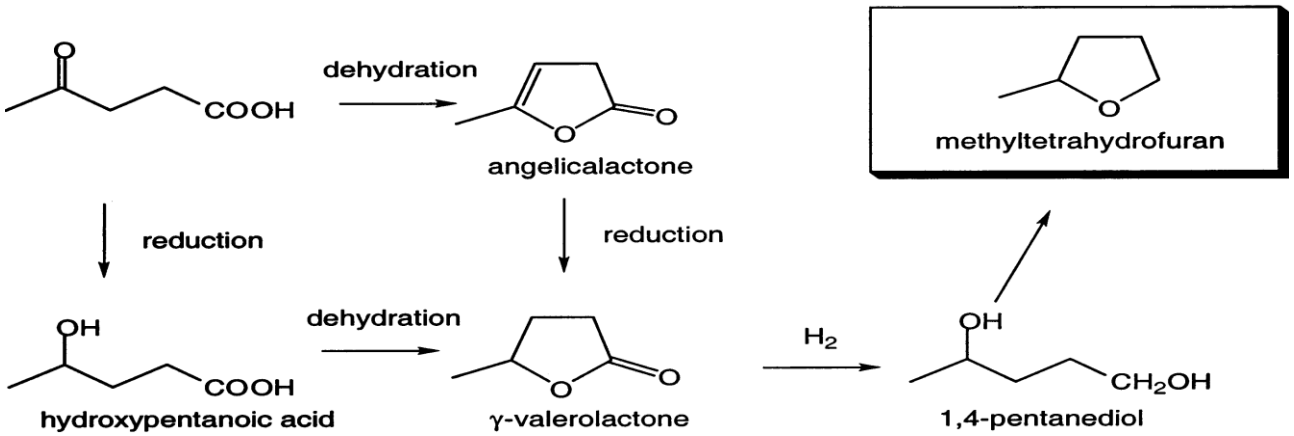


Figure 1.15: *Reaction route for HMF production ([20])*

A central intermediate to LA-based MTHF processes is ϒ-valerolactone (GLV). It holds prime importance as a renewable carbon source for green solvents and transportation fuels. It can be further converted to 1,3-pentadiene (piperylene) through 1,4-pentanediol (PDO). PDO on dehydrogenation in the presence of acid produces MTHF (20, 21).

DALA is a broad spectrum, biodegradable herbicide that shows high activity towards dicotyledonous weeds. More recently, it has been found to be useful as an insecticide and as a component in photodynamic therapy as a cancer treatment. A variety of synthetic routes for DALA have been reported. DALA can be prepared form N-substituted amino acids, and through a stepwise build of the carbon chain. More elegant strategies used cyclic starting materials such as furfurylamine, 5-hydroxy-2-pyridone, N-methoxycarbonyl-3-piperidinones, and 5-hydroxymethyl-2-furfural. The starting material for the preparation of DALA is levulinc acid, which requires the formation of a C-N bond at the C5 carbon. The most common approach for activating the C5 position toward amination is the bromination of levulinic acid in an alcohol medium. In figure 1.16, the conventional synthesis of DALA starting from the bromination of levulinic acid to get 5-bromolevuinic acid is shown; to introduce the key amino group, it undergoes treatment with potassium phthalimide, giving an intermediate component. Its acid hydrolysis gives the desired product.

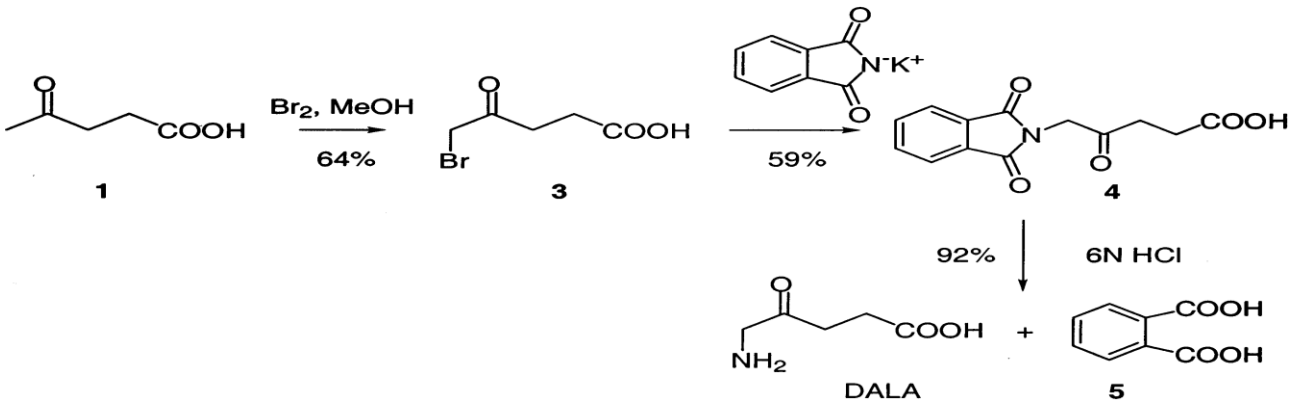


Figure 1.16: *Reaction pathway for DALA production* (21)

The major difficulties with this approach are the relatively low yields in the first two-stages and the generation of a large amount of waste products (21).

Diphenolic acid (DPA) is another material that has found wide application in the production of polymers and other materials. Figure 1.17 reports its structural formula.

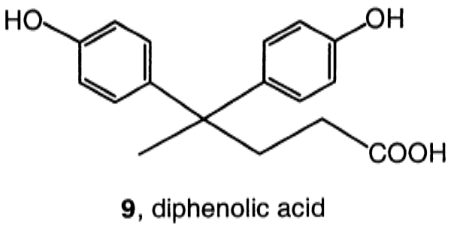


Figure 1.17: *Chemical structure of DPA (22)*

It is structurally analog to bisphenol A (BPA). It can serve in many of the same applications as BPA for the synthesis of industrially essential epoxy resins and polycarbonates that are used in the production of reinforced plastics and other chemical materials. It can be a direct replacement for BPA in polycarbonates, epoxy resins, polyacrylates, and other polymers. It contains a carboxyl group, absent from BPA, which confers as additional functionality that is useful in polymer synthesis. It can be easily synthesized from the condensation reaction of LA with two moles of phenol in the presence of Bronsted acid (22).

1.5 Aim of the thesis

This thesis aims to investigate the possibility of improving the production process of levulinic acid from lignocellulosic biomass (mainly corn stover), and to find out the best-operating conditions that guarantee the highest levulinic acid production. Generally, levulinic acid is produced by the acid hydrolysis of biomass. In this thesis, the effectiveness of a hydrothermal pretreatment process on the main product's yield is discussed. Experimental measurements will be first carried out, and the results obtained will be used to develop a flowsheet of a possible industrial process.

Chapter 2

Materials and Methods

This research aims not only to assess the yield of levulinic acid obtained from the acid hydrolysis of corn stover, but also to verify if, by introducing a hydrothermal pretreatment of the biomass, it can be improved. In this chapter, all the materials, the chemicals, and the experimental procedures applied are presented. Finally, the methods and the instrumentations used to analyze the products are discussed.

2.1 Chemical compounds

The biomass used for the investigation is a lignocellulosic biomass. In particular corn stover, obtained from the Idaho National Laboratory was used as feedstock. This feedstock was reduced to 0.23 mm and 2.68 mm before its utilization. The measured moisture content was 4 wt%. Table 2.1 reports the composition analysis of the corn stover feedstock, measured according to the protocol for the determination of structural carbohydrates, lignin and ash in biomass.

Table 2.3: Composition of Corn stover

	wt.%
Cellulose	28.15
Hemicellulose	22.51
Lignin	26.95
Ash	6.51
Other	15.88

Moreover, in order to check the functionality of the acid hydrolysis' reactors, some additional experiments were carried out testing pure cellulose biomass.

Based on a literature review on the hydrothermal pretreatment and the following acid hydrolysis of corn stover, the presence of the following chemicals is analyzed in the products: acetic acid, lactic acid, formic acid, hydroxymethylfurfural (HMF), levulinic acid, and furfural; these are used as standard to create the calibration curve for the high-pressure liquid chromatography (HPLC) and the total organic carbon/total nitrogen (TOC-TN) analysis of products. Table 2.2 reports the purity of each chemical and its brand.

Table 2.2: Brand and concentration of chemicals

	Brand	Concentration
Levulinic acid	Acros Organics	98+%
Formic Acid	Fisher Science	88%
Lactic acid	Acros Organics	90% (solution in water)
Furfural	Alfa Aesar	98%
HMF	Fisher Science	98%
Acetic Acid	Fisher Science	99.9%

2.2 Experimental set-up

In this paragraph, the equipment used during the hydrothermal pretreatment and the following acid hydrolysis of biomass is presented.

2.2.1 Operating conditions of hydrothermal pretreatment

The hydrothermal pretreatment is carried on using the apparatus shown in figure 2.1 and schematized in figure 2.2. It consists of a high-pressure liquid peristaltic pump, an electrical tubular furnace, a water heat exchanger, a back-pressure regulator that keeps the pressure constant, and a thermocouple. The reactor is a 0.54'' ID, 65 ml volume, 48.5 cm length, high-pressure stainless steel tube. The temperature of the reaction zone is measured using a 1/16'' thermocouple placed inside the biomass bed.

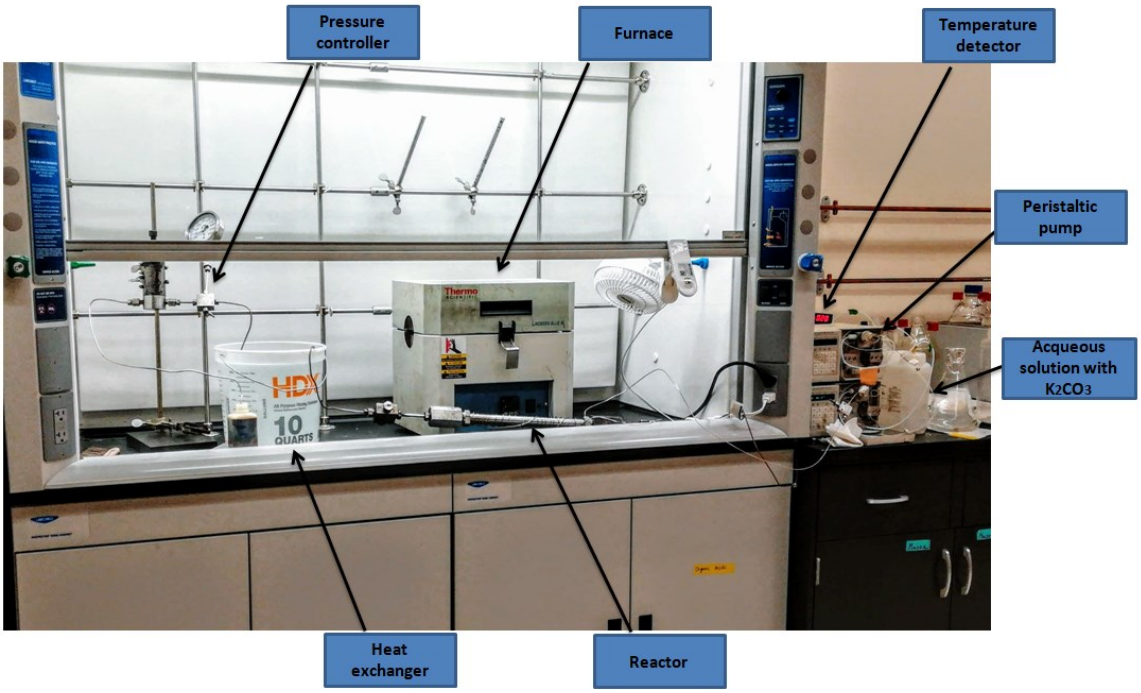


Figure 2.2: *Equipment for hydrothermal pretreatment*

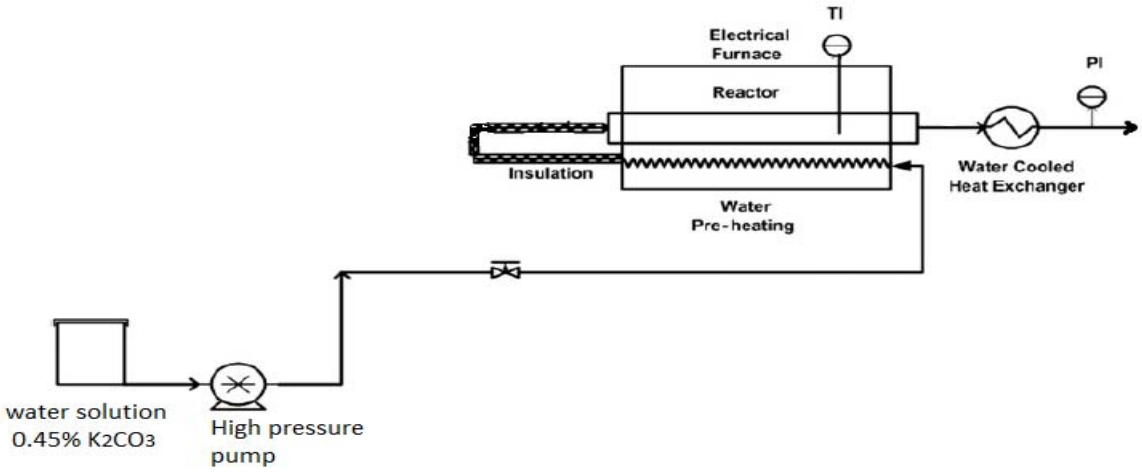


Figure 3.2: *Scheme of the pretreatment process (23)*

Table 2.3 shows the operating conditions of the process adopted in this work. As reported, this pretreatment can be divided into three steps, each of which takes place for 20 min: the preheating, the reaction, and finally the cooling phase.

Table 2.3: Operating conditions for the hydrothermal process

Temperature of Reaction [°C]	190	
Pressure of Reaction [atm]	34.02	
Biomass [g]	11	
Catalytic solution	0.45% (w/v) K_2CO_3	
Flowrate [ml/min]	Preheating	2.5
	Reaction	2.5
	Cooling	9.9
Time/step [min]	20	

As reported in table 2.3, only the reaction step is carried out in isothermal condition (190°C); this is because, during the preheating, the furnace heats the reactor until it arrives at 190°C, in 20 min; instead during the cooling phase, the solution flowrate is increased at 9.9 ml/min in order to reach room temperature in 20 min. In figure 2.3 is reported the temperature profile.

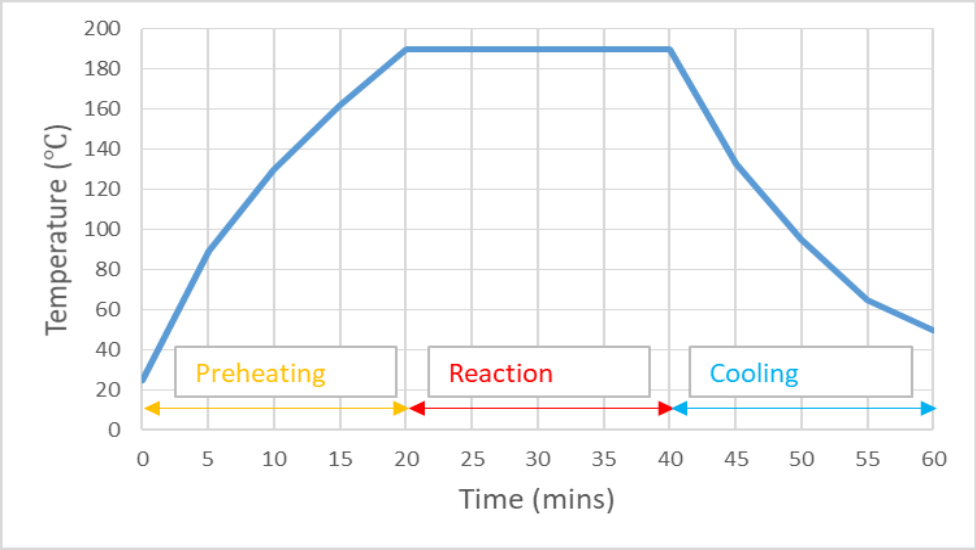


Figure 2.4: *Temperature profile for hydrothermal process*

The pretreatment process aims to change the chemical and physical structure of biomass, to increase the accessibility of enzymes during the following hydrolysis as shown in figure 2.4

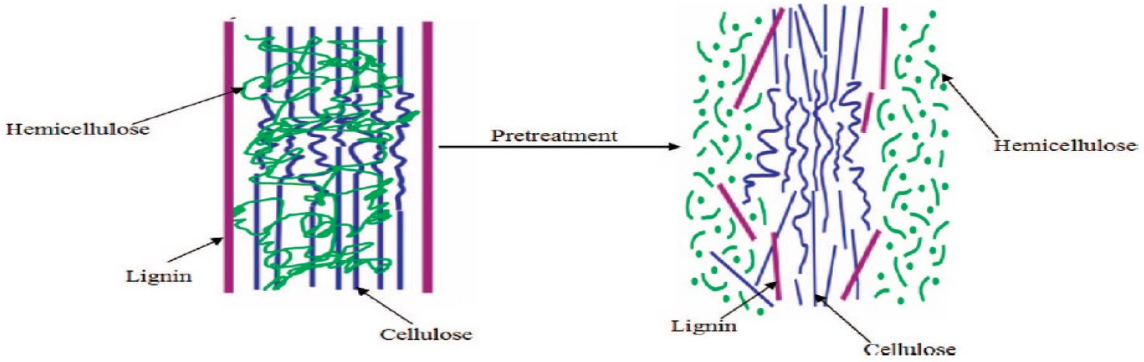


Figure 2.5: *Structural composition of lignocellulose biomass before and after the pretreatment*

To reach this goal an aqueous solution with 0.45% (w/v) K_2CO_3 was used, which was continuously pumped through the biomass; a stainless steel frit (2μm pore size) was placed in the reactor outlet to reduce the loss of biomass in the liquid stream. It was decided to use an alkaline solution to selectively remove the lignin and hemicellulose and to increase both the yield and the production rate of monomeric sugar.

To perform the pretreatment, the reactor was filled with 11 g of raw biomass from one side; the hydrothermal process starts when the reactor is put inside the furnace, and the preheating step begins feeding 2.5 ml/min of the preheated solution, continuously pumped through the biomass. Afterward, the reaction can start; it takes place, isothermally, for 20 min. This condition is achieved by manually controlling the furnace temperature using a fan. The last step is cooling; it takes place for 20 min, increasing the flowrate up to 9.9 ml/min to stop the reaction by reaching room temperature. The liquid coming out from the reactor is cooled down using a bucket full of cold water, and it is collected for the following analysis. In table 2.4 is reported an average value of hydrolyzed liquid, collected for each step.

Table 2.4: *Amount of hydrolyzed liquid collect during pretreatment.*

Step	Amount [ml]
Preheating	60
Reaction	50
Cooling	200

2.2.2 Operating conditions of acid hydrolysis

The biomass left from the hydrothermal pretreatment was withdrawn and then hydrolyzed. This process aims to convert the pretreated biomass, which is mainly composed of cellulose, into levulinic acid following the pathway reported in figure 2.5.

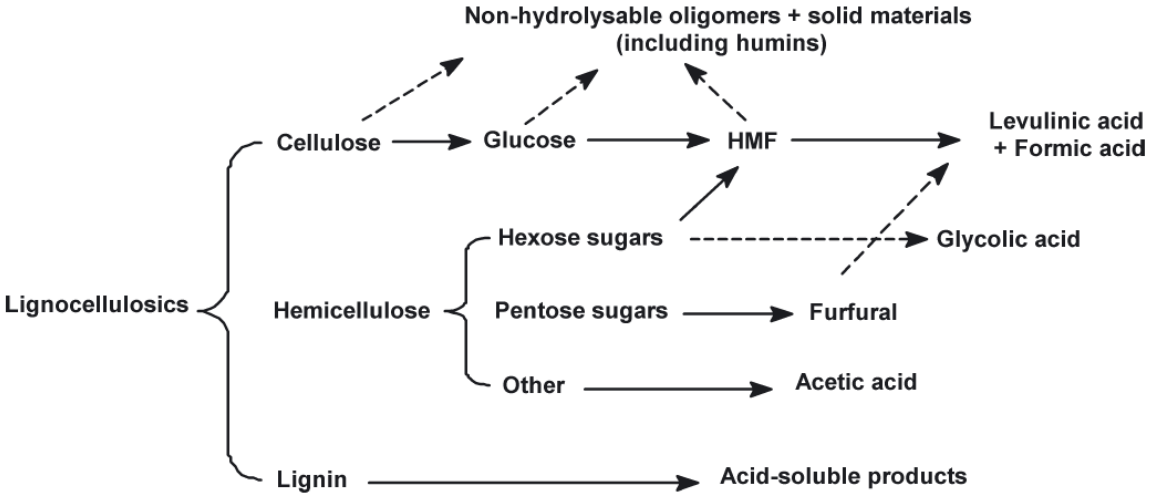


Figure 2.6: *Pathway for the production of levulinic acid (14)*

This reaction was carried on in isothermal conditions using a batch stainless steel reactor heated up through a sand bath, model 9-D TECHNE with temperature controller, as reported in figure 2.6.

Figure 2.7: *Sandbath for acid hydrolysis process*

Four reactors are built, using Swagelok components, to carry out more experiments contemporarily by changing the reaction time or keeping it constant; their configuration and size are shown, respectively, in figure 2.7 and table 2.5.

Figure 2.8: *Reactors for acid hydrolysis*

Table 2.5: Size of reactors

R [cm]	1.05
H [cm]	15
Volume of cylinder measured [ml]	51.5
Upper connection measured [ml]	1.35
Lower connection measured [ml]	1.35
Total volume measured [ml]	54.2

To check the temperature during the reaction, one of the reactors has a thermocouple, and its size is reported in table 2.6.

Table 2.6*:* Size of the reactor with a thermocouple

R [cm]	1.05
H [cm]	15
Volume of cylinder measured [ml]	51.5
Connection with thermocouple measured [ml]	5

Lower connection measured [ml]	1.35
Total volume measured [ml]	57.85

Process conditions are defined by choosing the operating temperature. As already said, the acid-hydrolysis process is carried out in isothermal condition. According to literature review, the temperature usually changes depending on the biomass feedstock used, but it ranges between 170-220 °C, so it was decided to work at 190°C. To keep water in its subcritical condition, a software form NIST was used to define the thermophysical properties of a fluid system; this is called IAPWS-95 (International Association for the Properties of Water and Steam), it is based on experimental data of the thermodynamic properties of water. It has the form of the fundamental equation explicit in Helmholtz free energy (f), as reported in equation 2.1.

$$f(\rho, T) = f^0(\rho, T) + f^r(\rho, T) \tag{2.1}$$

As shown in Eq. 2.1 the Helmholtz free energy has density (ρ) and temperature (T) as independent variables. Moreover, it is commonly split into f^0 that represents the properties of the ideal gas at given T and ρ and f^r that takes into account the residual fluid behavior.
The Helmholtz free energy of the ideal gas is given by equation 2.2

$$f^0(\rho, T) = h^0(T) - RT - Ts^0(\rho, T) \tag{2.2}$$

For the ideal gas, the enthalpy (h^0) is a function of temperature only, and the entropy (s^0) depends both on temperature and density.
The residual part of Helmholtz free energy is used to describe the real thermodynamic behaviour of fluid in the whole fluid region, and it must be determined empirically.
Usually, the Helmholtz free energy is used in its dimensionless form $\varphi = f/(RT)$, so equation 2.1 turns into equation 2.3

$$\varphi(\delta, \tau) = \varphi^0(\delta, \tau) + \varphi^r(\delta, \tau) \tag{2.3}$$

Where $\delta = \rho/\rho_c$ is the reduced density and $\tau = T_c/T$ is the inverse reduced temperature with ρ_c and T_c the critical density and the critical temperature, respectively (24).

For a given temperature, this software calculates the range of pressure at which the water is in its liquid phase, and it defines its density at those conditions of pressure and temperature.

Figure 2.8 reports the saturation pressure of water in function of temperature.

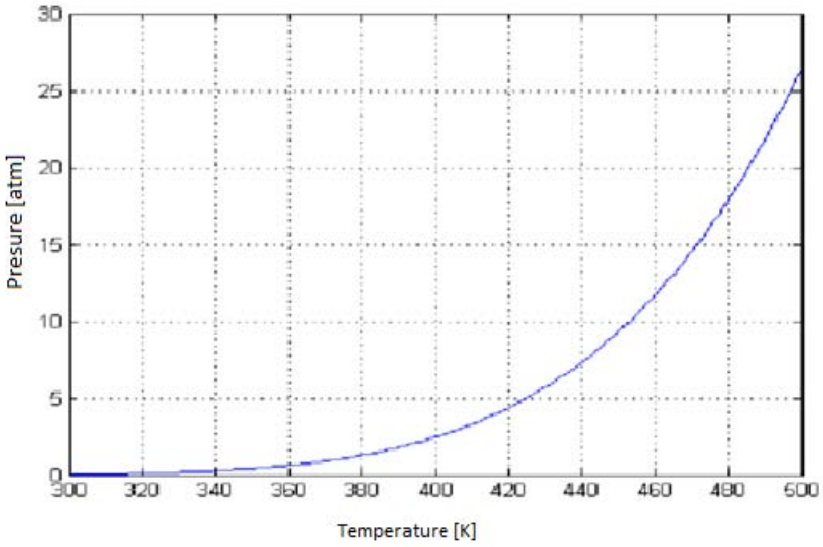


Figure 2.9: *Saturation pressure of water respect to temperature*

As shown in figure 2.8, at 190°C, or 463.15 K, the saturation pressure of water is around 12.38 atm. Therefore it is decided to work at 34.02 atm to be as far as possible from the changing phase's point. Table 2.7 reports the operating conditions of acid hydrolysis.

Table 2.7: Property of water at 190°C

Property	Value
Temperature [°C]	190
Pressure [atm]	34.02
Density of water [g/ml]	0.87

Each reactor was filled with an amount of dry biomass equal to 2% of the volume of solution. Table 2.8 reports other operating conditions used for the acid-hydrolysis process.

Table 2.8: Operating conditions for acid hydrolysis

	Reactor without thermocouple	Reactor with thermocouple
Total volume [ml]	54,2	57,85
Volume of acid solution [ml]	47,56	50,77
Dry biomass [g]	0,951	1,01

As already said, both raw and pretreated corn stover is hydrolyzed to assess the effect of the pretreatment. Besides, to test the functionality of these reactors, some experiments using pure cellulose were also run. The aim is to optimize the levulinic acid production by analyzing the effect of time and acid concentration on it. In table 2.9, a schematic resume of different sets of the experiments done is summarized, reporting also their operating conditions.

Table 2.9: Resume of acid-hydrolysis experiments and their operating conditions.

Biomass	Operating conditions (T= 190 °C, heating Time = 15 min)	Total number of experiments	Reaction Time (min)
Cellulose	5% H2SO4	6	45, 75, 105
Pretreated biomass	no acid	1	105
Raw biomass		1	105
Cellulose	2% H_2SO_4	13	15, 45, 75, 105, 135, 165
Raw biomass		29	5, 15, 45, 75, 105, 135, 165, 195, 225
Pretreated biomass		24	5, 15, 45, 105, 165

In literature, the possibility to carry out the acid hydrolysis using different types of acid solution (HCL, HBr, H_2SO_4) is reported. However, in this work, it is decided to run all the experiments using sulphuric acid (72% w/w).

Resuming the procedure, the acid hydrolysis is carried out, filling the reactors with 47.56 ml of acid solution (or 50.77 ml if reactor with a thermocouple is used), and 0.951g of dry

biomass (or 1.01 g in the other case). Reactors are sealed and put inside a hot sand-bath, fluidized through airflow. After 15 min of heating, the hydrolysis reaction starts and is kept running for different reaction times, as reported in table 2.7. After that, reactors are removed and placed directly in a cold water bath to stop the reaction. Liquid and solid products are then analyzed.

2.3 Determination of Structural Carbohydrates and Lignin in biomass

The chemical composition of raw corn stover and pretreated biomass was determined following the analytical procedure for the determination of structural carbohydrates and lignin in biomass (25).

As known, carbohydrates and lignin make up a major portion of biomass samples. Structural carbohydrates, namely cellulose and hemicellulose, are bounded in the matrix of the biomass, while non-structural carbohydrates can be easily removed using extraction or washing steps. This procedure uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The lignin fractionates into acid-insoluble material, that may also contain ash and protein, and acid-soluble material, which are measured by UV-Vis spectroscopy. During hydrolysis, the polymeric carbohydrates are hydrolyzed into the monomeric forms, which are soluble in the hydrolysis liquid. They are then measured by HPLC.

To realize these experiments, the following equipment was used:

- Analytical balance;
- Convection dry oven, with temperature control of 105 ±3°C;
- Muffle furnace, set at 575±25°C;
- Water bath set at 30±3°C;
- Autoclave for liquids, set at 121±3°C;
- Filtration setup, equipped with a vacuum pump;
- HPLC system equipped with refractive index detector and the Biorad Aminex HPX-87P column with ionic form H+/CO3- deashing guard column;
- UV-Visible spectrophotometer;

The reagents used for these experiments were:

- Sulfuric acid, 72% w/w (specific gravity 1.6338 at 20°C);
- Calcium carbonate;
- Water, purified, 0.2 μm filtered;
- High purity standards: D-cellobiose, D(+)glucose, D(+)xylose, D(+)galactose, L(+)arabinose, and D(+)mannose.

Firstly, the crucibles are placed in the muffle furnace at 575°C for at least four hours, and then they are weight. Some pressure tubes are then with 300 ± 10 mg of the sample of biomass and 3.00 ± 0.01 mL of 72% sulfuric acid; using a Teflon stir rod, the sample is thoroughly mixed. Tubes are placed in a water bath for 60 min. Using a stir rod, the sample is stirred every 5 to 10 min without removing the sample from the bath, in order to uniform the hydrolysis. Later, tubes are removed from the bath, and the acid is diluted to 4% concentration by adding 84 ± 0.04 mL of deionized water. Then, the Teflon caps are screwed securely and the sample is mixed by inverting the tube several times.

A set of sugar recovery standards (SRS) that takes through the remaining hydrolysis is prepared, and it is used to correct for losses due to the destruction of sugars during dilute acid hydrolysis. SRS should include D-(+)glucose, D-(+)xylose, D-(+)galactose, -L(+)arabinose, and D-(+)mannose; sugar concentrations should be chosen to most closely resemble the concentration of sugars in the test sample; in this case, 10 mg of each sugar are used, and 10 ml of deionized water and 348 μL of 72% sulphuric acid are added, and transferred in a pressure tube capped tightly. Finally, all the tubes are placed in an autoclave for one hour at 121°C; after completion of the autoclave cycle, the hydrolyzates slowly cool down to near room temperature before caps are removed.

2.3.1 Analysis of the sample for acid-insoluble lignin

The autoclaved hydrolysis solution is vacuum-filtered through one of the previously weighed filtering crucibles and the filtrate is collected in a filtering flask. It is important to transfer an aliquot (approximately 50 ml) into a storage bottle that is later used to determine acid-soluble lignin as well as carbohydrates (see section 2.3.3). Deionized water is used to quantitatively transfer all remaining solid out of the pressure tube into the filtering crucible. After this, the crucible and acid-insoluble residue are dried at 105°C for a minimum of four hours. Later, the

sample is removed from the oven and cooled in a desiccator, and the weight of the crucible is recorded. The difference in weight of the crucibles gives the amount of acid-insoluble lignin.

2.3.2 Analysis of the sample for acid-soluble lignin

First of all, a background of deionized water is run using a UV-Visible spectrophotometer. Then, the absorbance of the sample of hydrolysis liquor obtained from the previous step is measured at an appropriate wavelength on the same instrument. A dilution of 1.33 and 2, respectively for pretreated biomass and raw biomass sample, was necessary to bring absorbance into the range of 0.7-1.0. Table 2.8 reports the absorptivity constants for acid-soluble lignin measurement.

The amount of acid-soluble lignin (%ASL) is calculated according to the equation 2.4 :

$$\%\mathrm{ASL} = \frac{\mathrm{UV_{abs}} \times \mathrm{Volume_{hydrolysis\ liquor}} \times \mathrm{Diluition}}{\varepsilon \times \mathrm{ODW_{sample}} \times \mathrm{Pathlengh}} \times 100 \qquad (2.4)$$

Where :

- $\mathrm{UV_{abs}}$ is the average UV-Vis absorbance for the sample at an appropriate wavelength, in our case it is equal to 0.8 and 0.7 for raw and pretreated biomass samples, respectively;
- $\mathrm{Volume_{hydrolysis\ liquor}}$ is the volume of filtrate, 86.73 ml:
- $\mathrm{Dilution} = \frac{\mathrm{Volume_{sample}} + \mathrm{Volume_{diluting\ solvent}}}{\mathrm{Volume_{\ sample}}}$, it is equal to 2 and 1.33 for raw and pretreated biomass samples, respectively;
- ε is the absorptivity of biomass at a specific wavelength, equal to 30 L/(g cm)
- $\mathrm{ODW_{sample}}$ is the weight of sample in milligrams; it is 288 mg and 300 mg for raw and pretreated biomass samples, respectively;
- Pathlength is the pathlength of UV-V in cm; in this case, it is equal to 1 cm.

2.3.3 Analysis of the sample for structural carbohydrates

First of all, a series of calibration standards containing the compound to be analyzed is prepared. These five standards are reported in table 2.10.

Table 2.4: Standards for the analysis of structural carbohydrates

	Tiny std			Low std			Med std			Med-High std			High std		
	Original conc	Dilution	conc (mg/ml)	Original conc	Dilution	conc (mg/ml)	Original conc	Dilution	conc (mg/ml)	Wt	vol	conc (mg/ml)	Wt	vol	conc (mg/ml)
Cellobiose				4,004	0.6 in 25	0,096	2,598	5 in 10	1,29	0,26	100	2,59	0,4	100	4
Glucose	0,2	0.6 in 50	0,048	4	0.6 in 25	0,096	2,604	5 in 10	1,3	0,26	100	2,6	0,4	100	4
Xylose	0,2	0.6 in 50	0,048	4,003	0.6 in 25	0,096	2,605	5 in 10	1,3	0,261	100	2,6	0,4	100	4
Galactose	0,2	0.6 in 50	0,048	4,001	0.6 in 25	0,096	2,603	5 in 10	1,3	0,26	100	2,6	0,4	100	4
Arabinose	0,2	0.6 in 50	0,048	3,999	0.6 in 25	0,095	2,596	5 in 10	1,29	0,26	100	2,59	0,4	100	3,99
Mannose	0,2	0.6 in 50	0,048	4,004	0.6 in 25	0,096	2,604	5 in 10	1,3	0,26	100	2,6	0,4	100	4

Approximately 20 ml of each liquor hydrolysis sample obtained in the previous step are transferred in a 50 ml flask; each sample is neutralized to pH 5-6 by adding calcium carbonate. It is important to add this base slowly and continuously monitoring the pH by a pH-paper. After reaching pH 5–6, the calcium carbonate addition is stopped, the sample is allowed to settle and decant off the supernatant. The pH of the liquid after settling is approximately 7. The sample is prepared for HPLC analysis filtering the decanted liquid through a 0.2 μm filter into an autosampler vial. Finally, the calibration standard and the samples are analysed by HPLC using a Biorad Aminex HPX-87P column equipped with the appropriate guard column. HPLC conditions are:

- Injection volume: 10–50 μL, dependent on concentration and detector limits;
- Mobile phase: HPLC grade water, 0.2 μm filtered and degassed;
- Flow rate: 0.6 mL/minute;
- Column temperature: 80–85 °C;
- Detector temperature: as close to column temperature as possible;
- Detector: refractive index;
- Run time: 35 minutes;

2.4 Determination of Ash in Biomass

The amount of inorganic material in biomass, either structural or extractable, should be measured as part of the total composition. The ash content is a measure of the mineral content and other inorganic matter in biomass, and it is used, in conjunction with other procedures, to determine the total composition of biomass samples. This analysis is run following the protocol Determination of Ash in Biomass from NREL (26).

To run this analysis, the following apparatus is used:

- Muffle furnace, equipped with a thermostat, set to 575± 25 °C;
- Analytical balance;
- Ashing crucibles, 50 mL, porcelain;
- Convection drying oven

Firstly, two crucibles are put into the muffle furnace at 575 °C for a minimum of four hours. After that, the crucibles are removed and placed directly into a desiccator to cool down at room temperature and then weighed. 1 g of dry biomass is weighed and divided into the two crucibles, then placed into a furnace again for 24 hours; thereafter, the crucibles are removed and placed directly in a desiccator to cool down, and weighed them again. The amount of ash can be calculated by the formula 2.5:

$$\%\text{Ash} = \frac{\text{Weight}_{\text{crucible+biomass after pretreating}} - \text{Weight}_{\text{crucible}}}{\text{Weight}_{\text{dry biomass}}} \times 100 \quad (2.5)$$

2.5 Analysis of product from hydrothermal pretreatment and acid hydrolysis

From the hydrothermal pretreatment, a solid product is obtained, which is the pretreated biomass, and three liquid products are collected from the preheating, reaction, and cooling steps. Instead, from acid hydrolysis we get a liquid rich in levulinic acid and a solid residue.

2.5.1 *pH, TOC-TN, Elemental Analysis, and HPLC*

Initially, the pH of all the three liquids is checked using a standard pH-meter. The higher is the amount of lignin and hemicellulose removed, the lower is the pH of the solution.
After that, the concentration of total organic carbon and total nitrogen content in the liquid samples is analysed, in order to determine an overall balance of carbon.

The TOC analysis, measured with a TOC /TN analyser, uses catalytic combustion at a temperature of at least 850°C in an oxygen atmosphere, followed by infrared detection for the release of CO_2.

For TN analysis, this analyzer uses catalytic combustion followed by chemiluminescence detection of NO_3 and NO_2.

The solid sample is analyzed through the Elemental Analysis. Through it, a solid is analyzed for its elemental composition, defining the mass fraction of carbon, hydrogen, nitrogen, and sulphur, from which the structure of the sample can be defined.

This analysis is carried via the combustion of the sample in excess of oxygen, and products (mainly CO_2, water, and NO_2) are collected. Through their amount, the composition of the sample can be determined.

The products of acid hydrolysis are liquid hydrolysate and a solid residue.

The liquid is filtered and analyzed with High-Pressure liquid chromatography. Firstly, standards for the quantification of all the chemicals are prepared, as reported in table 2.1. Table 2.11 reports the volume of each component (V_1) used to create five standards having a different concentration (Std_1, Std_2, Std_3, Std_4, and Std_5) and a volume of 50 ml each.

Table 2.5: Standard for HPLC analysis

STANDARD	Assay	Density (g/ml)	Solution concentration (mg/ml)	Std 1 (mg/ml)	V1 (ml) = C2*V2/C1	Std2 (mg/ml)	V1 (ml)	Std3 (mg/ml)	V1 (ml)	Std4 (mg/ml)	V1 (ml)	Std5 (mg/ml)	V1 (ml)
Lactic Acid (w/w)	90	1,2	1080		23,62		11,8		4,72		0,47		0,11
Formic Acid (w/v)	0,88	1,22	880		28,98		14,5		5,79		0,57		0,14
Acetic Acid (w/v)	0,999	1,049	999	10	25,53	5	12,8	2	5,1	0,2	0,51	0,05	0,12
HMF (w/w)	0,98	powder			510,2		255		102		10,2		2,55
Furfural (w/w)	98	1,159	1135,82		22,45		11,2		4,49		0,44		0,11
Levulinic Acid (w/v)	0,98	1,134	980		26,03		13		5,2		0,52		0,13

The HPLC is a technique based on the separation of several components according to the chemical affinity between the fixed-phase placed inside the column and a mobile-phase that flows through it. So, different components have different residence time, and an IR or UV-Vis detector recognizes and quantifies each component producing a chromatogram. An example of chromatogram obtained for the products investigated is reported in figure 2.9.

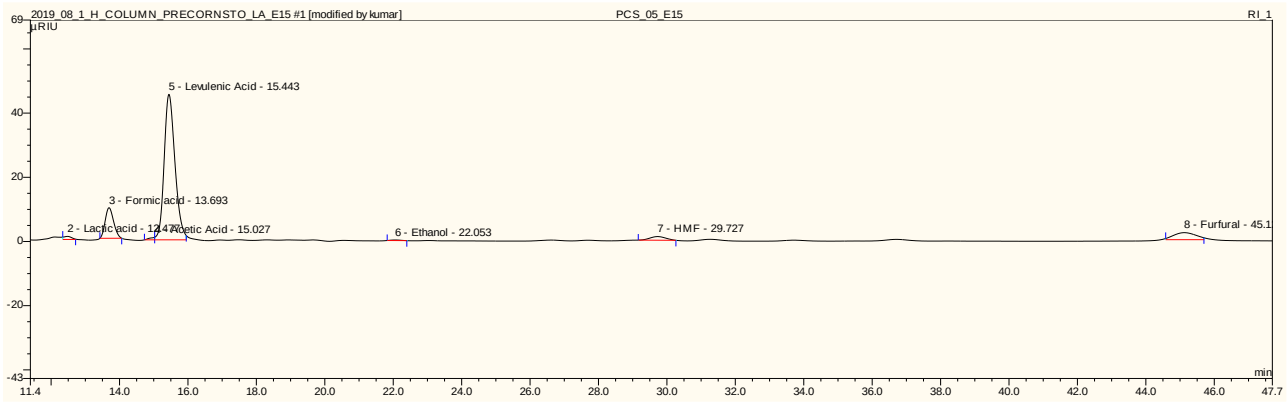


Figure 2.10: *Example of chromatogram generated from HPLC*

The composition of the solid residue is defined through the Elemental analysis, after being washed with water and centrifuged, to neutralize from acid residue.

Chapter 3

Experimental results

In this chapter, the experimental results of levulinic acid production are reported. Two different operating scenarios will be analyzed. Firstly, we will examine the acid hydrolysis of raw biomass and then the acid hydrolysis of pretreated biomass. The results will be compared to verify which one produces a higher yield of levulinic acid.

3.1 The importance of acid

The first task is to investigate the usefulness of acid in this process step. For this reason, the hydrolysis of raw corn stover was first carried out without the addition of any acid solution, but just water, as a control. The idea is to check if pure water, in subcritical condition, is strong enough to break down the chain of cellulose, producing single monomers of glucose, which are later converted into levulinic acid. A single experiment is hence run, analyzing the results of 105 min as reaction time. Table 3.1 reports the concentrations, of different components, in the products.

Table 3.1: Chemical concentration of row biomass' hydrolysis

Time [min]	**Concentration [mg/ml]**					
	lactic acid	formic acid	acetic acid	levulinic acid	HMF	furfural
105	0,61	0,05	0,08	0,01	0,07	0,33

As reported in the previous table, the concentrations of each component are quite low, especially for levulinic acid. Its yield, calculated with respect the total amount of cellulose (28.15 wt%) contained in the dry biomass, is given by equation 3.1:

$$\text{yield}_{\text{levulinic acid}} = \frac{c_{\text{levulinic acid}} \times V_{\text{solution}}}{m_{\text{tot}} \times m_{\text{glucose}} \times x_{\text{dry}}} \times 100 \qquad (3.1)$$

Where, $c_{\text{levulinic acid}}$ is the concentration of levulinic acid, in this case equal to 0.01 mg/ml; V_{solution} is the volume of solution (47.56 ml); m_{tot} is the total biomass (951 mg), while m_{glucose} is the amount of glucose contained in the biomass (28.15%) and x_{dry} is the dry fraction of the biomass (96%).

From Eq. 3.1, it is found that the yield of levulinic acid is 0.067%. It is clear that this value is quite low, especially if compared to 71.6% that is the maximum theoretical yield we should get from acid hydrolysis of cellulose, according to what is reported by Rackemann and Doherty (27). It is therefore concluded that acid addition is necessary to perform the hydrolysis.

3.2 Acid hydrolysis of pure cellulose using an acid concentration of 2% and 5%

In this paragraph, the effect of acid in the hydrolysis process is investigated. Generally, cellulose can be hydrolyzed in pure water by hydrogen atoms of the H2O molecule; however, this is a prolonged reaction because of the resistance of the cellulose to hydrolysis. The reaction can be speeded up using elevated temperature and pressure, or it can be catalyzed by acid (concentrated or dilute). Figure 3.1 reports a general reaction's mechanism to convent cellulose into glucose and by-products, such as disaccharides. This reaction involved the acid-catalyzed hydrolysis of cellulose. It begins by modifying the link between two glucose units, forming the corresponding conjugated acid. The cleavage of this C–O bond and breakdown of the conjugate acid to the cyclic carbonium ion then takes place, which adopts a half-chair conformation. After a rapid addition of water, free sugar is liberated. Because sugar competes with the water, a small amount of disaccharides are formed as by-products (28, 29).

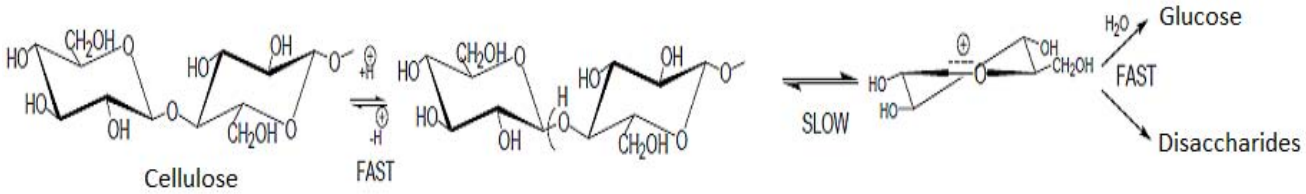


Figure 3.1: *Acid hydrolysis' reaction of cellulose*

The majority of research into levulinic acid production from sugars, cellulose, and biomass has been conducted with mineral acid catalysts. Most of them analyzed the effectiveness of

HBr, HCl, H_2SO_4. Some research indicates that the effectiveness of dilute acid is linked to the strength of their primary dissociation constant (pKa); other studies admit that the strength of the acid is not the most crucial characteristic of the catalyst because its effectiveness may be dependent on both the feedstock and concentration. However, the reaction's yield increases with acid concentration up to a critical concentration limit, which depends on other processing conditions and the feedstock; generally, too aggressive conditions lead to a higher prevalence of side reactions and polymerization of products and intermediate, with optimum concentration found to vary between 3.5 and 10 wt% (14).

In this work, experiments are carried out initially with pure cellulose, comparing the final yield with the theoretical one reported in the literature. All the experiments are done using only H_2SO_4 with two different concentrations (5 vol% and 2 vol%), and results will be compared at the end.

Firstly, the effect of a solution 5% H_2SO_4 acid on levulinic acid's yield is analyzed as a function of the reaction time (45, 75 and 105 min).

Figure 3.2 reports the average concentrations of each chemical, measured with HPLC.

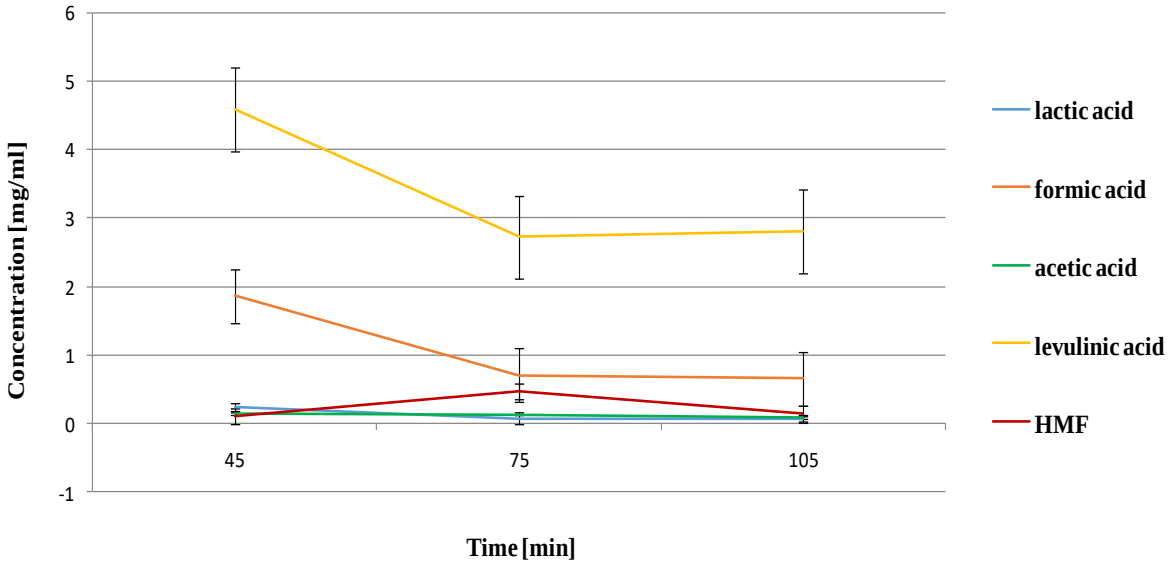


Figure 3.2: *Concentration of chemicals from acid hydrolysis (5% H2SO4) of cellulose*

The yield of levulinic acid with respect to the total biomass has been calculated using equation 3.2, and results are reported in figure 3.3.

$$\text{yield}_{\text{levulinic acid}}\,(\%) = \frac{\text{mg}_{\text{levulinic acid}}}{\text{mg}_{\text{total dry biomass}}} \times 100 \qquad (3.2)$$

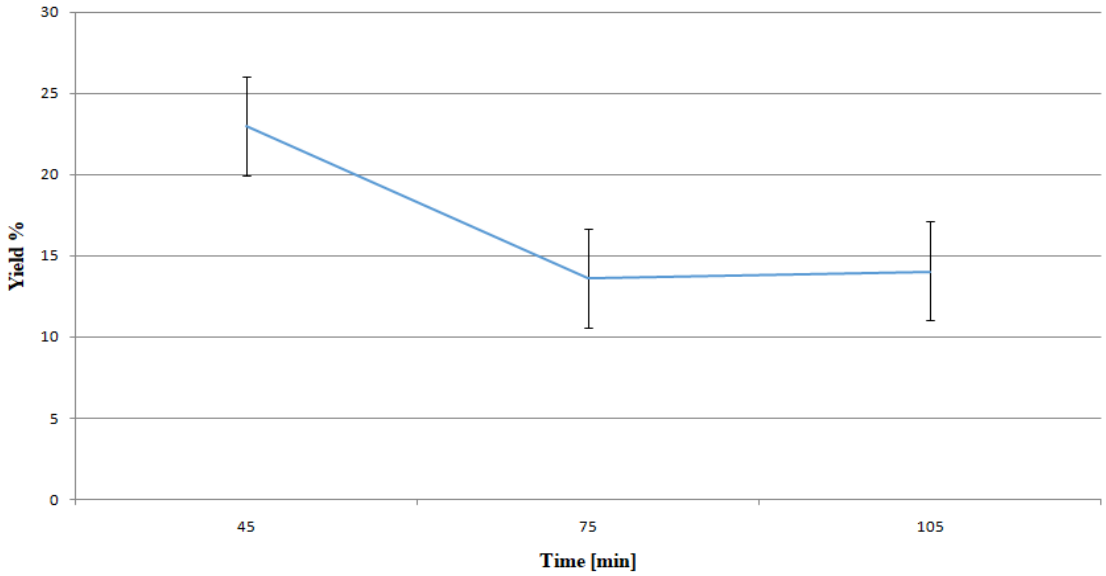


Figure 3.3: *Yield of levulinic acid from cellulose*

As reported, the yield of levulinic acid achieves a good value (25%) if the reaction is stopped at 45 min, and then it decreases with time, reaching about 15% after 75 and 105 min. Afterward, the same experiment is carried out using a solution with 2% of acid. Figure 3.4 reports the concentration of each chemical in the product.

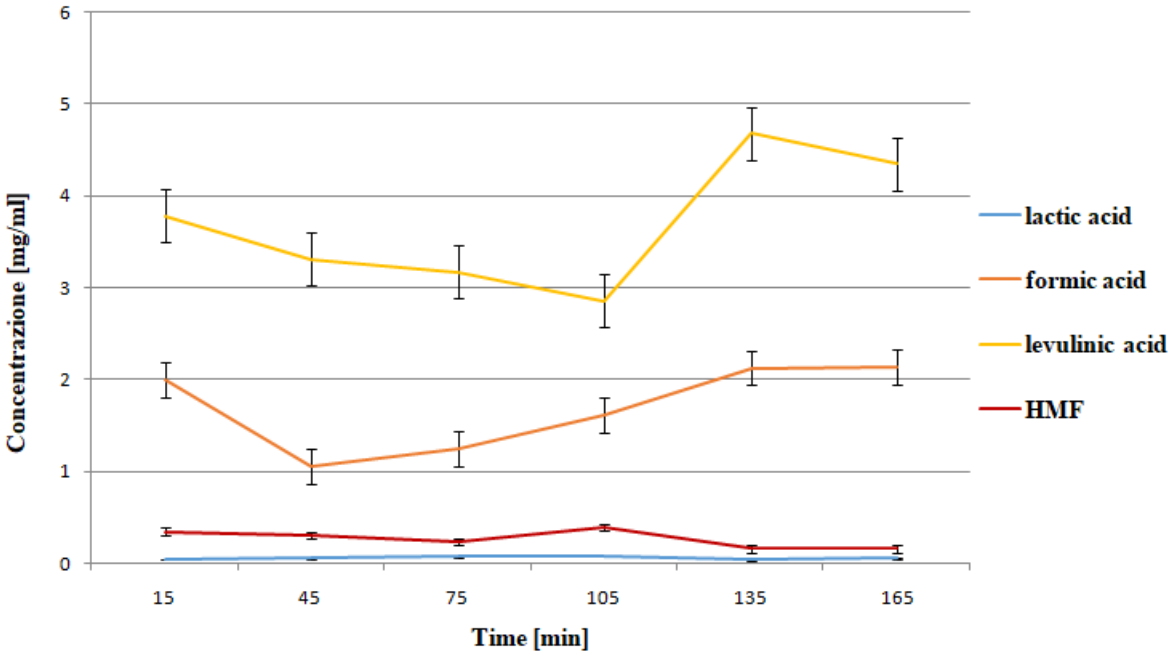


Figure 3.4: *Concentration of chemicals from acid hydrolysis (2% H_2SO_4) of cellulose*

The yield of levulinic acid is calculated using the same previous equation 3.2, and the results are plotted in figure 3.5. The values of yield achieved in these conditions are in the same range as those obtained with 5% acid concentration.

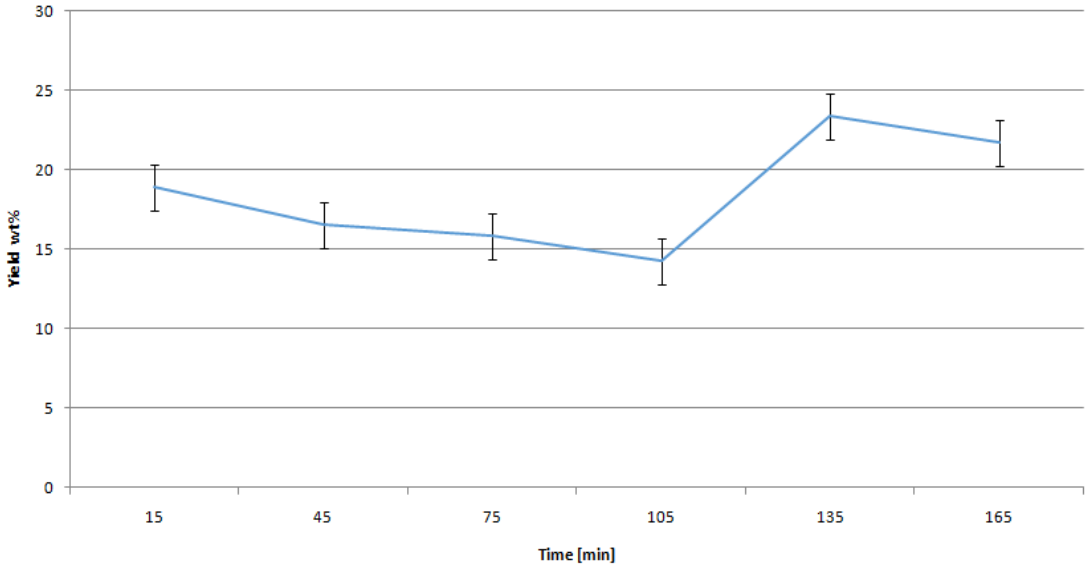


Figure 3.5: *Yield of levulinic acid from cellulose*

Comparing figures 3.2 and 3.4, in both cases, the concentration on levulinic acid is quite high; knowing that HMF is a precursor of levulinic acid formation, and observing that its

concentration is low in both cases, we can conclude that the reaction took place in its best way. The other chemicals are present with a concentration lower than 2%.

From the comparison of figures 3.3 and 3.5, as already said, even if usually reaction's yield increases with acid concentration, too aggressive condition may lead to a higher prevalence of side reactions and re-polymerization of products and intermediates (14). If we compared those values to the maximum theoretical yield get from the acid hydrolysis of pure cellulose, that is 71.6%, it is clear that we obtain almost the 34% of the maximum yield in the best conditions. Other processes, especially those that use the Biofine technology, can reach 70-80 % of the theoretical yield.

Hence, considering that in this specific situation, there are no evident advantages of working with the higher acid concentration solution, and to avoid corrosive effects, it is decided to keep using the solution of 2% of acid in all the following experiments.

3.3 Acid hydrolysis of Raw corn stover

The same experimental procedure is applied starting from the raw corn stover feedstock; products were analyzed with high-pressure liquid chromatography to find out the concentration of chemicals we are analyzing. Results are reported in figure 3.6.

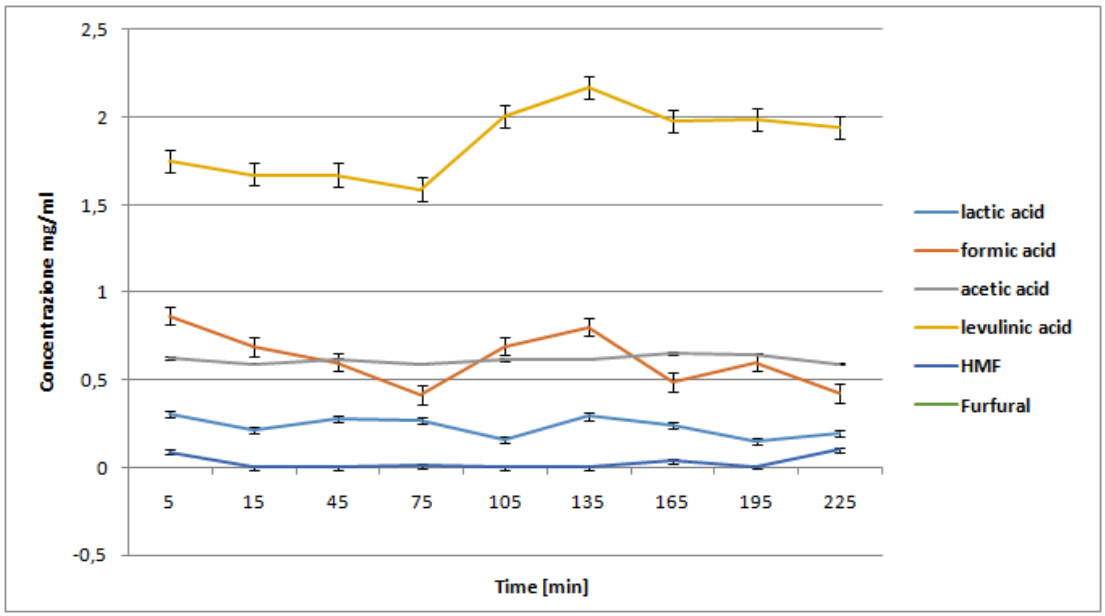


Figure 3.6: *Concentration of chemicals from acid hydrolysis (2% H2SO4) of row corn stover*

Since corn stover is a lignocellulosic biomass, its cellulose content is lower than the pure cellulose, so the concentration of levulinic acid we get is lower than the previous case.
The yield of levulinic acid is calculated with respect to the total dry biomass, so we have to consider that the raw corns stover had 4% of moisture. The results are presented in figure 3.7.

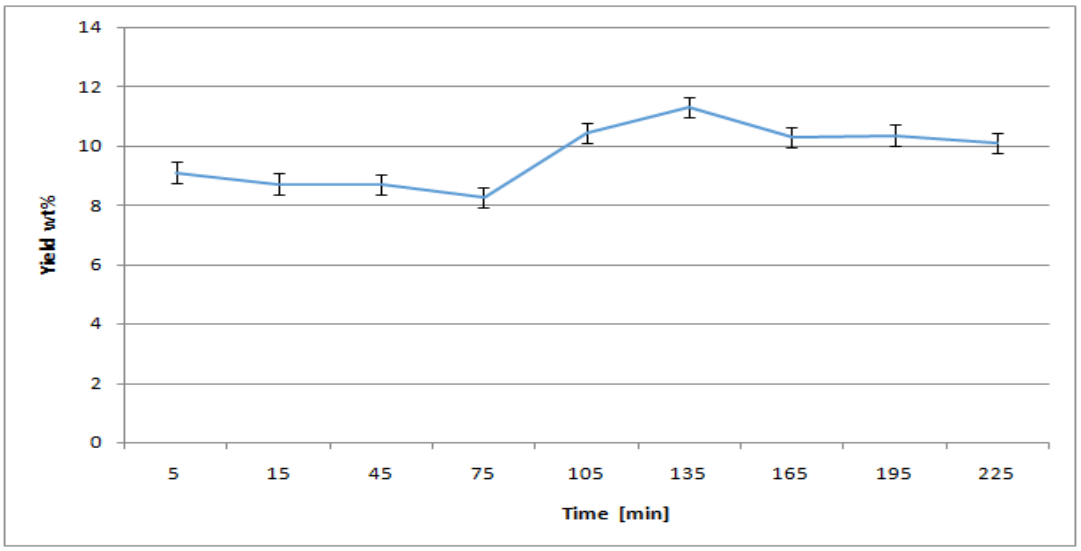


Figure 3.7: *Yield of levulinic acid from row corn stover*

The yield of levulinic is almost two times less than the one obtained from the acid hydrolysis of pure cellulose. An explanation could be that different factors can limit the effectiveness of acid hydrolysis of raw corn stover; among them, we find the crystallinity of cellulose, the degree of polymerization, the moisture content, the available surface area and the lignin content (12). In this research, we consider one of those independent parameters, namely the lignin content, because it can be directly measured and manipulated by a pretreatment process. The other variables were instead not investigated in this thesis. For this reason, the possibility of employing a pre-treatment step on the biomass to possibly break the lignin structure and increase the levulinic acid yield, was assessed.

3.4 Acid Hydrolysis of pretreated biomass

As already said, lignocellulosic biomass is mostly composed of cellulose, hemicellulose, and lignin. This set of experiments aimed to increase the yield of levulinic acid by modifying the chemical and structural composition of biomass, through a thermochemical pretreatment, before its acid hydrolysis.

Indeed, its structural composition prevents the contact between β-glycosidic linkage and cellulose enzymes, reducing the final yield of the process. So, the pretreatment aims to alter both the structural barriers (removal of lignin and hemicelluloses) and the physical barriers (pore size distribution, surface area, and degree of polymerization), which help in improving the accessibility of enzyme for the subsequent hydrolysis (23). In order to maximize the selectivity in removing lignin and hemicellulose, it was chosen to run the pretreatment using a solution with potassium carbonate and to work at 190°C, because the solubility of hemicellulose starts around 160-170°C (12).

At the end of the pretreatment process, we get a hydrolyzed liquid, richer in lignin and hemicellulose, and a pretreated biomass, whose composition was analyzed following the laboratory protocol for the determination of structural carbohydrates and lignin in biomass, as reported in chapter 2. In table 3.2, the compositions of raw corn stover and pretreated corn stover are compared. It can be seen that the lignin and xylan content is reduced by 8.97% and 13.77%, respectively, while glucan content increases by 39.5%.

Table 3.2: Chemical composition of a row and pretreated biomass

	% Lignin	% Glucan	% Xylan	%Mannan	%Galactan	%Arabinan
Row corn Stover	26.95	28.15	22.51	0	0	0
Pretreated Corn Stover	17.98	62.01	8.74	0	0	0

From the acid hydrolysis of pretreated biomass, we expect a higher yield of levulinic acid than the one obtained from the hydrolysis of raw corn stover. Table 3.3 reports the amount of total pretreated biomass used for these experiments and its dry fraction.

Table 3.3: Amount of total pretreated biomass feeds the reactors

Dry biomass (2%) [g]	0.951
Dry fraction of pretreated biomass	16.82 %
Total pretreated biomass [g]	5.653

To keep constant the concentration of the acid solution (2% H_2SO_4) inside the reactor, it is calculated the amount of liquid contained in the pretreated biomass, considering its liquid fraction, as reported in table 3.4.

Table 3.4: Amount of liquid contained in the pretreated biomass.

Liquid fraction of pretreated biomass	83.17%
Total pretreated biomass [g]	5.653
Liquid in biomass [g]	4.70

Using the dilution law reported in equation 3.3, it is defined the initial concentration (C_1) of acid solution to keep constant the final one, as reported in equation 3.4

$$C_1V_1 = C_2V_2 \tag{3.3}$$

$$C_1 = \frac{C_2V_2}{V_1} = 2.21\ \%\ \text{of}\ H_2SO_4 \tag{3.4}$$

Where V_2 is the final solution volume (47.56 l), C_2 is the final desired concentration (2%) and V_1 is the initial volume without considering the amount of liquid contained in the biomass (47.86 ml).

In this case, the range of reaction times investigated was enlarged, ranging from 5 min to 165 min.

In figure 3.8, the concentrations of all the chemicals analyzed in the product are reported.

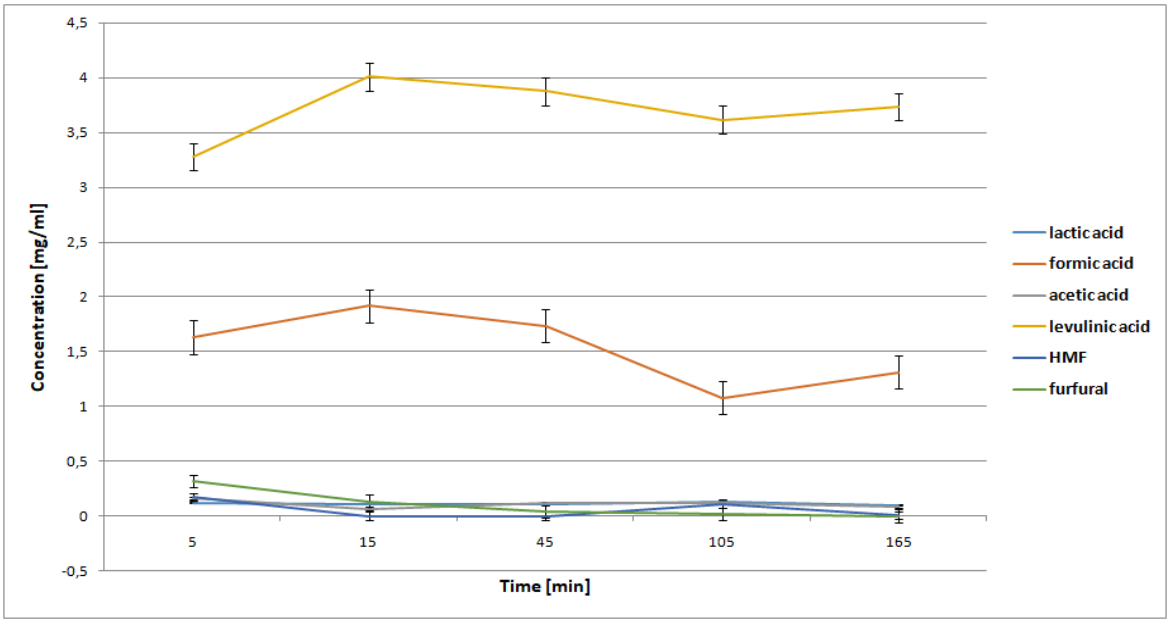


Figure 3.8*: Concentration of chemicals from acid hydrolysis (2% H_2SO_4) of pretreated corn stover*

The yield of levulinic acid is calculated, as usual, with respect to the total amount of dry biomass. Figure 3.9 reports its trend.

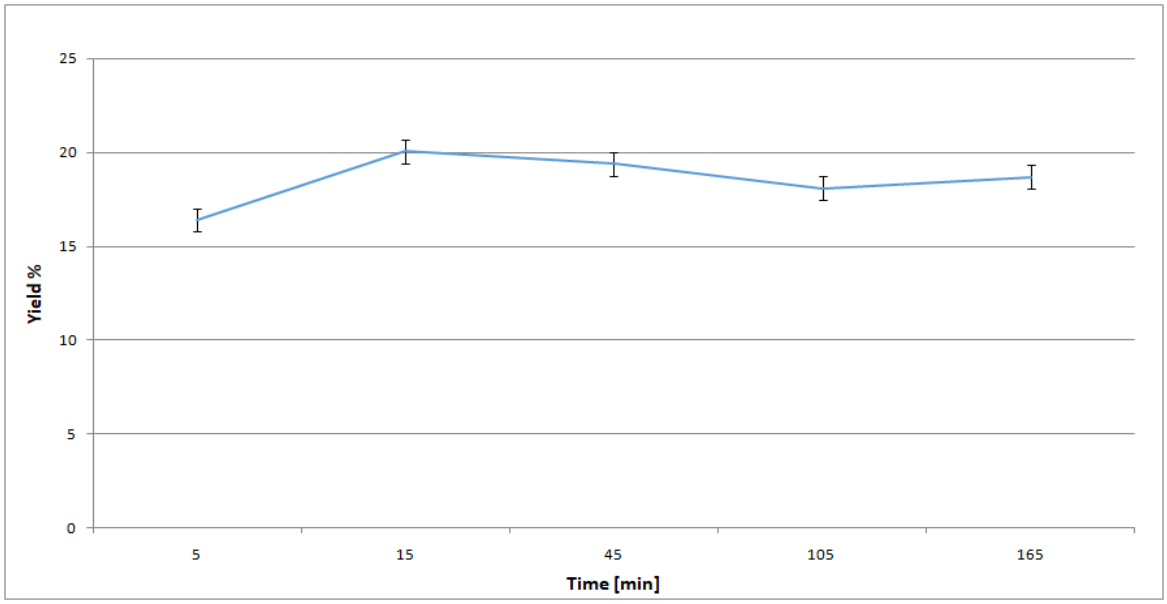


Figure 3.9: *Yield of levulinic acid from pretreated corn stover*

By comparing figures 3.7 and 3.9 is clear that the yield we get from the pretreated biomass is almost double than the one obtained from raw corn stover. In addition, the yield does not seem to be affected by the reaction time within the range investigated. So we can conclude that the pretreatment process can be used to increase the yield of levulinic acid and to reduce the reaction time to 5 min.

3.5 Balance of carbon

Finally, the mass balance of carbon, based on elemental analysis and Total-Organic-Carbon/ Total-Nitrogen analysis (TOC-TN) on both liquid and solid products, is presented.

First of all, we consider the pretreatment process. Through the elemental analysis, the composition in terms of nitrogen, carbon, hydrogen, and sulphur of both the raw corn stover and the pretreated biomass left after the reaction are defined; at the end of each pretreatment, the 62.38% of total biomass is lost in the hydrolyzed liquid, while only the 37.62% is left inside the reactor; in table 3.5, are reported the results of its analysis.

Table 3.5: Elemental analysis of both raw and pretreated biomass

	Nitrogen (%)	Carbon (%)	Hydrogen (%)	Sulphur (%)
Raw corn stover	0.504	43.952	5.491	0
Pretreated corn stover	0.161	39.521	5.102	0

Instead, TOC-TN analysis was applied to the hydrolyzed liquid collected during the preheating, the reaction, and the cooling steps. In table 3.6, the average values of several measurements are reported. It is essential to underline that the volume of samples collected in each step is not constant, so, we have considered an average value.

Table 3.6: TOC-TN analysis of liquid from pretreatment process

	Preheating	Reaction	Cooling
TOC-TN [mg/L]	15276.00	20215.67	2718.17
Volume [ml]	60	50	200

From these data, it is possible to calculate the amount of carbon contained in the liquid hydrolyzate through equation 3.5:

$$X_{gr,C}(\text{liq hydro}) = \sum_i TOC_i \times V_i = 2.47\text{g of carbon} \tag{3.5}$$

Considering a reference base of 100g of initial biomass, instead of 11g, $X_{gr,C}(\text{liq hydro})$ is equal to 22.45 g of carbon.

At the end of pretreatment, the biomass left is 37.62 g, which contains 14.86 g of carbon.

Finally, from the acid hydrolysis of the biomass, we collect a liquid analyzed with TOC-TN. In Table 3.7, we report all the data used to calculate its carbon content through equation 3.6.

Table 3.7: TOC-TN of liquid from acid hydrolysis

TOC-TN [mg/L]	5677
Volume [ml]	47.56

$$X_{gr,C}(\text{liq hydro}) = TOC \times V = 0.27\text{g of carbon} \tag{3.6}$$

Considering that the inlet biomass is 37.62g, instead of 0.951g, the carbon content is $X_{gr,C}$ = 10.68g.

The final solid residue, obtained from the 0.951g of biomass, is around 0.371g. It is called humin, or pseudo-lignin, and it is formed by the condensation of lignin and furans from monosaccharides in acid reaction condition. The elemental analysis defines its composition, which is reported in table 3.8.

Table 3.8: Elemental analysis of solid residue

	Nitrogen (%)	Carbon (%)	Hydrogen (%)	Sulphur (%)
Solid residue	0.255	33.000	3.851	0

Its carbon content is around 0.122 g of carbon.

However, considering that the inlet biomass is 37.62 g of biomass, instead of 0.951 g, the solid residue becomes 15 g, and its carbon content arises up to 4.94 g of carbon.

In figure 3.10, the material balance of the whole process, referred to 100 g of corn stover, is summarized.

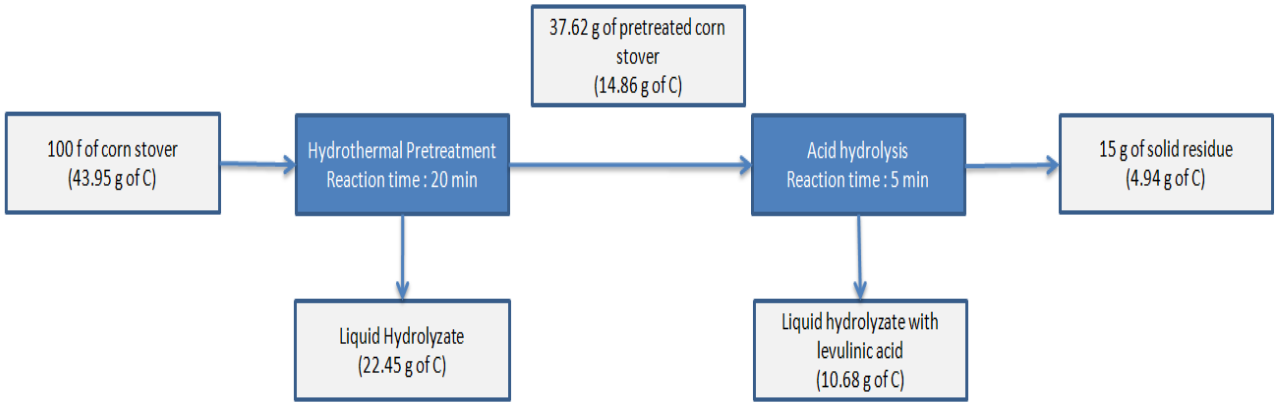


Figure 3.10: *Balance of carbon*

It needs to be noticed that there is a little unbalance in the carbon's balance because a small amount of carbon can be lost during the process. As shown in figure 3.9, 51% of the initial carbon is lost in the liquid hydrolysate during the pretreatment, while only 33% undergoes the hydrolysis reaction to produce levulinic acid and co-products. So just 24% of the initial carbon is contained in the liquid hydrolyzed produced by the hydrolysis reaction. In order to make the process more efficient and economically competitive, the carbon lost during the pretreatment can be converted into lactic acid; it is another useful product that has many

applications in the food industry as preservative and flavoring agents. Furthermore, the carbon contained in the solid residue can be used to produce electrode material, through the carbonization process, for electrochemical applications.

3.6 Discussion

Despite its great potential as a basic platform chemical, levulinic acid has never been produced in a significant volume because of its low yield and the high cost of equipment for its separation and purification. The maximum theoretical yield of levulinic acid from hexoses and cellulose is 64.5 wt% and 71.5 wt%, respectively, due to the co-production of formic acid. In the results published so far, levulinic acid yield was around 2/3 of the theoretical(27).
Levulinic acid can be synthesized by a variety of methods, each of which may use a different type of catalyst, such as homogeneous or heterogeneous. However, its final yield also depends on the chosen operation conditions, like temperature, pressure, acid concentration, reaction time and type of biomass.
The Biofine process represents one of the most celebrated technologies currently claimed for levulinic acid industrial production. This process involves the use of diluted sulphuric acid as a catalyst (1.5-3% according to the type of feedstock), and it takes place in two separate hydrolysis reactors. In the first step, biomass and sulphuric acid solution are mixed and continuously supplied to a small diameter tubular reactor that operates at a temperature within the range of 210 to 220°C and a pressure of 25 bar. The residence time in this first reactor is 12 seconds, in order to depolymerize the polysaccharides into their soluble monomers. The outflow mixture is then fed to the second continuously stirred tank reactor that operates at 190-200°C and only 14 bar (17). The efficiency of those two reactors and the use of polymerization inhibitors that reduce the char formation make the process more competitive because it achieves a total yield of 50-57% of levulinic acid, with respect to the cellulose content. Instead, Qi Fang (2001) reports another type of experiment based on levulinic acid production from kernel sorghum grains' flour, which was blended with 2%, 5% and 8% aqueous solution of sulphuric acid. This mixture was heated up to 160°C or 200°C in a pressurized reactor after a short pretreatment in order to increase the yield of levulinic acid. At 200°C with 8% of sulphuric acid concentration was achieved a maximum yield of 32.6%, based on sorghum content, which is mostly composed of carbohydrates (30, 31).

The processes we carried out is quite similar to the one performed by Qi Fang because both are based on the pretreatment of biomass and its acid hydrolysis, however, we worked with milder operating condition. Figure 3.11 reports the yield of levulinic acid calculated considering the cellulose content in the biomass.

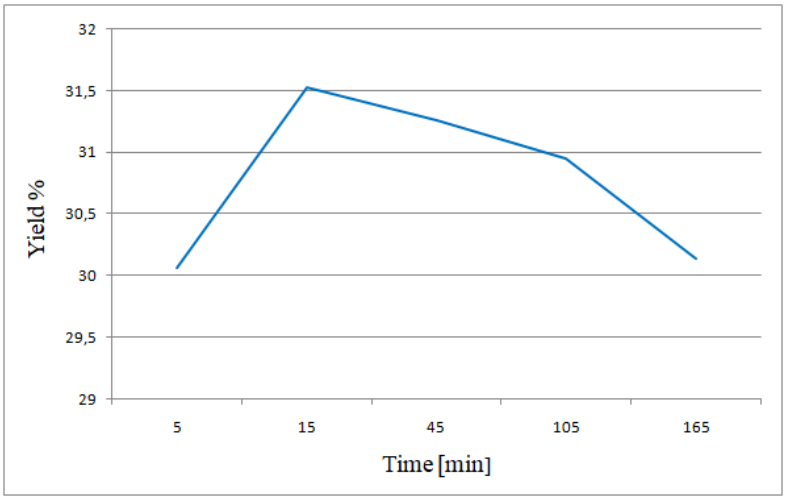


Figure 3.11: *Yield of levulinic acid*

According to figure 3.11, the highest yield of levulinic acid is 31.5% and it can be reached working at 190°C and with 2% of acid concentration. These conditions make a greener environment that can reduce the corrosive effect of the aqueous solution. Furthermore, as opposed to Qi Fang's research, results show that higher acid concentration turn on side reaction that reduced the total yield.

According to literature review, several other experiments were carried out using other types of biomass and other operating conditions resulting in a wide range of levulinic acid's yields, which are summarized in table 3.9.

Table 3.9: Yields of levulinic acid (27)

Feed	Cellulose content %	Acid concentration	Operating condition (°C)	Yield (mol%)
Fructose		3.6–7.2% HCl	95	~81
Glucose		5% H_2SO_4	170	80.7
Starch		6% H_2SO_4	200	66.4
Paper	85	>5% H_2SO_4	< 240	59.8
Pulp residues	80	1–5% H_2SO_4	1st stage: 210–230 2nd stage: 195–215	70-80

Wheat straw	40.4	3.5% H_2SO_4	210	68.8
Sorghum grain	73.8	8% H_2SO_4	200	45.6
Bagasse	42	4.5% HCl	220	82.7
Water hyacinth	26.3	10% H_2SO_4	175	53

Our experiments reach the maximum yield of 42.07 mol% based on cellulose content, working at 190°C, 34 atm with 2% of acid solution. Because of corn stover is made of low fibre content and the high presence of spongy vascular tissue in the stem (32), it was possible to work in environmental-friendly conditions, avoiding corrosive effect, but strong enough to guarantee consistent result with respect those reported in table 3.9.

Chapter 4

Economic assessment and process simulation

In this chapter, a preliminary assessment on the economic feasibility of a hypothetical industrial process for levulinic acid production based on corn stover is presented. Initially, it is reported the overall block flow diagram of the process, and each block is then individually analysed, reporting the thermal and electrical energetic costs and the final revenues.

4.1 Process flow diagram

A possible industrial process for levulinic acid production is made of different units, as reported in the block flow diagram (BFD) of figure 4.1

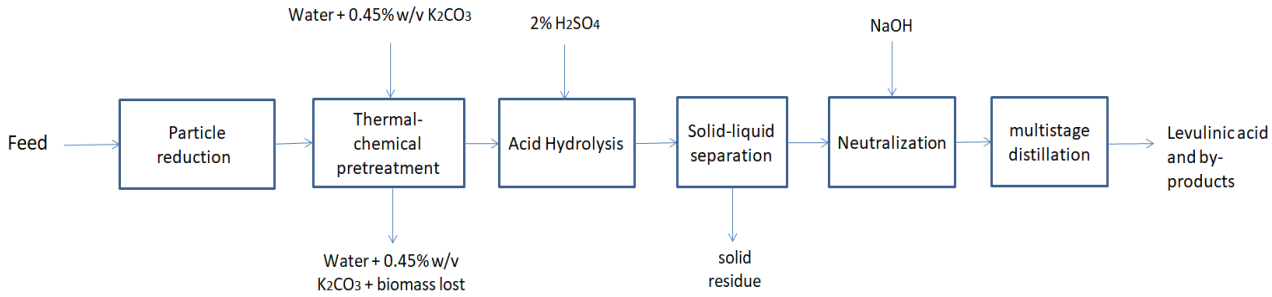


Figure 4.11: *Block diagram for levulinic acid production*

As reported in figure 4.1, the raw feedstock is firstly ground to specific particle size to increase the efficiency of the downstream reactions. Afterwards, it undergoes a hydrothermal pretreatment followed by the acid hydrolysis. Both of them are supposed to be carried out in continuous. Then, a solid-liquid separation occurs to remove the residual solids while, after neutralizing the liquid solution with sodium hydroxide, levulinic acid is separated by co-products through a distillation process.

4.1.1 Size reduction

It is supposed an inlet feed of 1000 kg/h of corn stover. Table 4.1 reports its initial composition.

Table 4.6: Initial composition of biomass

Component	Value [%wt]
Lignin	26.95
Hemicellulose	22.51
Cellulose	28.15
Ash	6.51

As reported by Perlack and Turhollow (2002), corn stover costs about €39.32-47.08/dry ton (33). Here, a value of 39.32 €/ton was taken. Size reduction is an essential pretreatment of biomass for energy conversion. It increases the total surface area, the pore size of the material and reduces cellulose crystallinity, which is required for bioconversion of lignocellulosic feedstock. To reduce biomass to a specific size, it is chosen to use a hammer mill, because it can grind many types of materials. It is relatively cheap, easy to operate, has low energy consumption, and it produces a wide range of particles. It operates at high speeds. The rotor shaft may be vertical or horizontal, generally the latter, and the shaft carries hammers. The grinding action results from impact and attrition between particles of the material being ground, the housing and the grinding elements (34). Energy consumption of grinding biomass depends on initial particle size, moisture content, material properties, feed rate of the material and machine variables. Here, it was quantified according to what reported by Sudhagar Mani and Tabil (2004) (32). These authors used a hammer mill with 22 swinging hammers attached to a shaft powered by a 1.5 kW electric motor. The shaft rotated at a speed of 60 r s^{-1}. Perforated metal screens covering the discharge opening of the mill retained coarse material for further grinding while allowing the properly sized materials to pass as finished product. A cyclone system is added to avoid dust generation. This experimental setup is reported in figure 4.2.

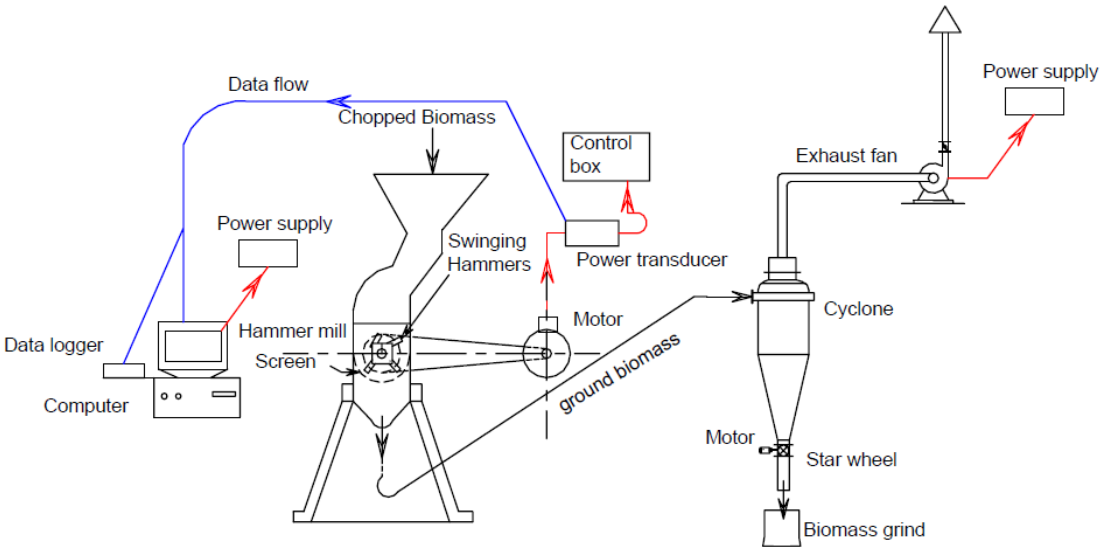


Figure 4.12: *Experimental set up for energy consumption determination of corn stover (32)*

Their results show that the average specific energy consumption to grind corn stover with 6%wt moisture content, using a screen opening of 3.2 mm, is around 6.96 kWh t^{-1}. The particle size ranges from 2.0 to 0.08 mm, and the sieving time is around 10 min. A regression analysis was performed by the same researchers, to predict the specific energy requirement for grinding biomass using the same screen size but having a lower moisture content and a simple linear model better fitted with experimental data. Figure 4.3 reports the linear regression for a different type of biomass.

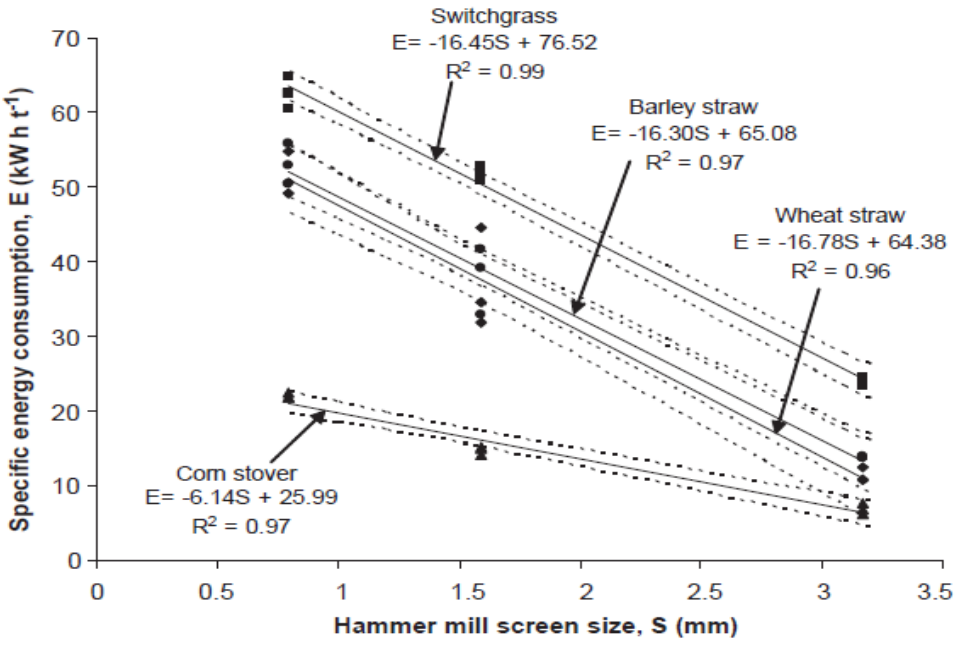


Figure 4.13: *Linear regression results for energy consumption determination of biomass (32)*

Equation 4.1 reports the formula used to calculate the energy consumption of corn stover grinding, derived from linear regression:

$$E = -6.14S + 25.99 \quad (4.1)$$

Where E is the specific energy requirement (kWh t^{-1}) and S is the hammer mill screen size (3.2 mm).

The corn stover used for our experiments has a dimension between 0.23 and 2.68 mm and a moisture content of 4%, so its energy consumption can be calculated using equation 4.1 keeping constant the screen size, and resulted equal to 6.34 kWh t^{-1}. Therefore, assuming the price of electricity around 0.05 €/kWh (35), the total cost for grinding the corn stover feedstock is around 0.317 €/h.

4.1.2 Pretreatment

The pretreatment is assumed to be a continuous process that is carried out with a reaction time of 1 hour. At laboratory-scale, the treatment is based on a solution made of water and potassium carbonate, which continuously flows through biomass, and is then cooled down. This process takes place at 190°C and 34 atm. Industrially, it involves different types of equipment such as heat exchanger, to heat the solution and to cool it down, and a pump to move the solution through the biomass.

The operative cost of each heat exchanger, to heat the solution (from 25°C to 190°C) and to cool it down (from 190°C to 25°C), depends on the amount of utility fluid used.

Due to the high-temperature gap, it is chosen to realize a heat integration, to reduce the total cost of the process, preheating the inlet solution till 180°C using the outlet flow's heat. The enthalpic balance can be used to find out the actual temperature of the outlet solution:

$$Q_{solution,in} = Q_{solution,out} \quad (4.2)$$

Considering that the inlet flowrate solution is equal to the outlet, the balance reported in equation 4.2 evolves as reported in equation 4.3

$$C_{p,solution,in} \times \Delta T_{solution,in} = C_{p,solution,out} \times (T_{inlet} - T_{outlet})_{solution,out} \quad (4.3)$$

Table 4.2 reports all the data used into the previous balance:

Variable	Value
$C_{p,solution,in}$ [J/g°C]	4.18
$\Delta T_{solution,in}$ [°C]	155
$C_{p,solution,out}$ [J/g°C]	4.43
$T_{initial,solution,out}$ [°C]	190

It is found out that $T_{final,solution,out}$ is equal to 43.74°C. Through the heat integration, it is decreased the temperature gap and the total cost of service fluid needed to heat the solution and to cool it down, so that a hot utility is needed to heat the inlet solution from 180°C to 190°C, while a cold fluid to cool down the outlet from 43.74°C to 25°C.

To heat the solution from 180°C to 190°C it was considered to use high-pressure steam, available at 41 barg and 254 °C.. To quantify its mass flowrate, it is applied the enthalpic balance reported in equation 4.4:

$$Q_{solution} = Q_{steam} \tag{4.4}$$

As shown by equation 4.4, the amount of heat absorbed by cold fluid ($Q_{solution}$) is the same as that one released by hot one (Q_{steam}). Developing the thermal balance, equation 4.4 becomes:

$$M_{solution} \times C_{p,solution} \times \Delta T_{solution} = M_{steam} \times \lambda_{steam} \tag{4.5}$$

Where $M_{solution}$ is the mass flowrate of the solution, derived by scaling-up the laboratory experimental data; $C_{p,solution}$ is the specific heat of the solution, assumed to be the one of water, since it is the main component; $\Delta T_{solution}$ is the difference between the outlet and inlet temperature after the heat integration; λ_{steam} is the latent heat of the steam. Table 4.3 reports the values of these data used in equation 4.5.

Table 4.3: Variables for heat exchanger's power consumption

Variable	Value
$M_{solution}$ [g/h]	11.72×10^5

$C_{p,solution}$ [J/g°C]	4.43
$\Delta T_{solution}$ [°C]	10
$Q_{solution}$ [kW]	14.43
λ_{steam} [kJ/kg]	1800

The resulting mass flowrate of steam required for heating the solution is 28.86 kg/h. To evaluate its cost is taken the price of high-pressure vapour of 29.97 $/t, as reported by Coulson & Richardson (35). Considering a $/€ conversion of 1.12, the total operating cost for heating the solution is 0.77 €/h.

Considering to use cooling water as service fluid to cool down the same solution and assuming 10 °C as temperature variation, the flowrate of cooling water needed can be calculated applying the thermal balance, as done previously, reported in equation 4.6

$$M_{solution} \times C_{p,solution} \times \Delta T_{solution} = M_{cool.\ water} \times C_{p,cool.\ water} \times \Delta T_{cool.water} \quad (4.6)$$

The cooling water mass flowrate is equal to 19.35 t/h. Considering the price of 0.01 €/t ([4]), its total cost is around 0.19 €/h.

In order to quantify the energy consumption of the pump, equation 4.7 is used.

$$P_a = \frac{\dot{V} \times H \times \rho \times g}{\eta} \quad (4.7)$$

Where $\dot{V}$ is the volumetric flowrate, H is the height of water's column, g is the gravity acceleration, ρ is the density of water and η is the efficiency of the pump. Table 4.4 reports the values of the data used in equation 4.7.

Table 4.4: Variables for pump's power consumption

Variable	Value
$\dot{V}$ [m^3/s]	3.2×10^{-4}
H [m]	340
g [m/s^2]	9,81
ρ [kg/m^3]	1000
η	0.9

P_a[kW]	1.21

Considering the cost of electricity, the pumping cost is around 0.06 €/h.

It is also essential to quantify the costs related to process water and potassium carbonate consumption. Considering the price of process water report in the Culson & Richardson equal to 0.5 €/t, the total water cost is around 0.58 €/h. The concentration of potassium carbonate is 0.45%w/v, and knowing that 1kg is paid 0.84 € (35), its total cost is estimated to be 4.43 €/h.

4.1.3 Acid hydrolysis

After the pretreatment, the biomass left into the reactor undergoes the hydrolysis reaction using an acid solution (2% H_2SO_4). In laboratory-scale, this is a batch process. However, industrially, this reaction is carried out continuously with 5 mins of residence time, and it involves two types of equipment because the acid solution needed to be heated up at 190°C, and pumped at 34 atm through the biomass. Besides, it is essential to quantify the costs related to acid consumption. Table 4.5 reports the sulphuric acid's flowrate needed and its relative cost.

Table 4.5: Determination of acid flowrate

Volumetric flowrate of solution [L/h]	18813,95
Fraction of acid	2% v/v
Flowrate of acid [L/h]	376,27

Considering that 1kg of pure acid is paid around 0.059€ (35), the total cost of its consumption is around 39.9 €/h. Furthermore, it is possible to calculate the cost of water consumption, as done for the pretreatment reaction, which is around 9.40 €/h.

It is important to evaluate the energy consumption for heating the hydrolyzed solution at 190°C. As done for the pretreatment, it is introduced a heat integration in order to decrease the temperature gap by preheating the inlet flow using the distillation column's residue flow (§ 4.1.6) which is at 244°C. So, it is assumed to preheat the inlet solution up to 180°C. The remaining duty is given using high-pressure steam used as service fluid. Applying equation

4.4, it is evaluated its mass flowrate needed. Table 4.6 reports data used in the thermal balance.

Table 4.6: Variables for heat exchanger's power consumption

Variable	Value
$M_{solution}$ [g/h]	7.83×10^5
$C_{p,solution}$ [J/g°C]	4.43
$\Delta T_{solution}$ [°C]	10
Q [kW]	9.10
λ_{steam} [kJ/kg]	1800

The mass flowrate needed is around 19.29 kg/h, and considering its price, the total cost for this heat exchanger is around 0.51 €/h.

Applying equation 4.7, it is possible to estimate the pumping cost related to the acid hydrolysis. Table 4.7 reports the variables' values used for the calculation.

Table 4.7: Variables for pump's power consumption

$\dot{V}$ [m^3/s]	5.2×10^{-3}
H [m]	340
g [m/s^2]	9.81
ρ [kg/m^3]	1000
η	0.9
P_a[kW]	19.37

The total cost for pumping the solution is around 0.96 €/h.

4.1.4 Liquid-solid separation

After the hydrolysis, the liquid containing levulinic acid and by-products is separated from the solid residue formed because of side reactions. It is proposed to use ad industrial filter-press usually utilized in water treatment plants to separate the sludge from liquid. The advantages

are its high separation efficiency and the high dry fraction of the sludge; however, the drawbacks are the short life of the filter and the high maintenance required. It is made of several plates alternated by frames, and this creates a space where the sludge is stoked while the solution is pumped through at high pressure. The operating time is supposed to be around 15 min. The technical features of filter-press, reported in table 4.8, refer to model F500 AS 10 (Filtri Fazzini s.r.l)

Table 4.8: Technical features of filter press F500 AS 10.

Filtering flowrate [L/day]	480-960
Working pressure [bar]	4-8
Hydraulic engine [kW]	2.2
Shaking-Plate engine [kW]	0.13

As reported in table 4.8, the filtering flow rate of a single unit is lower than the total coming from the acid hydrolysis; for this reason, multiple-parallel filter-presses are required, at least 20 units.

Considering the price of electricity and the operating time, the cost for a single hydraulic and shaking-plate engine is 0.11 €/h and 0.007 €/h, respectively.

The total solid recovered is around 146,36 kg/h, and it can be used in electrochemical applications to produce electrodes, and the sales gain is around 11.72 €/h.

4.1.5 Neutralisation

The liquid solution obtained from the previous filtration must be neutralised before being fed into the distillation column to separate levulinic acid from the other by-products. Neutralisation process is done using pure NaOH. To entirely neutralise the solution is essential to satisfy equation 4.8 :

$$\mathrm{mol}OH^- = \mathrm{mol}H^+ \tag{4.8}$$

Knowing that the density of H_2SO_4 is 1.89 kg/L and knowing the volumetric flowrate of acid, it is possible to define the mass flowrate of the acid solution, that is equal to 711.17 kg/day. Through the molecular weight of sulphuric acid (98.079 g/mol) the molar flowrate is

calculated. Equations 4.5 and 4.6 report the dissociation reaction of sulphuric acid and NaOH in water.

$$H_2SO_4 \rightarrow 2H^+ + SO_4^- \quad (4.9)$$

$$NaOH \rightarrow Na^+ + OH^- \quad (4.10)$$

As shown in equation 4.9, one mole of acid produces two moles of H^+ ions, and, according to equation 4.8, this corresponds to the molar flowrate of OH^- ions. According to equation 4.10, the flowrate of NaOH is hence calculated and reported in table 4.9

Table 4.9: Molar flowrate of sodium hydroxide to neutralize the solution.

Molar flowrate [mol/day]	Values
H_2SO_4	7250,96
H^+	14501,93
OH^-	14501,93
NaOH	14501,93

Through the molecular weight of sodium hydroxide, the mass flowrate of NaOH is calculated, which is equal to 580.03 kg/h. Because of 1kg of pure sodium hydroxide is paid 2.39 €, its total cost is 1386.28 €/h.

4.1.6 Distillation

The final step of the process is the distillation column to separate levulinic acid with the highest possible purity. This is simulated using a software called Aspen Plus. Figure 4.4 reports the flowsheet through which the separation is achieved.

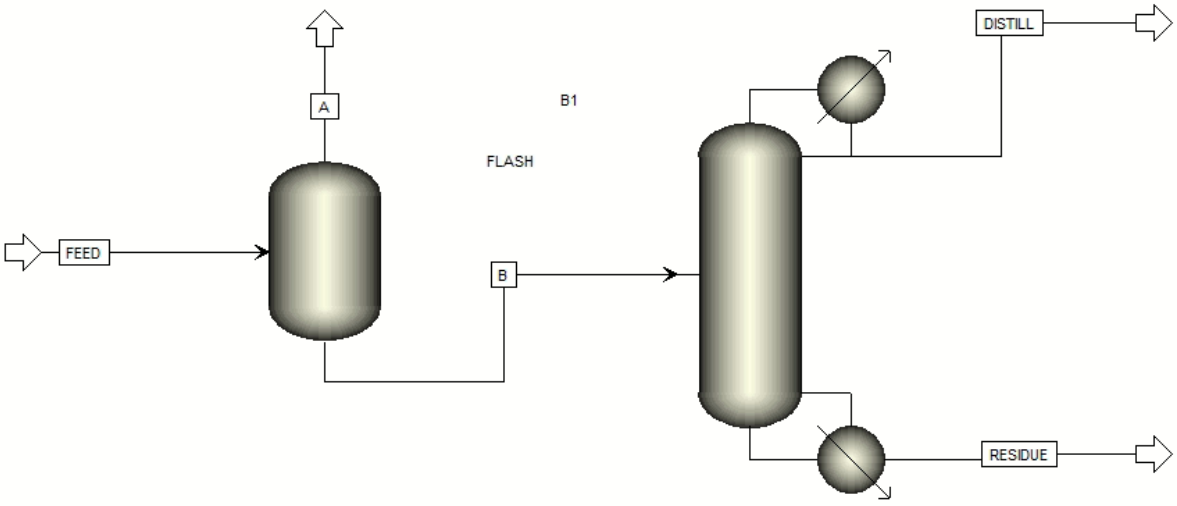


Figure 4.14: *Distillation flowsheet*

As shown in the previous figure, the flowsheet is composed of a flash unit (FLASH) and a distillation column (B1).

The feed heading to the flash comes from the neutralization process, so its operating conditions are 190°C and 34 atm. Table 4.10 reports its composition.

Table 4.10: Composition of the feed stream

Component	[kg/h]
Water	18704.64
Formic acid	30.24
Acetic acid	1.152
Lactic acid	1.728
Furfural	5.976
Levulinic acid	70.12
Total Feed	18814.08

As reported in table 4.10, HMF is neglected into feed composition because it is assumed that its lower flowrate (1.24 kg/h) does not affect much the calculation and simulation results.

ELECNRTL is chosen as thermodynamics model because it is the most versatile electrolyte method which can handle aqueous solution system, considering components and reactions for electrolyte systems. The reactions of ice and salt formation are not considered, instead, equation 4.11, 4.12 and 4.13 report the dissociation reactions taken into account.

$$C_2H_4O_2 + H_2O \leftrightarrow CH_3COO^- + H_3O^+ \quad (4.11)$$

$$CH_2O_2 + H_2O \leftrightarrow CHO_2^- + H_3O^+ \quad (4.12)$$

$$2H_2O \leftrightarrow OH^- + H_3O^+ \quad (4.13)$$

According to the previous equation, only acetic acid, formic acid and water can generate ions. Flash is carried out in adiabatic condition to reduce the feed pressure to 1 bar. As shown in figure 4.4, the flash unit generates a liquid (B) and a vapour (A) streams both at 100°C and 1 bar. In table 4.11 is reported mass flow and composition of those streams.

Table 4.11: Mass flow and composition of liquid (B) and vapour (A) flash's streams

	A	**B**
Mass flow [kg/h]	3226,27	15587,68
Water	3221,18	15482,64
Formic acid	4,75	23,25
Acetic acid	0,12	1,044
Lactic acid	0,001	1,72
Furfural	0,16	5,81
Levulinic acid	0,05	70,07
H_3O^+	0	0,92
OH-	0	$5{,}286 \times 10^{-8}$
HCOO-	0	2,19
CH_3COO^-	0	0,001
Mass Fraction		
Water	0,99	0,99
Formic acid	0,001	0,001
Acetic acid	$3{,}749 \times 10^{-5}$	$6{,}698 \times 10^{-5}$
Lactic acid	$4{,}969 \times 10^{-7}$	0,0001
Furfural	$5{,}058 \times 10^{-5}$	0,0003
Levulinic acid	$1{,}590 \times 10^{-5}$	0,004
H_3O^+	0	$5{,}94 \times 10^{-5}$
OH-	0	$3{,}39 \times 10^{-12}$

HCOO-	0	0,0001
CH_3COO-	0	7,54 x10^{-8}

As shown, stream A is mainly composed of water and a little amount of formic acid (15%) is lost in the vapour stream, while all the other components remain in the liquid phase.
The liquid stream is fed into the column to purify levulinic acid as much as possible. The distillation column is made with 5 ideal stages, with a total condenser at the first, and a kettle reboiler at the fifth. Initially, as degree of freedom, it is chosen to define a reflux ratio of 2 and a bottom flowrate of 70 kg/h. The feed stage is fixed to be the third from the top and the column is assumed to work at 1 bar, without any pressure drop between stages.
From the initial set up, table 4.12 reports the heat duty for both the condenser and reboiler.

Table 4.12: Heat duty of condenser and reboiler

	Condenser	Reboiler
Temperature [°C]	100	244.24
Heat duty [kW]	-1219.31	1219.58

In order to optimize the operating conditions, it is realized a sensitivity analysis to investigate the effects of reflux ration on levulinic acid concentration. Figure 4.5 reports the result of this analysis.

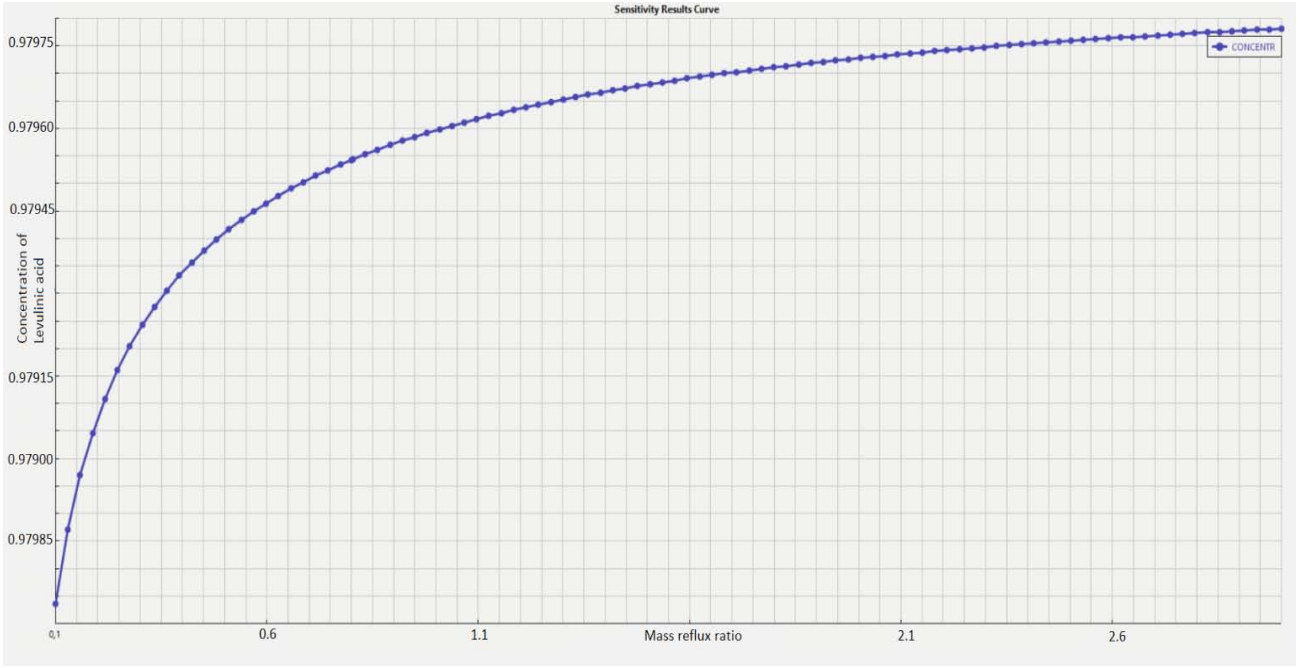


Figure 4.15: *Sensitivity analysis of reflux ration on levulinic acid concentration*

As shown in figure 4.5, a higher value of reflux ratio has a positive effect on levulinic acid concentration, however, it remains quite constant around 0.979. Furthermore, increasing the reflux ratio also increases the heat duty of the condenser and reboiler too. For this reason, it is chosen to reduce the reflux ratio at 0.8, keeping a high quality of levulinic acid and bounding the cost relates to heat duties.

Table 4.13 reports the concentration and mass fraction of distillate and residue for running the simulation keeping constant the bottom flowrate at 70 kg/h and with the new set up for reflux ratio.

Table 4.13: Mass flow and composition of distillate and residue

	Distillate	Residue
Temperature (°C)	100	243,90
Pressure (bar)	1,01	1,01
Mass Flow [kg/h]	15517,68	70,00
Water	15482,62	0,014
Formic acid	23,24	$7{,}34 \times 10^{-5}$
Acetic acid	1,044	$1{,}00 \times 10^{-5}$
Lactic acid	0,315	1,413
Furfural	5,813	0,0045

Levulinic acid	1,50	68,56
H_3O^+	0,93	$5,48 \times 10^{-10}$
OH-	$5,312 \times 10^{-8}$	$1,06 \times 10^{-16}$
HCOO-	2,20	$1,29 \times 10^{-9}$
CH_3COO-	0,0011	$1,492 \times 10^{-14}$
Mass Fraction		
Water	0,99	0,0002
Formic acid	0,001	$1,04 \times 10^{-6}$
Acetic acid	$6,72 \times 10^{-5}$	$1,43 \times 10^{-7}$
Lactic acid	$2,03 \times 10^{-5}$	0,020
Furfural	0,0003	$6,50 \times 10^{-5}$
Levulinic acid	$9,69 \times 10^{-5}$	0,98
H_3O^+	$5,99 \times 10^{-5}$	$7,83 \times 10^{-12}$
OH-	$3,42 \times 10^{-12}$	$1,51 \times 10^{-18}$
HCOO-	0,0001	$1,85 \times 10^{-11}$
CH_3COO-	$7,62 \times 10^{-8}$	$2,13 \times 10^{-16}$

As reported in table 4.13, the distillate stream is mainly composed of water and most of the other products. Instead, the residue contains levulinic acid with 98% of purity. Table 4.14 reports the new values of heat duty relates to the condenser and reboiler.

Table 4.74: Heat duty of condenser and reboiler with a reflux ratio of 0.8

	Condenser	Reboiler
Temperature [°C]	100	243.9
Heat duty [kW]	-731.858	731.858

In this study, it is assumed that the total cost of the distillation process only depends on the energy consumption related to the condenser and the reboiler. So comparing table 4.12 and 4.14, it is clear that reducing the reflux ratio, also decreases the total cost of the distillation.
In order to define the effective costs of the column, we assume to use high-pressure steam and cooling water as service fluids, for reboiler and the condenser respectively. Applying the thermal balance reports in equation 4.4, and following the same procedure used to determine

the energy consumption of pretreatment and hydrolysis' heat exchangers, it is found out the mass flowrate of vapor needed at the reboiler. Table 4.15 reports the values of those variables used in the thermal balance.

Table 4.15: Reboiler's energy consumption

Variable	Value
$Q_{reboiler}$ [kW]	731.86
λ_{steam} [kJ/kg]	1800

The mass flowrate of vapor used in the reboiler to heat the solution to 243.9 °C, is around 1463.72 kg/h with a total cost of 39.04 €/h.
In the same way, table 4.16 reports data used to calculate the amount of cooling water needed at the condenser to cool down the solution.

Table 4.16: Condenser's energy consumption

Variable	Value
Q_{water} [kW]	731.6
$C_{p,cooling\ water}$ [J/g°C]	4.18
$\Delta T_{\ cooling\ water}$ [°C]	10

The flowrate of cooling water is 63007.58 kg/h, with a total cost of 0.63 €/h.
According to simulation results, it is calculated the hypothetical gain derived by considering only the levulinic acid, disregarding the other components, because their purifications would require more separation equipment, increasing the total cost. Table 4.17 reports the gain related to levulinic acid.

Table 4.17: Hypothetical gain from levulinic acid.

Component	Mass Flow [kg/h]	Price [€/kg]	Gain [€/h]
Levulinic acid	68,56	164	11243.84

The price of levulinic acid is taken from the Merck Chemical Industry (36).

4.2 Cost analysis

This analysis aimed to summarize the cost and the revenues in order to find out whether the process is profitable or not, and which is the most expensive step among grinding biomass, pretreatment, acid hydrolysis, separation, neutralization and distillation. Table 4.18 summarizes each contribution, which are the items taken into account to quantify the total cost of each step.

Table 4.18: Cost for each process' step

	Items	Cost [€/h]
Grinding biomass	Corn stover	39.32
	Grinding	0.32
Pretreatment	Water	0.59
	Potassium carbonate	4.43
	Heat exchangers	0.96
	Pump	0.06
Acid hydrolysis	Sulphuric acid	39.96
	Water	9.41
	Heat exchanger	0.51
	Pump	0.96
Separation solid-liquid	Multiple-parallel filter-presses	2.33
Neutralization	Sodium hydroxide	1386.28
Distillation	Distillation column	39.67
TOTAL COST		1524.81

Figure 4.6 shows the percentage contribution to the total cost of each process steps.

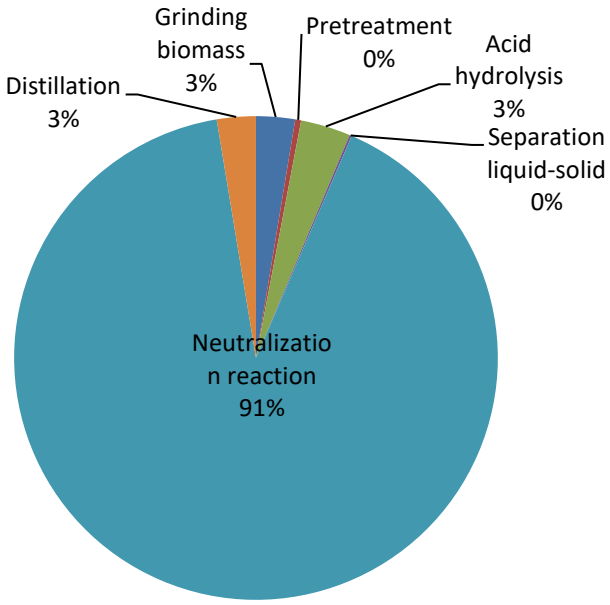


Figure 4.16: *Percentage of each process step on the total cost*

As reported, the higher cost is given by the neutralization reaction (93%), while the other process' contributions are negligible.

In figures 4.7 and 4.8 are reported the single cost items, discriminated in terms of mass or energy costs, related to acid hydrolysis and pretreatment, respectively.

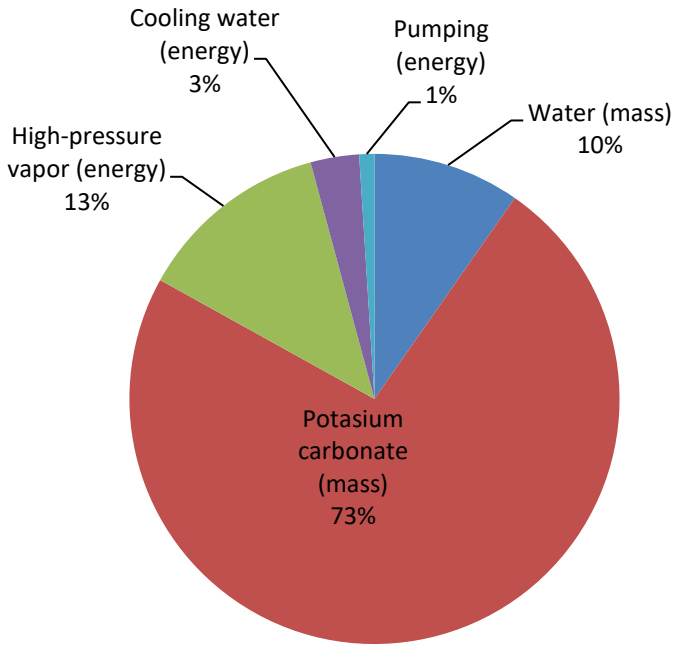


Figure 4.17: *Pretreatment cost's contributions*

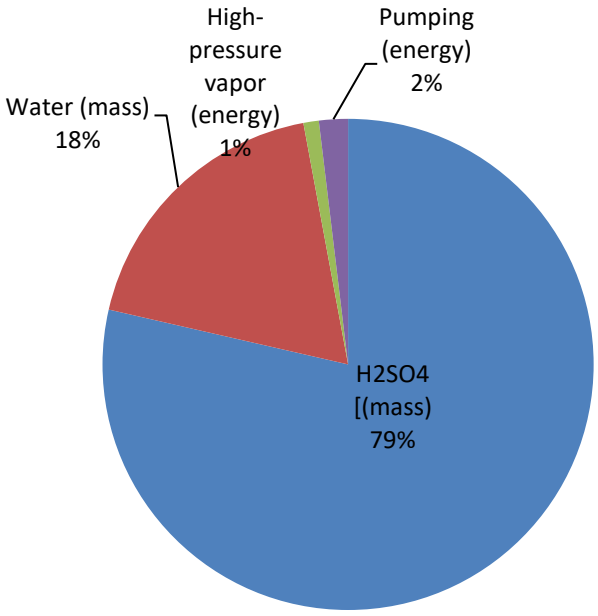


Figure 4.18: *Hydrolysis costs' contributions*

As shown, the higher cost contribution for the pretreatment and hydrolysis reactions is given by the potassium carbonate and sulphuric acid used in these reactions.
Equation 4.14 is used to give an initial economic assessment of the hypothetical feasibility of the process:

$$\text{Gain} = \text{revenue} - \text{costs} \tag{4.14}$$

The total revenue is based on two contributions, the first linked to levulinic acid contained in the residue of the distillation column, as reported in table 4.17; the second is related to the solid residue, obtained during the liquid-solid separation and equal to 11.72 €/h. The total costs are reported in table 4.18. So equation 4.14 can be written as reported in equation 4.15

$$(11243.84 + 11.72) - 1524.81 \cong 9730.67 \ €/\text{h} \tag{4.15}$$

The final result shows that the plant is able to generate a profit of 9730.67 €/h. However, some considerations must be done:

- The total calculated revenues do not consider the gain linked to the products contained in the distillate;

- Even though distillation column does not represent the highest contribution to the total cost, it can be considered to increase the final profit more, by operating a vacuum distillation. In such way, the boiling temperature at the reboiler would be reduced and a cheaper service fluid could be used. Further analysis of this scenario must be done to verify the advantages of heat integration.
- The higher cost contribution is given by the chemicals, namely potassium carbonate and sulphuric acid, used as catalysts in the pretreatment and hydrolysis reaction, respectively.

According to this consideration, a more detailed analysis, also considering the capital and operating cost and the finantial investement, would be necessary to get a clear idea about the real profitability rate allowed by this industrial plant.

Conclusions

This research aims to optimize levulinic acid production from lignocellulosic biomass, defining the best-operating conditions and investigating an alternative way to produce this green component, which has received the interest of the scientific community, due to its properties and its wide range of applicability. Commonly, levulinic acid is produced by acid hydrolysis of biomass. In this thesis, the possibility of introducing a thermochemical pretreatment before the biomass hydrolysis, and the related advantages and drawbacks, on the final yield of levulinic acid, are discussed. The pretreatment aims to change the physical structure of biomass to improve the conversion of cellulose into levulinic acid during the hydrolysis. Several experiments were performed both on raw and pretreated corn stover and, comparing the final yields, the effectiveness of the pretreatment is discussed. While the maximum theoretical yield of levulinic acid from pure cellulose is around 71.5% due to the formation of coproducts, the experimental results showed that the pretreatment can improve the total yield from 10% to 20% based on the total biomass, while also reducing the reaction time from 135 min to 5 min.

Based on the experimental results obtained at lab-scale, a preliminary economic assessment was carried out to investigate the feasibility of a hypothetical industrial process for levulinic acid production. Only the operation costs (thermal and electrical duties, feedstock supplies) of the entire process were taken into account at this level. The comparison between the total costs and revenues shows that this plant is able to generate a profit of 9730.67 €/h. However, some consideration must be done with respect to the simplifications introduced in the analyses:

- The total revenues were calculated only considering those linked to levulinic acid, neglecting the economic potential of other by-products (e.g. formic acid) because their purification and separation and the related costs were not taken into account. Nonetheless, they could further increase the economic potential of the process;
- The higher contribution to the overall cost comes from the chemical, namely potassium carbonate and sulphuric acid, used to increase the selectivity of pretreatment and hydrolysis reaction towards the levulinic acid production.
- Even though the distillation column does not bring the highest cost contribution, the final profit could be reduced by operating a vacuum distillation. In such a way, the

boiling temperature, at the reboiler, would be reduced and a cheaper service fluid could be used. Further analysis of this scenario should be done to verify the advantages of implementing the heat integration.

Overall, the proposed process appears promising, although a more detailed economic analysis, including capital investment and other financial indexes would be needed to draw more precise conclusions on the actual profitability.

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