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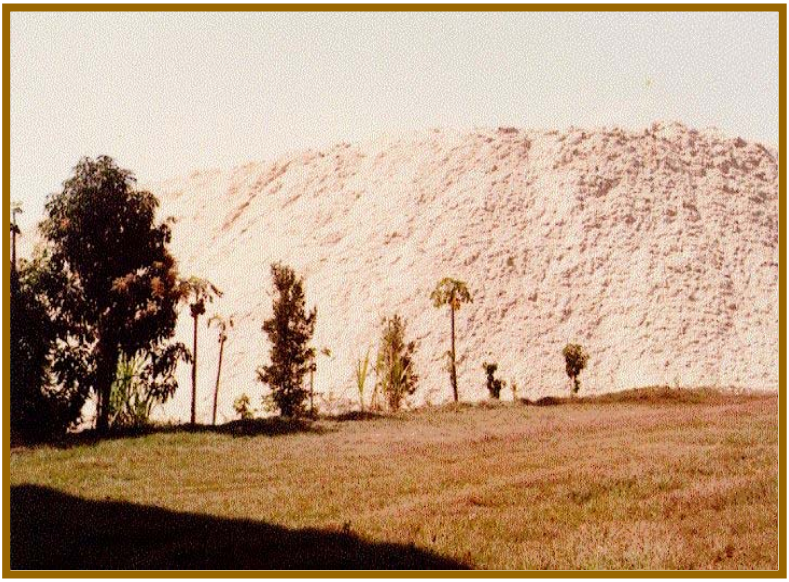

BIOMASS OF LIGNOCELLULOSIC COMPOSITION FOR FUEL ETHANOL PRODUCTION WITHIN THE CONTEXT OF BIOREFINERY

Nei Pereira Jr.
Maria Antonieta Peixoto G. Couto
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Nei Pereira Jr is a Full Professor of the Biochemical Engineering Department of the School of Chemistry of the Federal University of Rio de Janeiro (UFRJ). He has the degrees of Chemical Engineer (UFRJ, 1977); *MSc* in Technology of Biochemical Processes (UFRJ, 1982) and *PhD* in Biotechnology (Manchester University, UK, 1991). Since 1991 he has leading Research & Development & Innovation projects in the Laboratories of Bioprocess Development of UFRJ, where he has already supervised 67 theses (44 *MSc* and 23 *PhD*). Presently, he is supervising 08 *MSc* and 12 *PhD* students in the theme "Biotechnology of Lignocellulosic Materials", whose involvement started in 1987. He has published more than 200 works (full papers and congress manuscripts) and 07 patents, one of them concerning the ethanol production from sugar cane bagasse via cellulose enzymatic hydrolysis. He also develops cooperative works with national and international teaching and research institutions, as well as with enterprises. He holds scholarships from the Brazilian Council for Research (CNPq) and from the Rio de Janeiro State Research Foundation (FAPERJ) in recognition to his contributions to the development of Science and Technology in Brazil. He was recently awarded with the following prizes: PETROBRAS Inventor 2005, 2006 and 2007; Golden Thesis (2006), granted by the School of Chemistry by achieving the supervision of 50 theses in its Graduate Program on Technology of Chemical and Biochemical Processes, and the national prize of the Brazilian Association of the Chemical Industry (ABIQUIM) Outstanding Researcher 2006.

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PRESENTATION

This second volume of the **Series on Biotechnology** aims to present to graduate students and people involved with Biotechnology issues to the fascinating theme associated to the utilization of **Lignocellulosic biomass for the production of fuels and chemicals within the context of Biorefinery**. This subject has been compulsorily in the agenda of the main events of all Biotechnology related areas, and has been considered one of the most important themes of modern Science & Technology.

The conversion of lignocellulosic biomass to useful products has long been recognized as a desired and worthwhile endeavour but unfortunately has been neglected over the years. The virtually unlimited resources of lignocellulosic biomass in the form of residues and the constant rising costs of the edible raw materials have now generated renewed interest in the development of innovative cost-efficient processes for converting plant-derived lignocellulosic biomass to liquid fuels.

On top of that and in tuning with the world tendencies for searching for substitutes of fossil fuels, ethanol is in a short and middle term the most likely candidate. Ethanol is a simple alcohol produced from the hydrolysis and fermentation of the soluble and non-soluble carbohydrate fractions in plant biomass material. Today, the most common substrates used for ethanol production worldwide are valuable and edible soluble sugar and starch from agricultural crops, primarily sugarcane and corn, respectively. The great majority of the ethanol produced in Brazil and in the United States today is manufactured by mature and established production processes, which include milling, saccharification (in the case of starchy feedstocks), fermentation and distillation. The classic ethanol production processes use only a small portion of the sugar cane and corn biomass. The lignocellulosic fibrous stalks remaining after the ethanol process are burnt for energy/power production or accumulated in close proximity to the milling site. The residual worthless, non-edible lignocellulosic wastes, such as residual sugarcane bagasse and straw, cereal straws, corncobs and cornstover, hold great potential as alternative feedstocks for ethanol production, without competing for land use. Nonetheless, ethanol production from these residues requires more advanced pretreatment and hydrolysis technologies, since the polysaccharides are much more difficult to be hydrolysed economically into fermentable sugars.

Thus, the manuscript intends to introduce the reader to the technological aspects involved in the use of lignocellulose as feedstocks for chemical and biochemical processes, providing important data about lignocellulosic biomass generation in Brazil, their composition, fractionation (pretreatments), evolution of biomass processing featuring enzymatic hydrolysis, hexose and pentose fermentation and strategies for second generation ethanol production from the abundant residual plant biomass. We hope it can be useful for students who have awakened themselves for this important subject.

The Authors

"When the United Nations held a conference on Science & Technology for development, in Vienna in the summer of 1979, Biotechnology was missing from the discussions. Today it would be impossible to consider Science & Technology policies in any country without paying close attention to the role of Biotechnologies. In nearly all policy deliberations, Biotechnologies have come to occupy a crucial role, both from the perspective of basic research, where experimentation with genetic manipulation and enzymatic processes are some of the hottest items on any agenda, as well as from the perspective of economic competition, where Biotechnology is considered one of the key growth industries in the international marketplace. Few, however, have sought to examine the biotechnological challenge from the particular perspective of the industrial potential and capacities of developing countries".

Jacobsson, Jamison & Rothman
The Biotechnology Challenge (1986).

CONTENTS

Biomass of Lignocellulosic Composition for Fuel Ethanol Production and the Context of Biorefinery

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INTRODUCTION

The growth of human population has originated a rising and associated energy demand which is mostly supplied by non-renewable sources, especially by petroleum. However, the progressive worldwide exhaustion of this fossil carbon source is each time a less denied reality, in the way that the most pessimistic previsions confirm its total depletion in about 41 years (ODAC, 2007). Due to extraction difficulty, consumption increment and scarcity, its prices will continue to increase, values above 100 US$ per barrel are foreseen by the end of this decade (MAPA, 2005).

The environmental protection is another factor which has brought great concern to modern society. The burning of fossil fuels and the deforestation emit great quantities of gases in the atmosphere, especially CO_2 (anthropic emissions). The rising emissions of this gas and others as methane (CH_4), nitrous oxide (N_2O), hydrofluorinecarbonates (HFCs), perfluorcarbonates (PFCs) and sulphur hexafluoride (SF6) in the atmosphere have caused serious environmental problems, accentuating the global warming. Due to the quantity in which it is emitted, the CO_2 is a gas that most contributes to the global warming. Its persistence in the atmosphere can take decades. This means that the emissions of nowadays have long term effects, resulting in climate system impacts along the centuries, which already affects in present time, as follows: increasing of the global temperature, rise of the ocean level, reduction of the artic ice layer, adverse impacts on agriculture and loss of biodiversity. These environmental impacts, fundamentally occurring with the burning of fossil fuels, are a reality with which society has to live, to control and to adapt itself, needing to be conscious of the importance of this problem, and requiring changes in various consumption and behaviour habits.

Fortunately, great progresses could be seen in the manner of looking for alternative energy sources, particularly the biomasses, and for the implementation of actions as for reducing CO_2 concentration in the atmosphere, such as: preservation of native forests, implementation of agroforestal systems and recuperation of degraded areas. The participating nations of the convention

1* author for correspondence

on climate changes, which took place in June 1992 in Rio de Janeiro, compromised themselves by proposing actions to reduce greenhouse gas (GHG) emissions. The convention came into force in 1994, and from that time, the countries have met to discuss the topic and try to look for solutions for this serious problem which can compromise life in our planet.

1997 in *Kyoto* was established an important agreement known as the *Kyoto* Protocol, in which were defined mechanisms and limits for the reduction of GHG emissions to 5.2% between 2008 and 2012, in relation to the verified levels of 1990. The negotiations of the protocol were extended until 2004 when Russia ratified the document. With its ratification, becoming a treatise, measures to aim the reduction of these gases come into force and, together with other inconveniences of petroleum, create a great opportunity for the use of alternative energy sources, produced from renewable materials, collectively denominated BIOMASSES.

With this treatise a worldwide carbon market has already been created. The countries which cannot reduce their GHG emissions can buy credits from other countries which contribute to reduce these gases from the atmosphere in a larger quantity as they emit. This is one of the principal aspects of the *Kyoto* treatise, which transforms environmental concerns into economic worries.

Brazil finds itself in a sufficient privileged position to assume the leadership in the integral utilization of BIOMASSES, since it is one of the major planet potentials in renewable feedstocks by the great availability of agricultural cultures in large extension, with prominence for the cane industry; by intense sun radiation, plenty of water, climate diversification and it is the pioneer in the production of biofuel in large scale, the ETHANOL. Moreover, the country unifies conditions to be the principle receiver of investments, arising from the carbon market in the segment of production and use of bioenergy, having in its environment its greatest richness and owning enormous atmospheric absorption and regeneration capacity.

In Brazil, the fuel ethanol is produced from sugar cane juice, a material directly fermentable by possessing its main substrate (sucrose) in a form of direct utilization from the producing biological agent, the yeast *Saccharomyces cerevisiae*, and therefore eliminating previous hydrolysis procedures. Even when sugar and alcohol, in lots of cases, are produced concomitantly, it does not seem to be rational that considerable fertile soil extensions should be used for the production of fuel in detriment to the food production, of major social importance. On the other side, it would not be strategically secure, for a long time, to depend only on sugar cane for the ethanol production when Brazil has, as alternative sources, starchy feedstocks and abundant residues generated by the agricultural/agro-industrial and forestall sectors.

Additionally, studies indicate that the projected demand for ethanol for 2013 in Brazil will be 32 billions of liters, what corresponds to almost twice of the current production (PEREIRA, 2006). Several factors contribute to this increase, including: the growing of bifuelled car selling (*flex fuel*); the worldwide increase of ethanol demand and consequently of the Brazilian exportations; the increment on gasoline demand, which is mixed with 20-25% of anhydrous ethanol, and the implementation of the biodiesel mixture, produced with ethanol, in the diesel oil.

However, it can be foreseen that the harvested sugar cane in the same period will not be sufficient to supply such demand, ratifying the need of producing ethanol based on other sources.

In this context, research and development have been intensified in a more diversified way for the utilization of renewable feedstocks in substitution of fossil sources. Emphasis has been placed on the utilization of abundant agricultural (those produced in the field, resulted from the harvesting activity) and agro-industrial (those generated in the industrial process units) residues, both of **lignocellulosic composition**. The utilization of these residues, denominated RESIDUAL BIOMASSES, is of great interest and relevance, constituting one of the most important topics of modern Biotechnology, since there is no additional demand for extension area for agricultural activities. The aim is to transfer them from the position of solid residues to valuable feedstocks for the production of fuels and a variety of chemical substances, within the context of what has been called by BIOREFINERY. Progresses in this area indicate that the utilization of renewable feedstocks, including their residues, should revert the worldwide dependence on fossil sources.

AGRICULTURAL AND AGRO-INDUSTRIAL RESIDUES GENERATED IN BRAZIL

Analyzed for a long term, the economical viability of ethanol produced from sucrose and from starch is conditioned to the availability of feedstocks and to the problem of land occupation. In accordance to some authors, the use of annual cultures for energetic purposes (grains & cereals and sugar cane) reduces the soil fertility and its availability for food production. There are competitive demands for soil resources for the production of biomasses and for their alternative uses. In reality, biomass is a precious resource, unlimited and has diverse uses. Therefore, the expansion of these cultures gives reason to great controversies, because such activities can become a social-economical problem if there will not be a concomitant establishment for an appropriated agricultural policy. Besides, with the fast growth of the current ethanol market, the agricultural surplus can become exhausted, causing an ascendant pressure on feedstock prices. Therefore, a lot needs to be done that the ethanol from sugar cane and/or cereals, or even the biodiesel from vegetable oils might become, in short terms, real substitutes of petroleum derivative fuels (SCHUCHARDT and RIBEIRO, 2000; LAL, 2005; RAMOS, 2000). Besides the great search for ethanol, another factor which should be considered is the commercial sugar market, for which worldwide demand grows 3.4 millions of tons/year. Brazil, in spite of detaining 36% of the world market, can only currently amplify its sugar exportation to 1.7 millions of tons without compromising the alcohol supplies on the Brazilian market (PEREIRA, 2006).

A great feedstock source for bioconversion into ethanol is concentrated in lignocellulosic materials, normally in the form of residues, and even though some of them have been used as solid fuel, for animal feeding, and as recycling material, there are enormous surpluses. They are found in abundance and in great use availability (BRITO, 2000). Approximately, 350 millions of tons of agricultural and agro-industrial residues are produced yearly in Brazil (Figure 1), being originating mainly from sugar cane, followed by soya, which present the major generation amount (Figure 2).

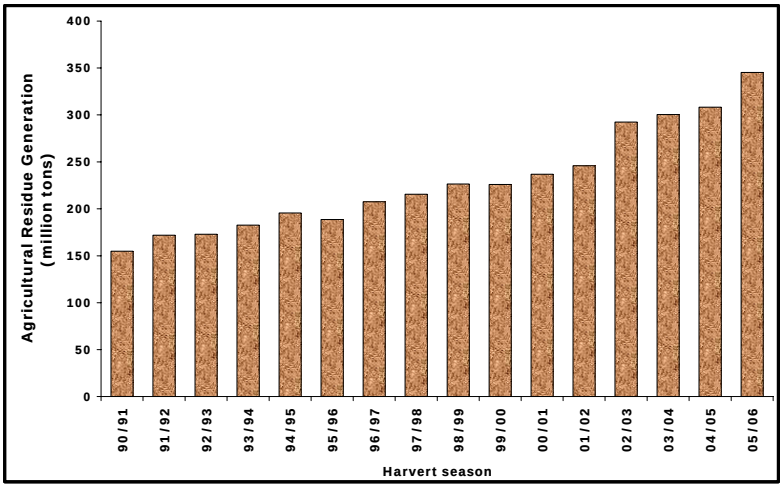

Figure 1: Production of Agricultural Residues in Brazil
Source: Adapted from CONAB (2006).

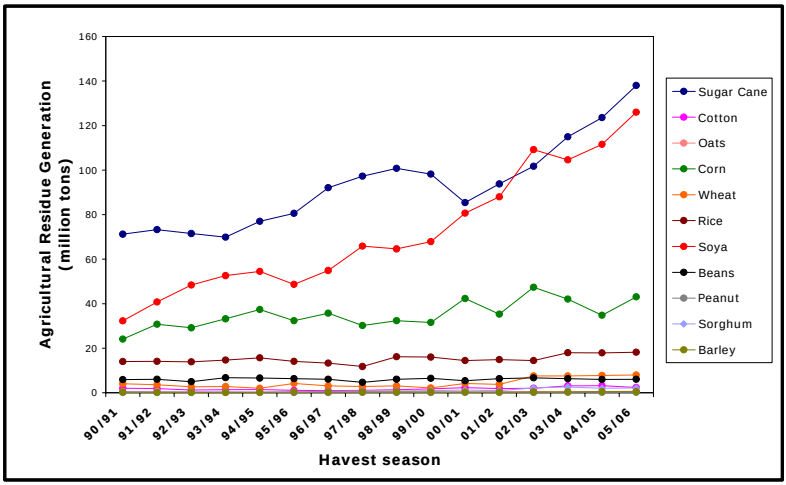

Figure 2: Production of Residues from the Main National Cultures
Source: Adapted from CONAB (2006).

Because of a vast biodiversity found in its territory, Brazil owns a great variety of residual biomasses of which utilization will be of great economical and social interest, since they possess a very low economical value and they are seen as additional cost inside the production process, due to the need of its final disposal (RAMOS, 2000; LAL, 2005; VIANNA JR. e VIEIRA, 2002).

As an example, the generation of cane bagasse in 2004 was approximately 101.8 million tons (MAPA, 2006). This agro-industrial residue is mostly burnt for energy generation in sugar mills. However, the viability of new technologies for residual biomass utilization could deviate part of this bagasse for the production of fuels and other chemicals of major economic value. Therefore, it is important to encourage investments for the development of new technologies to obtain energetic gains from the renewable resources, which are produced in great quantity in the country (RAMOS, 2000).

The contained energy in one ton of bagasse, with 50% of humidity, corresponds to 2.85 GJ (BIODIESEL.BR, 2006). The bagasse is the macerated stem, not including the straw and the quills, which present 55% of the accumulated energy in the cane plantation. This fabulous potential is rarely used, and, in the majority of the cases, is burnt on the field. Bagasse can offer energetic use outside of the sugar mills and can be used as fodder. Also, it has application in the production of cellulose/paper, structural elements and, more recently, its utilization has been contemplated as feedstock for the production of ethanol, through hydrolysis and fermentation technology.

Not all produced residues can or should be used for the production of bioenergy. The indiscriminate removal of residues can cause decline in soil quality with lasting adverse environment impacts. The return of agricultural residues to the soil improves its quality through its impact on reducing erosion risk, recycling of nutrients, improvement of soil structure and stabilization, water retention, energy supply for soil microbiological processes, and increasing on agronomical productivity. In view of these factors, in general only 40-50% of the agricultural residues can be used for different kinds of activities (LAL, 2005; VIANNA JR & VIEIRA, 2002).

Ethanol production technologies from lignocellulosic hydrolysis of agricultural and agro-industrial residues are in development, being realized in developed countries, portentous research investments for this aim, and will reach commercial period of probation in a few years. It is estimated that in 2020 about 30 billion liters of alcohol can be obtained from lignocellulosic source only in USA (KIM and DALE, 2004; MAPA, 2005).

BIOMASSES OF LIGNOCELLULOSIC COMPOSITION

The lignocellulosic materials are the most abundant organic compounds in the biosphere, participating in approximately 50% of the terrestrial biomass. The term lignocellulose structure is related to the part of the plant which forms the cellular wall (half-lamella, primary e secondary walls), composed of fibrous structures, basically constituted of polysaccharides [cellulose (40-60%) and hemicellulose (20-40%)]. These components are associated to a macromolecular structure containing aromatic substances, denominated lignin (15-25%) (SUN & CHENG, 2002). In a general way, it can be affirmed that those materials possess in their compositions approximately, 60-70% of polysaccharides (in a dry basis), which contain in their monomeric units valuable glycosides (sugars). A schematic representation of the lignocellulose complex is shown in Figure 3.

Cellulose is a polysaccharide, polymer of D-glucose, forming chains of β-1,4 bonds, and maintaining a linear and plane structure. Cellobiose, disaccharide 4-*O*-(β-D-glycopyranosil-D-glucopyranose), is the repeated polymer

unit. In natural celluloses, the chains are aligned in a way of forming complex organized fibrils, whether in crystalline or amorphous structures. These fibrils are established amongst them with inter and intra hydrogen bonds, which individually are weak, but collectively, they result in a great binding strength, giving to the cellulose a high resistance to the hydrolysis attack (Figure 4).

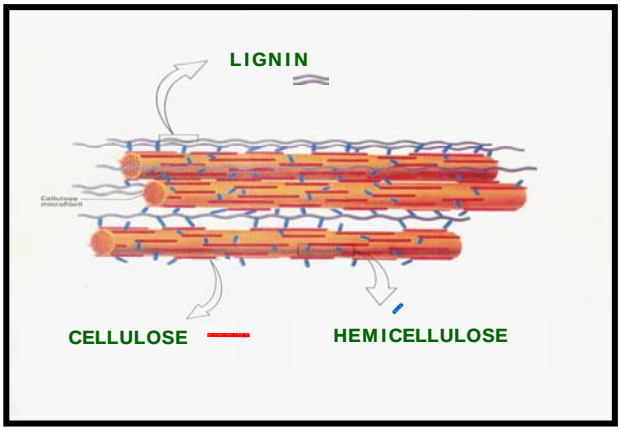

Figure 3: Scheme of the Lignocellulose Structure.
Source: DARNELL (1990).

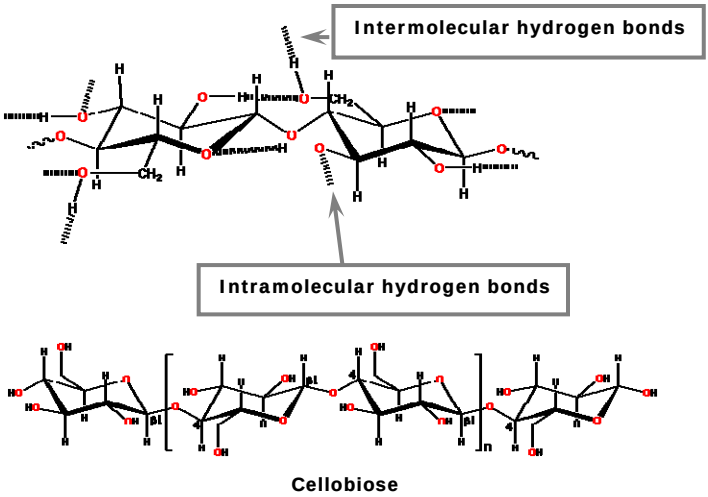

Figure 4: Chemical Structure of Cellulose.
Source: COUGHLAN (1985).

Hemicelluloses are closely associated with cellulose in plant tissues and together with cellulose they are the most abundant carbonic material in plants. These macromolecules, contrarily to cellulose, present heteropolysaccharic nature and a considerable degree of ramification, consequently not presenting crystalline regions. They are constituted, in their great majority, of a mixture of polysaccharides with a low molecular mass, as follows: xylans, arabinans, arabinoxylans, mannans and galactomannans. The fundamental units (monomers) are, basically, molecules of D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, D-glucuronic acid, D-galacturonic acid, α-D-4-*O*-methylglucuronic acid and also some oxidation products, as for example, acetates.

Differently to cellulose, the hemicellulose structure does not present a high crystallinity, therefore, being more susceptible to the chemical hydrolysis under milder conditions. The varieties of bindings and ramifications, just as the presence of different monomeric units, contribute to the hemicellulose structure complexity and its different conformations (JACOBSEN, 2000 and MALBURG *et al.*, 1992). Figure 5 shows the structures of hemicelluloses of angiosperm (A) and gymnosperm (B), of which the principal linear chains are constituted of xylans.

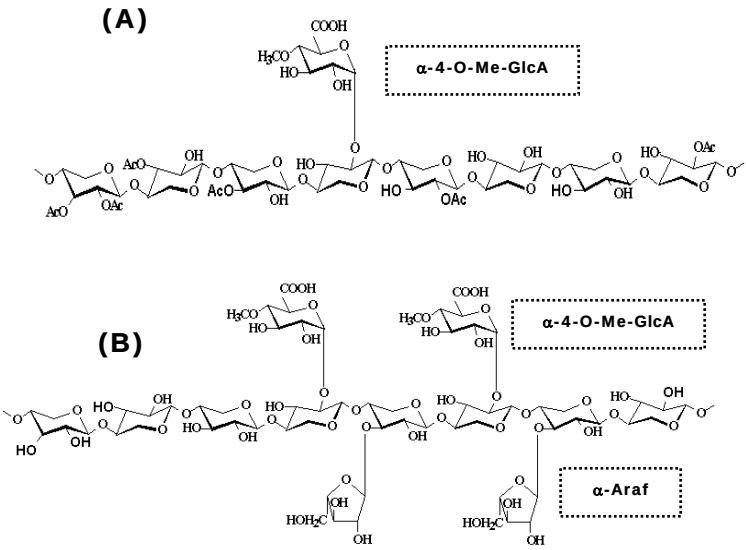

Figure 5: Angiosperm (A) and Gymnosperm (B) Hemicellulose Structures. Ac: acetyl group; α-Araf: α-Arabinofuranose; α-4-*O*-Me-GlcA: α-4-*O*-methylglucurinic Acid
Source: SUNNA and ANTRANIKIAN (1997).

Table 1 summarizes the main differences between the polysaccharides, components of lignocellulosic materials. The understanding of these characteristics is of fundamental importance, in order to define strategies for the use of these biomasses as feedstocks for ethanol production and other chemicals.

Table 1: Differences between Cellulose and Hemicelluloses

CELLULOSE	HEMICELLULOSES
Consists of glucose units	Consist of various units of pentoses and hexoses
High degree of polymerization (2,000 a 18,000)	Low degree of polymerization (50 a 300)
Forms fibrous arrangement	Do not form fibrous arrangement
Presents crystalline and amorphous regions	Present only amorphous regions
Slowly attacked by diluted inorganic acid in hot conditions	Rapidly attacked by inorganic acid diluted in hot conditions
Insoluble in alkalis	Soluble in alkalis

Lignin is a natural macromolecule composed by *p*-propylphenolic units with methoxyl substituents on the aromatic ring and, between these units, exist principally ether-type bounds. Lignin presents a highly complex structure (Figure 6), formed by polymerization of three different monomers: coumaric alcohol (I), coniferyl alcohol (II) and synapyl alcohol (III), which differ from one another by possessing different substituents in their aromatic ring. This structure is also responsible for the hardness of the cell wall, constituting in a binding material ("glue-like substance"), which holds the cellulosic fibers. Lignin possesses high molecular mass and presents about 25% of the photosynthesis biomass produced yearly on earth, retaining 50% more carbon than cellulose.

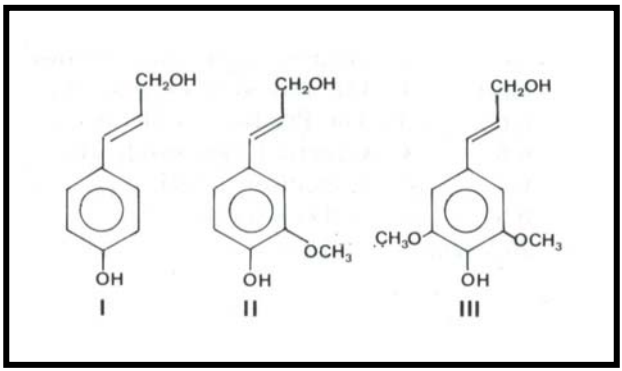

Figure 6: Primary Precursors of Lignin.

The lignin biodegradation is an oxidation process which involves a complex extracellular enzymatic system, produced principally by fungus species which live in wood, amongst them, the most studied is the fungus of the white rottenness *Phanaerochatae chrysosporium*. Some bacteria, especially the actinomycetes, are also able to degrade lignin. The main involved enzymes are lignin peroxidase, manganese peroxidase and lacase. These enzymes present great utilization potential for the pulp and paper industry, especially for the treatment of their recalcitrant effluent (ODIER & ARTAUD, 1992 and BREEN & SINGLETON, 1999).

Besides these three main components, there are others in minor proportions, such as resins, tannin, fat acids etc. Nitrogen compounds are found in small quantities, in general in the form of proteins. Amongst the mineral salts, the salts of calcium, potassium and magnesium are the most frequent (D'ALMEIDA, 1988 and WAYMAN & PAREKH, 1990).

Such as form and size of the cell wall of lignocellulosic materials vary from species to species, their chemical composition is distinctly in function of their origin. Table 2 presents the composition of some lignocellulosic materials, expressed in their three main components. In a general way, cellulose can be found in greater proportions, followed by hemicellulose and by lignin, finally. Even presented in minor quantities than the cellulose fraction, lignin offers sufficient limitation to delay or even to hinder completely the microbial attack on the material.

Table 2: Composition of Lignocellulosic Residues

Material	Composition (%)				Reference
	Cellulose	Hemicellulose	Lignin	Other	
Cane Bagasse	36	28	20	NR	2
Cane Straw	36	21	16	27	1
Maize Straw	36	28	29	NR	2
Corncob	36	28	NR	NR	2
Corn Straw	39	36	10	NR	3
Barley Straw	44	27	7	NR	3
Rice Straw	33	26	7	NR	3
Oat Straw	41	16	11	NR	3
Cotton Straw	42	12	15	NR	4
Peanut Shell	38	36	16	NR	4
Rice Shell	36.1	19.7	19.4	20.1	6
Barley Bran	23	32.7	21.4	NR	5
Pine Tree	44	26	29	NR	2
MSW	33	9	17	41	1
Willow	37	23	21	NR	2
Grass	32	20	9	39	1
Paper	43	13	6	NR	2
Cardboard	47	25	12	NR	2
Newspaper	62	16	21	1	1

MSW: Municipal solid wastes; NR: Not reported values; (1) SHLESER (1994); (2) OLSSON and HAHN-HÄGERDAL (1996), (3) AWAFO (1997); (4) GHOSH and SINGH (1993); (5) COUTO and SANROMÁN (2005); (6) CEN and XIA (1999).

Amongst lignocellulosic residues of greater importance, we can stand out: cane bagasse and straw, corn straw and stover, rice straw, wheat straw, processed-wooden wastes and municipal residues from paper. In the Brazilian context, it can be estimated that only the sugar-alcohol sector generates, approximately, 6.6 million tons of exceeded cane bagasse (surplus) and 76 million tons of straw (MAPA, 2006 and DEDINI, 2005). An estimation of the potential of these residues in the sugar-alcohol sector for ethanol production, for example, let us to conclude that, with the technological knowledge we have today, we could double the Brazilian production of this fuel, without needing to expand the extension of the agricultural areas. Considering even the food biomass residues, the total generated quantity in our country reaches the value of approximately 350 millions of tons/year (Figure 1). This is an enormous potential, which should not be neglected and point out that these materials should have a more rational use.

RESIDUAL BIOMASSES AND THE BIOREFINERY ASSOCIATED CONCEPT

The chemical industry has been discovering the value of the molecules contained in the lignocellulosic materials, and due to the abundance of their generation in the form of residues, the market starts to focus on their use. A promising area has been developed, and it is generally agreed to denominate it "**BIOREFINERY**".

Biorefinery is a relatively new term, referring to the use of renewable materials (*biomasses*) and their residues, in a most integral and diversified way for the production of fuels, chemicals and energy, with minimal generation of wastes and emissions.

The Biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum, and where an industrial segment works as a generating pole of raw materials to others. Industrial Biorefineries have been identified as the most promising route to the creation of a new domestic biobased industry. Saying it in a simple way, it means that products, secondary products and agricultural and agro-industrial residues sustain different types of processes.

The utilization of lignocellulosic biomass within the context of Biorefinery is based on two different platforms. Both platforms aim at providing building blocks to obtain a variety of valuable products. The **biochemical platform** is based on biochemical conversion processes of glucosides (sugars) extracted from the biomass by hydrolytic (chemical and/or enzymatic) processes. Yet, the **thermochemical platform**, as the name implies, is based on thermochemical conversion processes by reacting the raw material at high temperatures with a controlled amount of oxygen (gasification) to produce syngas ($CO + H_2$) or in the absence of oxygen (pyrolysis) to produce a bio-oil, which after hydrodeoxigenation process produces a liquid mixture of hydrocarbons similar to

those of petroleum crude oil. The following figure illustrates the two lines of using lignocellulosic feedstocks for the production of fuels and other chemical substances of industrial interest.

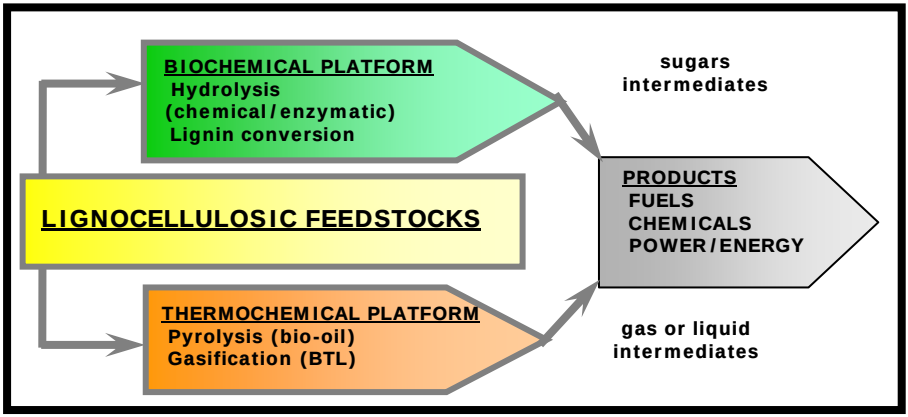

Figure 7: Utilization of Lignocellulosic Feedstocks within the Context of Biorefinery (BTL: *biomass-to-liquids*)

Figures 8, 9 and 10 describe schematically the Biorefinery concept, following the biochemical platform and its application around the agro-industry, having its lignocellulosic biomasses as a center of the production processes of a great variety of molecules. Such concept has been a target of heavy investments from the north-Americans, aiming at restructuring their industry, especially the alcohol industry. The idea of creating a "broad belt" of processes presents enormous logistical advantages, principally for the transportation of feedstock, products and services offer. In Brazil, this new industrial structure is still on seed stage, being increasingly studied.

The total hydrolysis of **cellulose** generates only glucose, which can be converted into a series of chemical and biochemical substances (Figure 8). We can say that the glucose, because of the existence of an exclusive and common metabolic pathway for the great majority of living beings, can be biologically converted into a wide range of substances such as: ethanol, organic acids, glycerol, sorbitol, mannitol, fructose, enzymes, and biopolymers, amongst others. It can be still chemically or enzymatically converted to hydroximethylfurfural, which is an important intermediate for the production of dimethylfuran (DMF) or furan-based polymers.

The hydrolysis product of the **hemicellulose** fraction is a mixture of sugars, as described before, with predominance, in most cases, of xylose. The traditional market for this sugar has been the production of furfural, a selective solvent, very reactive, being used in large scale in the purification of mineral, vegetal and animal oils, as well as in the vitamin A concentration of fish liver.

Alternatively, xylose can be hydrogenated to produce xylitol, which presents applications as a non-cariogenic sweetener, with the same sweetening power of sucrose and with metabolization in the humans independent of insulin.

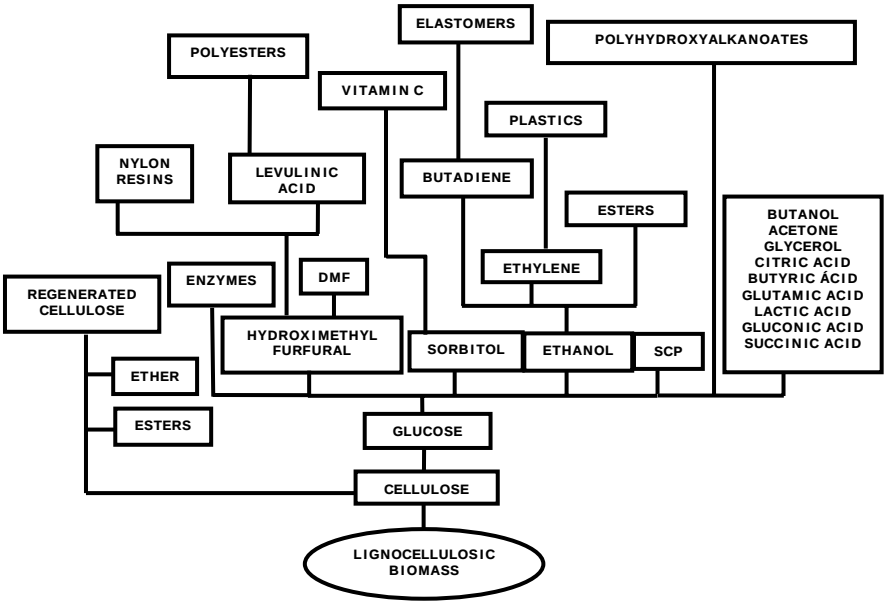

Figure 8: Lignocellulosic Feedsstock Biorefinery: **Cellulose Products**

Nevertheless, xylose can be biologically converted to single cell protein (SCP) and to a variety of fuels and solvents, such as: ethanol by yeasts with the ability to ferment this pentose (*Pichia stipitis*, *Candida sheratae* or *Pachysolen tannophilus*); xylitol, by microrganims with exclusively NADPH-dependent reductase activities on xylose, as for example, *Candida guilliermondii*, *Debaromyces hanseni* and *Candida tropicalis* (FOGEL *et al.*, 2005; VASQUEZ *et al.*, 2006); biopolymers (polyhydroxyalkanoates, polylactate etc); a series of organic acids (succinic, propionic, acetic, lactic and butyric); solvents (butanol and acetone) and other fuels/fuel additives (DMF, butanol, 2,3 butanediol).

As said before, the lignocellulosic biomass is the most abundant organic material on earth. Approximately, 50 millions of tons of **lignin** are generated worldwide per year, as residues from the production processes of the cellulose pulp and paper industry (OTTO DILLE, 2008). Most of the residual lignin is burnt to generate energy in this industrial segment. However, in view of its interesting functional properties, lignin offers useful perspectives to obtain high-value products, such as: carbon fibers, emulsifiers, dispersants, sequestrants, surfactants, binders and aromatics (LIGNIN INSTITUTE, 2006).

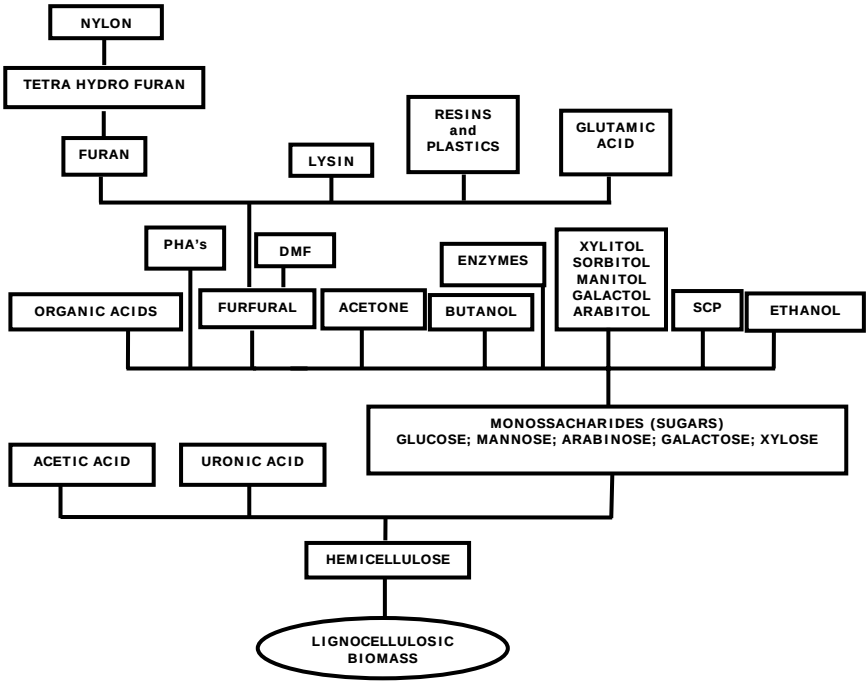

Figure 9: Lignocellulosic Feedsstock Biorefinery: **Hemicellulose Products**

The physical and chemical properties of lignin differ depending on the extraction technology (sulphite process, Kraft process, alkaline and organosolv process). For example, the lignosulphates[2] are hydrophilic and the *Kraft lignins*[3] are hydrophobic (van DAM *et al.*, 2006).

The industry started first using lignin in the 1880's, when lignosulfonates were used in leather tanning and dye baths. From that time onwards, lignin has even found applications in food products, serving as emulsifiers in animal feed and as raw material in the production of vanillin, which is extensively used as flavoring in food, as component in the pharmaceutics product formulation and also as fragrance in the perfume industry. The derivative product applications of

2 In the sulphite pulp production process, the wood chips are subjected to heat in acids and the lignin is sulphonated in the way that it becomes soluble in water and, can be separated from the insoluble cellulose. The soluble lignins in the water are denominated lignosulfonates.

3 In the pulp production with the *Kraft* process, the wooden chips are heated in a pressure vessel (digester) with boiling liqueur formed principally of an aqueous solution of sodium hydroxide and sodium sulphide.

lignin will expand literally, creating impacts in a lot of industrial segments (LIGNIN INSTITUTE, 2006).

Although hundreds of applications for lignin can be pointed out, its main use in the pulp and paper industry is as bio-fuel to replace fossil fuels in heat or power generation, and the lignin-depleted black liquor can be reused in the cooking operation. All those well-established industrial knowledge of the pulp and paper industry can be incorporated in the Biorefinery concept using other sources of lignocellulosic feedstocks.

By producing multiple chemicals, the Biorefinery takes advantage of the various components in biomass and their intermediates, therefore maximizing the value derived from the biomass feedstock. A Biorefinery could, for example, produce one or several low-volume/high-value chemical or biochemical products and a low-value/high-volume liquid transportation fuel such as bioethanol or dimethylfuran; and at the same time generating electricity and process heat, through combined heat and power technology for its own use, and perhaps enough for sale of electricity to the local utility. The high-value products increase profitability, the high-volume fuel helps to meet energy demands, and the power production helps to lower energy costs and reduce greenhouse gas emissions from traditional power plant facilities. Although some of these facilities already exist, the Biorefinery has yet to be fully realized.

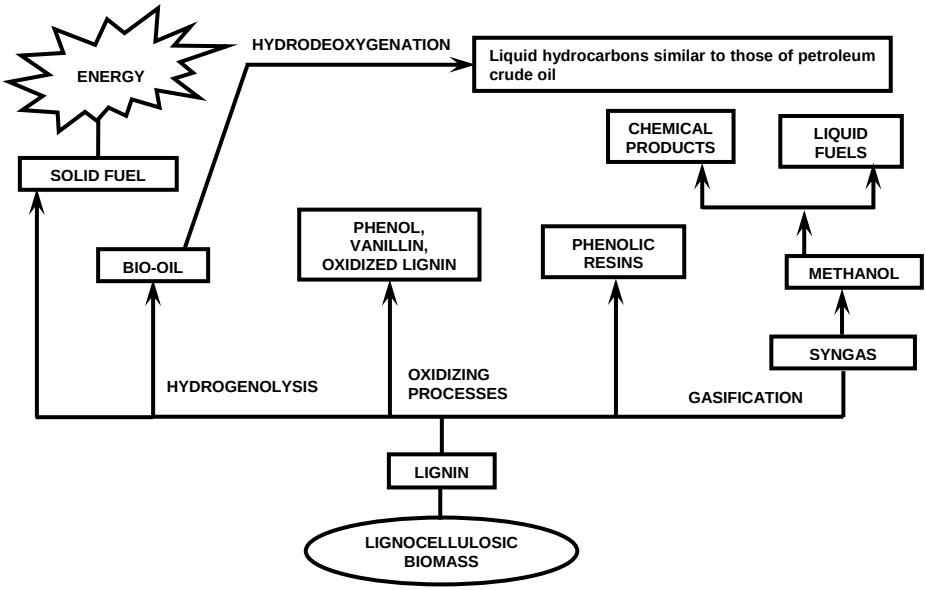

Figure 10: Lignocellulosic Feedsstock Biorefinery: **Lignin Products**

FRACTIONATION OF LIGNOCELLULOSIC BIOMASS COMPONENTS

To make possible the use of lignocellulosic materials as feedstocks for the production of ethanol and other chemicals following the biochemical platform, it is necessary to separate their main components. For this separation, a pretreatment stage is essential, which aims at fundamentally disorganizing the lignocellulosic complex. The pretreatment can be realized through physical, physical-chemical, chemical or biological processes, and can be either associated or followed by hydrolysis procedures of the polysaccharides (hemicellulose and cellulose) in their respective monomeric units (pentoses and hexoses).

The most adequate pretreatments are: the prehydrolysis or the steam explosion process, with a partial depolymerization and dissolution of the hemicelluloses. From the remaining material (cellulose + lignin), the cellulose can be separated, through lignin dissolution with alkalis or organic solvents (delignification), remaining the cellulose component with its increased digestibility for the enzymatic hydrolysis, or lignin can be separated through the cellulose hydrolysis with strong mineral acids (concentrated or diluted) in high temperatures (HARRIS, 1975). The latter has been abandoned, since toxic substances can be generated in the hydrolysis process.

Figure 11 shows a simplified scheme for the fractionation of the main components of the lignocellulosic materials.

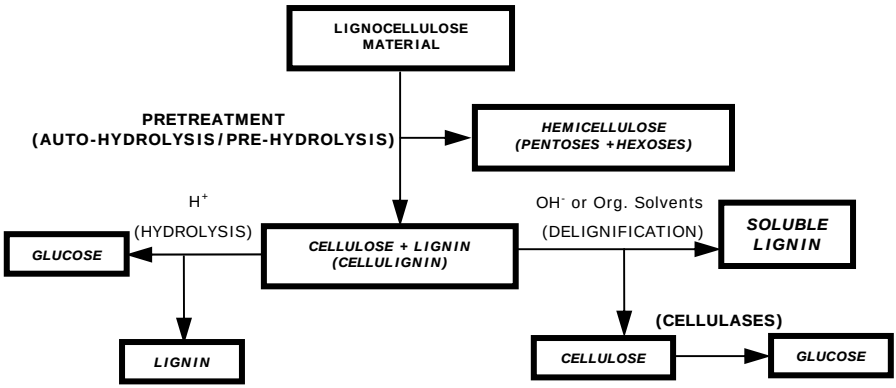

Figure 11: Fractionation of Lignocellulosic Components.
Source: SCHUCHARDT *et al.* (2001).

PRETREATMENTS

In the lignocellulosic biorefinery context, be it for the production of ethanol or other chemicals, pretreatment is understood as a process through

which the cellulose molecule becomes more susceptible to enzymatic hydrolysis by cellulases. In the literature, frequently the terms “prehydrolysis” and “autohydrolysis” are used as synonyms of pretreatment. The increasing enzyme accessibility to the cellulose molecule is due to the removal of the hemicellulose fraction, as well as to the partial lignin removal (acid soluble lignin), promoting a sort of “opening up” of the lignocellulose matrix. Additionally, as described further on, the usual pretreatment techniques involve a synergism between the heat action, the medium pH and the time of exposition under process conditions. This results in a decrease in cellulose crystallinity, and, consequently, making it more susceptible to the action of cellulases (RAMOS, 2003; LYND, 1996; LYND *et* al, 2002; MC MILLAN, 1994; MOSIER *et al.*, 2005; OGIER *et al.*, 1999; SUNG and CHENG, 2002).

The pretreatments can be divided in four types: physical (comminution of the material through fragmentation or grinding); physical-chemical (steam-explosion, catalyzed or not); chemical (acid hydrolysis in mild conditions, ozonolysis or oxidizing delignification) and biological (microbial or enzymatic), according to the agent which acts in the structural alteration (MC MILLAN, 1994; SUNG and CHENG, 2002).

Because of the heterogeneity of the lignocellulose, it is not possible to choose only one pretreatment as being considered the best. The choice will depend, basically, on the nature/source of the material which needs to be treated, as well as on the use of the hydrolysate material. Various pretreatment processes have been developed aiming at increasing the efficiency in the removal of the hemicellulose fraction (LYND, 1996). Thus, pretreatment processing conditions must be tailored to the specific chemical and structural composition of several sources of lignocellulosic biomass. As follows, the main available pretreatment technologies for utilizing lignocellulosic materials are described with more details.

Thermal Pretreatments

A quite efficient alternative for the extraction and partial hydrolysis of the hemicelulose is the technology of compression and fast decompression, carried out by the so-called “steam-explosion”, also denominated “autohydrolysis”. Its operation works through the impregnation of the lignocellulosic material in water, in a system under high pressure (7 to 50 atm) and temperature (160 to 190^{o}C) (SUN and CHENG, 2002). After that, the pressure is alleviated instantaneously. This change provokes a violent explosion, resulting in the rupture of the structural bindings of the lignocellulosic material (NEGRO *et al*., 2003). A wet solid material is obtained with the lignocellulosic complex disorganized (so-called cellulignin) and a liquid phase extractable by explosion, composed of: xylose, xylooligosaccharides and uronic and acetic acids. The partial hydrolysis of hemicellulose, in special of highly acetylated xylanas, results fundamentally from their acid characteristics, therefore the term “autohydrolysis”.

The structures of the hemicelluloses differ significantly as a function of their origins (HAMELINCK *et al.*, 2005; SUN and CHENG, 2002). The hemicelluloses of *hardwood* are composed in their greater part of highly acetylated heteroxylans, generally classificated as 4-*O*-methyl glucuronoxylans.

Hexosans, in the form of glucomanans are also present, but in much lower quantity. Due to the acid characteristics and the chemical properties, the xylans of *hardwood* are relatively labile (unstable) to the acid hydrolysis and suffer auto-hydrolysis in relatively moderate conditions. In contrast, the hemicelluloses of *softwoods* have a higher proportion of glucomanans and galactoglucomanans in part acetylated, and the xylans only correspond to a small fraction of their total structure. In consequence, the hemicelluloses of *softwood* (in its major part composed of hexosans) are more resistant to the hydrolysis processes than the hemicelluloses of *hardwood* (in its major part composed of pentosans) (RAMOS, 2003).

The steam-explosion is an operation of wide knowledge in the Brazilian sugar-alcohol sector, which uses it to increase the digestibility of the cane bagasse for consumption in the animal feeding. In such a way that, the adoption of this pretreatment technology for second generation ethanol production, would be easily incorporated by this industrial sector.

> In the last decades, various studies have been realized involving the use of chemical agents, aiming at increasing the process efficiency of the steam-explosion. In this case, the denomination "catalyzed steam-explosion" has been used. The main chemical agents used are: sulfuric acid, with concentration varying between 0.1 and 5% v/v and sulfurous anhydride (which in solution form sulfuric acid). When using sulfuric acid, previously to the steam-explosion, the material is soaked in the acid solution. After this phase, it is realized the steam-explosion process. In the case of the use of sulfurous anhydride, it is carried out by introducing in the vapor phase a rich stream with this gas. In both cases, the temperature range and the exposition time are not different from the simple steam-explosion (LYND, 1996; OGIER *et al.*, 1999 and HAMELINCK *et al.*, 2005).
>
> Other chemical substances can be used, as the carbonic gas, which in solution forms carbonic acid (HOHLBERG *et al.*, 1989) or ammonium, a known process as AFEX (*Ammonia Fiber Explosion*), which principle is based on the high solubility of the hemicellulose in alkaline environments (TEYMOURI *et al.*, 2005). However, it needs to be considered that, similarly, lignin presents high solubility in these alkaline environments, and it might be necessary a detoxification step of the medium generated from this process.
>
> In the steam-explosion technology, the recuperation of the pentoses in the liquid stream takes place in the range of 45 to 65%, and when catalysed it rises to 80-90%. The retention of the cellulose in the solid fraction, in both cases, is superior of 90% of the original structure.

Chemical Pretreatments

Various chemical pretreatments were studied aiming at the removal of the hemicellulosic fraction, the cleavage of the bindings between the lignin and the polysaccharides and the reduction of the cellulose crystallinity degree before the enzymatic hydrolysis of cellulose. Even thought a lot of these processes reach high efficiency, there exists the disadvantage of them requiring plants constructed with materials which have great resistance for drastic reaction

conditions, especially concerning the aspect of environment corrosivity. The main used chemical agents are: acids, alkalis, gases, oxidant agents, solvents, etc (HAMMELINK *et al.*, 2005; SUN and CHENG, 2002; OGIER, 1999).

The alkaline pretreatment is frequently used to increase the digestibility of lignocellulosic materials. This process was originally developed in the paper and cellulose industry in the pulping processes to attain paper with long fiber, being indicated, especially when working with straws, due of their lignin content. The normally used conditions in this pretreatment are: concentration of NaOH between 8 and 12% of the dry biomass to be treated, time of exposition between 30 and 60 minutes and temperature between 80 and 120ºC (MOSIER, 2005). The disadvantage of this process is related to the caustic soda price and the difficulty of its recuperation, what still involves prohibitive costs (HAMMELINK *et al.*, 2005; SUN and CHENG, 2002).

An alternative to the alkaline pretreatment is the simultaneous use of peroxide (*"alkaline peroxide medium"*). The delignification of the lignocellulosic materials with peroxide of hydrogen depends strongly to the pH, since its dissociation occurs in pH values around 11.5. Such dissociation results in the formation of highly reactive radicals, which act with the lignin molecule leading to its solubilization and oxidation. Some variations of this process involve two phases, the first using caustic soda and the second soda and peroxide. The oxidative delignification with peroxide occurs in low temperatures (25-40ºC), and as a general rule, the generated residues have a low pollutant load. Another agent which is being recognized by its high oxidant power and selectivity in breaking the structure of lignin is the peracetic acid. This acid promotes the opening of the aromatic rings of the lignin, generating dicarboxylic acids and their lactones (TEIXEIRA *et al.*, 2000). Similarly to the previous case, the process also can be operated in two phases, trying to minimize the costs with peracetic acid, since its price is high. (HAMMELINK *et al.*, 2005; SUN and CHENG, 2002; OGIER, 1999).

Notoriously, the advantage of the alkaline or the alkaline-oxidative pre-treatment is the low energetic demand. Nevertheless, these processes present some potential disadvantages. Strongly alkaline environments can degrade the hemicellulose in saccharinic acids, which are not substrates for fermentation processes and the oxidized degradation of lignin generates an accumulation of phenolic monomers and oligomers, which are inhibitors of the biological transformation processes. The process named *organosolv*, involving the use of diluted alkalis together with solvents (ethanol, for example) has been considered as a promising alternative for the delignification (SUN and CHENG, 2002). Nevertheless, such technology is still being studied and recent experiences of its application in Brazil shows that the problem of the generation of toxic inhibitors was still not solved.

As already described, the acidity of the medium is one of the fundamental aspects for increasing the efficiency of the pretreatment. Based on this, the acid pretreatment processes - in special those which employ diluted sulfuric acid - are more and more becoming a target of worldwide studies. The high reaction rates, the reduced consumption of acid and its low cost, when compared with alkalis, constitute in advantages of these processes. As mentioned previously, the disadvantages reside in the question of the corrositivity and, also, depending on the imposed operational conditions, in the formation of the

inhibitors. The acid concentration ranges from 0.1 to 5%, the temperature between 110 and 220°C, and the exposition time from 10 to 180 min. Several studies indicate that the pretreatment performed with more than one stage can reach high efficiency, causing a lower consumption of cellulases during the enzymatic hydrolysis phase (MOSIER, 2005; OGIER, 1999; LYND, 1996).

Biological Pretreatments

The biological pretreatments consist in the use of a "*pool*" of enzymes, aiming at the hydrolysis of the hemicellulose and the delignification. In the case of the hydrolysis of the hemicelluloses, in spite of the specificity of xylanases, where the action is carried out through the synergy of the β-xylosidase, endo 1,4-β-xylanases, acetyl-xylanaesterase, α-glucoronidase and L-arabinofuranosidase enzymes, there are problems related to the costs of those enzymes, which still consist in impediment for the implementation of these on an industrial scale. In this way, studies have been developed with the objective of producing enzymes from the xylanase complex (FERREIRA *et al*, 2006; DAMASO *et al.*, 2004). However, the principal focus has been to the paper and cellulose sector, in which a crescent interest can be observed to use such enzymes in the stage of pulping, in substitution of chlorine and chorine derivatives substances. This can be seen, principally, due to the irreversible tendency in favour of the total chlorine free bleaching (TCF systems) and elemental chlorine free (ECF systems) (VIIKARI *et al*, 1994).

The commercial development of hemicellulases is not so advanced than that of the cellulases; therefore the current commercial preparations of cellulase have been developed for the hydrolysis of pretreated biomass with diluted acid, where the hemicellulose is removed before the cellulose saccharification, as previously described. Nevertheless, with the development of non-acid pretreatments, in which the hemicellulose fraction stays intact or partially hydrolised, the hemicellulases will be compulsory required.

The present commercial cellulases, as of the *Trichoderma reesei* strains, tend to posses low hemicellulase activity and are not adequate for the complete conversion to the hemicellulose monomeric sugars. It is been expected that the development of low cost hemicellulases production, working in synergism with cellulases, will be intensively focused in the next future. Figure 12 shows the different enzymes of the xylanolytic complex.

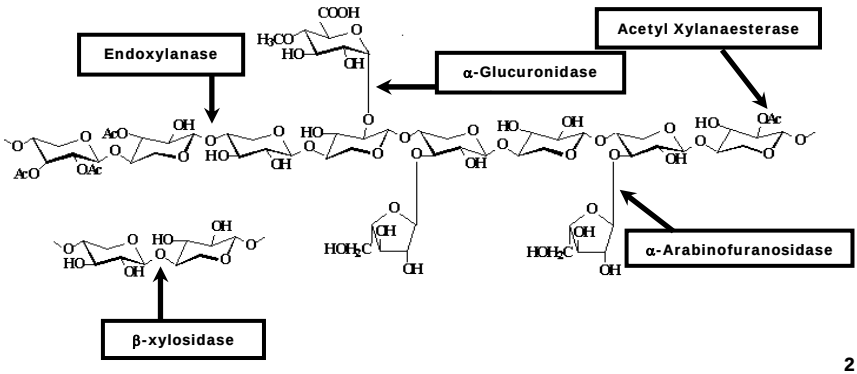

Figure 12: Enzymes Involved in the Hydrolysis of Xylans.

Lignin imposes challenges for the enzymatic cellulose hydrolysis, due to its non-productive binding with the cellulases, which results in a decrease in their catalytic power and inactivation. Berlin *et al.* (2005) proposed a new approach to improve the activity of the cellulases during the hydrolysis of lignocellulosic materials using enzymes which bind themselves weekly to the lignin. The authors show that cellulases of naturally-occurring microorganism, with similar catalytic activity, differ significantly in relation to their affinity for lignin and, therefore, affecting the performance of the enzymes on the native substrates. Palonen (2004) showed that the localization and the structure of lignin affect more the enzymatic hydrolysis than the absolute lignin quantity in the lignocellulosic complex. The study revealed, furthermore, that modifications of the lignin surface by oxidants treatments with laccase led to a rise of the cellulose hydrolysis. However, there still not exist sufficient studies about the transposition of a laboratorial scale to pilot plant which can show their technical and economical viability for substituting chemical pretreatments.

Other Technologies

Other pretreatment processes are being studied as well. Some studies propose the use of liquid hot water (*"LHW"*). This technology, called thermohydrolysis, involves the washing of the material with pre-heated water in high pressure, with temperatures of circa of 220°C and times around 2 minutes, but the efficiencies are still low, when compared with the steam-explosion or with the acid prehydrolysis (MOSIER *et al.*, 2005).

The use of irradiation with micro-waves has been a target of some researches (KITCHAIYA *et al.*, 2003). Commonly used conditions are irradiation of 240 W per 10 minutes. However, contrary to all described pretreatment technologies, irradiation is still studied on bench scale, and it remains uncertain its application on an industrial scale, in view of the inherent energetic demand for the process.

Relevant pretreatments are: **steam-explosion process** and **diluted acid hydrolysis** (GLASSER and WRIGHT, 1998; LYND, 1996; MOSIER *et al.*, 2005; SUNG and CHENG, 2002). It should be pointed out that, when the intention is to hydrolyse the cellulose fraction with enzymes, the steam-explosion pretreatment is one of the most adopted technological tendencies. In this case, the hydrolytic process is carried out in several stages, as follows: comminution of the lignocellulosic material, steam-explosion, followed by the removal of the hemicellulosic fraction (liquid phase). The liqueur from the prehydrolysis step contains some soluble pentoses and hexoses, lignin and oligomers of pentoses. The oligomers are then hydrolyzed to monomers, or at least to dimers or trimers, by any conventional technique. Alternatively, the steam-explosion process can be integrated (catalyzed steam-explosion), by using auxiliary inputs, as acidic gases (being SO_2 mostly used), which will result in a monomer-containing liqueur. Lastly, cellulases are used in the remaining solid (cellulignin) for the attainment of a glucose-rich medium (LYND *et al*, 2002). A delignification step might be necessary to improve the enzymatic efficiency. Some technological "bottlenecks" can be identified in the pretreatment using acid medium and high temperatures, as follows: formation of unwanted toxic compounds, derived from the sugar and lignin degradation, which can cause inhibition to the biological conversion

processes; and problems related to equipment corrosion when working in acidic medium with high temperatures.

Table 3 summarizes the principal characteristics of the more employed and more current pretreatment technologies.

Table 3: Characteristics of the Main Pretreatment Technologies for LC Feedstsocks

Characteristics	Pretreatment Technology			
	Steam-explosion	**Catalysed Steam-explosion**	**Diluted Acid Hydrolysis**	**Thermohydrolysis**
Typical operational conditions	Batch or Continuous 190-270°C 1 to 10 min	Batch or Continuous 160-200°C 1 to 10 min	Batch or Continuous 150-180°C 5 to 30 min	Batch 170-230°C 5 to 60 min
Consumption of chemical inputs	No	Yes	Yes	No
Recuperation of Pentoses	45 to 65%	> 80%	> 80%	> 80%
Formation of inhibitors	Yes, under severe conditions	Yes, under severe conditions	Yes, under severe conditions	Few
Reduction of the required particle size	Medium	Medium	High	Medium
Efficiency of the cellulose enzymatic hydrolysis	> 70%	> 80%	> 90%	> 80%
Waste Generation	Less significant	Moderate	Significant	Less significant
Corrosivity of the medium	Low	Low to moderate	Moderate to high	Low
Simplicity of the process (potential)	High	Moderate to high	Moderate	Not evaluated
State of arte	Various pilot plants	Various pilot plants	Various pilot and demonstrative plants	Bench scale

Sources: Adapted from LYND (1996) and OGIER *et al.* (1999).

FACTORS INFLUENCING THE PRETREATMENT

The comprehension of the mechanisms involved in the pretreatment processes is still not completely elucidated and the project of those processes is normally made on empirical basis. It can be affirmed that exist different levels of importance of the involved mechanism in function of the different pretreatment processes. In the lignocellulosic materials found in the nature, the cellulose is intimately associated with the hemicellulose and with other structural macromolecules, being the microfibrils rich in carbohydrates involved with a kind of “glue” constituted of lignin (LYND, 1996). Clearly, the primary function of the

pretreatment is to open up the multi-component matrix, in the way of turning cellulose more accessible to the biocatalysts, as described previously. In this way, several factors assume outstanding importance, according to the following summary:

Time x Temperature: in a first moment, it is possible to believe that, as higher the temperature is, as greater is the hydrolysis efficiency. Nevertheless, higher temperatures can lead to the degradation of free sugars to furfurals (furfural from pentoses and hydroxymethyl furfural from hexoses), reducing the efficiency of the pretreatment and increasing the toxicity of the hydrolysate. On the other hand, even with lower temperatures, the same thing can take place when the time of exhibition is very long. In a general way, higher temperatures implicate in shorter times and vice versa (MC MILLAN, 1994; MOSIER *et al.*, 2005). Additionally, it should be noticed that the greater the extension of the prehydrolysis step, the greater the cost is with heat to maintain the temperature and the slower the overall process.

Size of the particles: generally, the pretreatment efficiency when using processes with diluted acid is increased in systems with particles of smaller size. The size of particles affects the available surface area for further actuation of the hydrolysis agent, as well as the cellulose crystallinity and its degree of polymerization. On the contrary, when steam-explosion is used, especially with cane bagasse, it is more convenient to work with particles of bigger size (CADOCHE and LÓPEZ, 1989; LYND, 1996).

Structure of the cellulose molecule: the crystallinity and the polymeration degree of the cellulose molecules are closely associated to its reactivity. Greater efficiencies are reached with a lower degree of polymerization and crystallinity indexes (Mc MILLAN, 1994; MOSIER *et al.*, 2005).

Acidity of the medium: the proton concentration has an important role for the chemical reactions involved in the pretreatment stage. Even though the technology does not involve the addition of acid to the process, with the use of high temperatures the medium acidity is favoured by the releasing of endogenous organic acids, principally acetic acid, as well as by the protonation resulting from the water dissociation (LYND, 1996; MOSIER *et al.*, 2005).

Humity: The role of the water in the pretreatment processes should not be underestimated. The presence of water decreases the temperature of lignin degradation, facilitating its removal from the fibers. Moreover, the glycosidic linkages in the cellulose as well as in the hemicellulose are cleaved by hydrolysis, and in case of the hemicelulose - the most susceptible fraction to the hydrolysis - partial desacetylation and depolymerization take place. Similarly, the lignin also suffers from partial depolymerization. The cleavage of the glycosidic bonds can be incremented with the increase of the water deprotonation constant in high temperatures, what causes a drop in the medium pH. For example, at 220^{o}C, the medium pH reach 5.6, while at room temperature the value is equal to 7.0. Due to this effect, it is believed that, in high temperatures, the role of the water is more relevant than the role performed by the free organic acids (LYND, 1996).

Finally, it should not be neglected that the efficiency of the pretreatment processes is a result of the synergism between temperature, time and medium acidity. The combination of this factors defines the parameter "degree of severity", which is intrinsically associated with the toxicity and fermentability of the hydrolysates. Generally, there exists an optimum degree of severity, under which the hydrolysis efficiency will be lower, and above which there will be degradation of sugars and formation of other derivative inhibitors from lignin (Mc MILLAN, 1994).

DETOXIFICATION PROCESSES OF LIGNOCELLULOSIC HYDROLYSATES

Depending on the application of the hydrolysates and on the adopted pretreatment technology, their detoxification can be necessary. Preferably, the generation of inhibitors during the pretreatment stage should be minimized, since, in a lot of cases, the detoxification technology can lead to a partial loss of sugars originating from the hemicellulose hydrolysis (BRITO, 2000; MUSSATO and ROBERTO, 2004). Table 4 shows some usual detoxification procedures.

Table 4: Procedures for the Detoxification of Hemicellulose Hydrolysates

Procedures	**Effects**
Treatment with fluent steam.	Removal of volatiles (furfural, phenols, acetic acid).
Neutralization with CaO, NaOH, KOH; treatment with active coal; filtration.	Reduction in acetic acid concentration.
Neutralization (pH=6.5) or alkalinization (pH=10) with $Ca(OH)_2$, CaO or KOH; removal of the precipitated; addition of H_2SO_4 (pH=6.5).	Precipitation of acetate, heavy metals, furfural, tannins, terpenes, phenolic compounds
Ionic exclusion chromatography.	Aromatics removal.
Neutralization (pH=6.5) with $CaCO_3$; removal of the precipitated; treatment with active coal; filtration.	Clarification; removal of $SO_4^{=}$ and phenolic compounds.
Extraction with ether.	Removal of furfural.
Vacuum Evaporation.	Removal of acetic acid.
Extraction with ethyl acetate.	Removal of derivative compounds from the lignin degradation.

Recent studies developed in our laboratories on the prehydrolysis of cane bagasse showed that the tendency is to minimize, or even, to abolish the use of techniques of detoxification. Through the progressive acclimatization of yeasts in non-detoxified hydrolysates, Fogel *et al.* (2005) and Betancur (2005) reached good results in the production of xylitol and ethanol, respectively. The absence of further treatments after prehydrolysis potentially makes the lignocellulosic biomass utilization economically more competitive.

ENZYMATIC HYDROLYSIS OF CELLULOSE

The cellulose hydrolysis processes can be chemical or enzymatic. The first, of greater knowledge, is run under established conditions of temperature (pressure), exposition time, type and concentration of acid, as well as solid:liquid ratio, similarly to the prehydrolysis, which was described previously.

The option for the enzymatic hydrolysis of the cellulose comes from the absence of severe conditions, typically of the chemical hydrolysis. This technological strategy differs from the conception of old processes in which the chemical hydrolysis of cellulose and hemicellulose (polyssaccharides with different susceptibilities to the hydrolytic attack) was taking in one step. These processes generated hydrolysates with high toxicity, which hindered the metabolism of the microrganisms agents of the fermentative processes. Nevertheless, the cellulose chemical hydrolysis has been left behind and substituted by the enzymatic hydrolysis. Therefore, its description will not be emphasized here.

Celulases have in the nature a fundamental role, through the degradation of cellulose present in plant biomass to establish a basic link in the development of the carbon cycle. To face the challenge of degradating the cellulose, cellulolytic microorganisms produce a complex mixture of enzymes: *the cellulases*. These enzymes, which collectively present specificity for the glycosidic linkages β-1,4, are all necessary for the complete solubilization and hydrolysis of cellulose (amorphous and crystalline), existing a synergism in their way of acting. The soil surface is the principal habitat of the cellulolytic aerobic microrganisms.

The enzymes of the cellulolytic complex are classificated in three groups: **Endoglucanases**, which cleave the internal bindings of the cellulose fiber producing cellodextrins; **Exoglucanases**, which act in the external region of the cellulose producing cellobiose; and **ß-glucosidases**, which hydrolyse soluble oligosaccharides to glucose (LYND *et al*., 2002). Figure 13 outlines the action of these enzymes on cellulose.

The group of exoglucanases is constituted in its majority of 1,4-ß-D-glucan-glucanohydrolases enzymes (EC 3.2.1.74), also known as cellodextrinases and 1,4-ß-D-glucan-cellobiohydrolases enzymes (EC 3.2.1.91). Amongst both types, certainly the most referred in literature is the cellobiohydrolases (CBH).

The cellobiohydrolases are distinguished in two types: The enzymes of type I (CBH I) hydrolyse reducing terminals, while the ones of type II (CBH II) hydrolyse non-reducing terminals. These enzymes generally suffer inhibition by their hydrolysis product (cellobiose) (AWAFO, 1997).

The structure of cellobiohydrolases presents a region in the form of a “hook”, which function is to bind the cellulosic fiber, facilitating its access to the catalytic site. Additionally, it is referred that the CBH I possesses ten active subsites under catalytic domain, which function is to bind itself physically to the cellulose and initiate the chemical reactions which hydrolyse the chain to cellobiose.

The third and last great group of enzymes of the cellulolytic complex include the ß-glucosidases, or ß-glucohydrolases enzymes (EC 3.2.1.21). The ß-glucosidases have the property to hydrolyse soluble cellobiose and oligosaccharides (with less than seven monomeric units) to glucose. Equally to the cellobiohydrolases, they are also reported to suffer inhibition of their hydrolysis product (AWAFO, 1997).

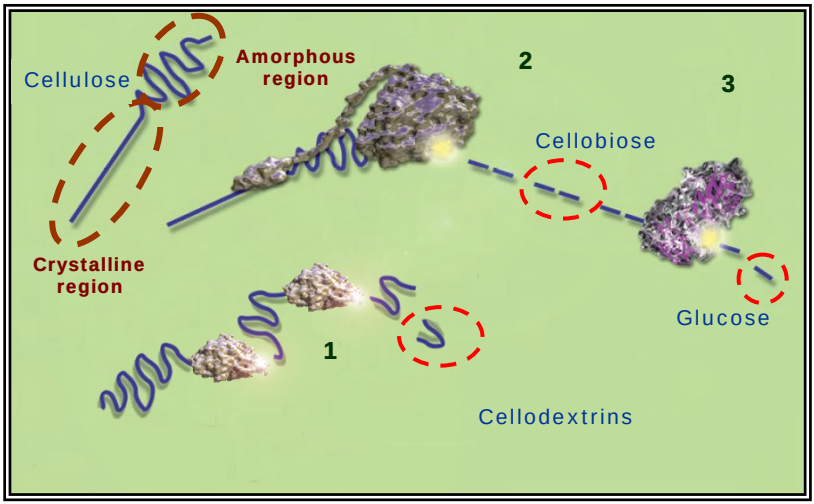

Figure 13: Enzymes involved in the Hydrolysis of the Cellulose.
(1): **ENDOGLUCANASE**; (2) **EXOGLUCANASE** and (3) **β-GLUCOSIDASE**

When acting together, the cellulases present a better yield than the sum of the individual yields when acting isolated. Such effect is known as synergy. There are at least three forms of synergy (LYND *et al.*, 2002):

- Endo-Exo synergy. The endoglucanases, acting in the amorphous regions of the fiber, provide reducing e non-reducing terminals for the action of the CBH I and CBH II, respectively;
- Exo-Exo synergy. The CBH I and CBH II acting simultaneously in the hydrolysis of the reducing and non-reducing terminals released by the action of the endoglucanases;
- Exo-BG synergy. The cellobiohydrolases liberate cellobiose, which is substrate for the ß-glucosidases.

The hydrolysis of the cellulose polymer by cellulases involves basically two phases: The adsorption of the cellulases on the surfaces of the cellulosic substrate (fiber) and the hydrolysis of the cellulose in fermentable sugars. For this, the following steps happen (AWAFO, 1997):

1. Diffusion of the cellulolytic complex from the bulk of the fluid to the location region of the cellulose substrate. In the case of insoluble substrate, the

diffusion happens in direction to the film immediately surrounding the solid substrate;
2. Adsorption of the cellulolytic complex to the available sites in the cellulosic substrate;
3. Formation of an active cellulase-substrate complex;
4. Hydrolysis of the glycosidic linkages of the cellulose polymer;
5. Diffusion of the hydrolysis products from the active sites "cellulases-substrates" to the bulk of the medium;
6. Desorption of the cellulase complex from the hydrolysed substrate.

ENGINEERING OF CELLULASES

Cellulases are produced, amongst other microorganisms, by different filamentous fungi, being the greatest producers, belonging to the species *Trichoderma, Penicillium and Aspergillus.* Although, generally, the levels of this enzymatic complex, being secreted from the fungus, fulfill in the nature to the necessity of the lignocellulose decomposition and the availability of sugars for their metabolism, the industrial use of the cellulases requires the attainment of enzymatic preparations with high activity and stability levels, being necessary to modify strains of naturally-occurring filamentous fungi in hypersecretors, using techniques of classic Genetic or Molecular Biology. Studies in this direction have been developed by different national and international laboratories of universities and enterprises, deserving distinction the hyperproducing strains of *Trichoderma reesei*.

Several approaches have been used to improve the performance of cellulases and to decrease the amount of the necessary enzymes for an efficient hydrolysis of lignocellulosic materials. The first goal for cellulases engineering has been the cellobiohydrolases, since they tend to constitute 60-80% of the natural cellulolytic complex (LYND *et al.*, 2002). Teter *et al.* (2004) demonstrated that the utilization of combined techniques of Genetic Engineering (*site-directed mutagenesis*, *site-saturation mutagenesis*, *error-prone PCR and DNA shuffling*) generated highly productive strains of cellobiohydrolase (*Trichoderma reesei* Cel7a), which surpassed the wild strain in the hydrolysis of pretreated agricultural residue of the corn processing.

Another approach that has been utilized is the insertion of heterologous genes which codify for the production of cellulases in already existing systems, so that the overall performance of the recombinant strain is improved. Bower (2005) introduced various bacterial genes which codify for the endoglucanase in *Trichoderma reesei*. One of them, the GH5A of *Acidothermus cellulolyticus*, was fusionated with the one of the cellobiohydrolase (CBH1) of *T. reesei*. The fusion product was expressed in *T. reesei* and demonstrated being more effective in the cellulose saccharification of the corn processing residue than that originating from the parental strain (time reduction of the cellulose hydrolysis from 10 to 6 hours).

These findings show that further developments should occur in the next future in order to make viable the production of cellulases, particularly in the same industrial plant (*dedicated cellulase production*) where the producing processes are in operation, simply because great quantities of enzymes will be required for the efficient hydrolysis of the abundant lignocellulosic material, be it for the production of ethanol or other chemicals. Process integration of the

production of cellulases with other producing processes in the same industrial installation is also within the context of Biorrefinery.

HEXOSE AND PENTOSE FERMENTATION

The ethanol production technologies of sugary (sugar cane juice) and starchy (corn) feedstocks are commercially established and carried out by the yeast *Saccharomyces cerevisiae*. However, as described before, the hydrolysates of the polyssaccharides of the lignocellulosic materials possess a mixture of hexoses (mainly glucose) and pentoses (mainly xylose), being the naturally-occuring strains of *Saccharomyces cerevisiae* unable to metabolizing xylose.

The fermentation of glucose occurs primarily when the glucose concentration is high or when oxygen is not available. The cells attain a maximum specific growth rate of about 0.45 hr^{-1} with a low biomass yield of 0.15 g dry mass per gram glucose consumed and a high respiratory quotient (the ratio of CO_2 production rate to O_2 consumption rate), resulting in a low energy yield of only about 2 ATP molecules per mole of glucose metabolized. The stoichiometry of this reaction is:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy}$$

The term “glucose fermentation” refers to the whole of the sugar’s breakdown from the glucose molecule itself to ethanol and carbon dioxide. In other words, glycolysis (glucose to pyruvate or the so called Embden-Meyerhof-Parnas pathway) plus the conversion of pyruvate to ethanol. Glucose fermentation can be divided into three phases. The first phase is the conversion of glucose to fructose 1,6-bisphosphate. This is referred to as the *activation phase* since it includes two reactions which use ATP molecules to add phosphate groups to the sugar, thus activating or destabilising the sugar by adding energy. The second phase, the conversion of fructose 1,6-bisphosphate to pyruvate, is the *ATP generating phase* as it includes two reactions that create ATP from ADP. As the generation of ATP is the whole point of the pathway this is clearly a key phase. The third phase, conversion of pyruvate to ethanol plus CO_2, is perhaps the most frequently misunderstood phase. It produces *no* extra ATP and the end products (ethanol and CO_2) are of no value to the cell. In fact they are both toxic and must be disposed of by excretion or by further chemical changes. The only reason for the third phase is the regeneration of NAD; it is usually referred to as the *NAD regeneration phase*. NAD is converted to NADH earlier in the pathway, in the reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase and is in short supply in the cell. Every glucose molecule passing through the fermentation pathway would result in the conversion of two NAD molecules to NADH and the supply of NAD would rapidly run out. If that happened the whole of the pathway would stop as that NAD requiring reaction could no longer take place. In converting pyruvate to ethanol the cell makes use of a reaction that converts NADH to NAD thus reversing the previous change and regenerating the NAD, and enabling the pathway to continue. Fermentation of one glucose molecule results in the generation of two ATP molecules from ADP. To start with, two ATP molecules are used up in the activation phase and these will need to be replaced before there is any overall energy profit. Two reactions (catalysed by phosphoglycerate kinase and pyruvate kinase) generate ATP from ADP, but each

of these reactions will take place twice for each glucose molecule passing through the pathway, the sugar having been split into two three carbon units by aldolase. Therefore, four ATP molecules are generated but two of these are simply replacing the ones lost in activation, and the total yield is two ATP molecules per glucose. This is not a very high yield, but fermentation has the great advantage of being anaerobic and therefore not dependent on the presence of an adequate oxygen supply.

Xylose-fermenting microrganisms use the pentose-phosphate shunt and the Embden-Meyerhof-Parnas pathway. Pyruvate is finally converted to different end-products, such as ethanol, organic acids, ketones and volatile products, depending on the microrganism and on the regulation of carbon flow through available metabolic routes, which can be controlled by the process variables.

Whereas in most bacteria the metabolism of D-xylose proceeds via direct isomerisation to D-xylulose catalyzed by xylose isomerase, in the great majority of yeasts and filamentous fungi the formation of xylulose occurs via a two-step reaction in which xylose is reduced to xylitol by NADPH-dependent xylose reductase, with subsequent oxidation of xylitol to xylulose by NAD^+-dependent xylitol dehydrogenase. Yeasts show different abilities in the utilization of xylose, this being closely related to their requirements for oxygen; some can consume xylose only aerobically, others are able to fermenting it *quasi*-anaerobically or under oxygen restrict conditions. Xylulose is, this way, incorporated into the pentoses-phosphate pathway, originating glyceraldehyde 3-P and fructose 6-P. Both are converted in piruvate through the glycolytic via, which gives origin to ethanol through two sequential reactions (decarboxilation and reduction). Figure 14 illustrates the two initial reactions of xylose catabolism.

Figure 14: Xylose Catabolism in Yeasts and Filamentous Fungi
Source: Malburg *et al.* (1992).

Even though certain naturally-occurring yeasts, as for example: *Pichia stipitis*, *Pichia segobiensis*, *Candida tenius*, *Candida shehatae* e *Pacchysolen tannophilus* (TOIVOLA *et al.*, 1984) are capable to ferment xylose to ethanol, the production rates are more reduced, compared to those of the alcoholic fermentation of glucose.

Aiming at the integration of these two processes (xylose and glucose fermentations), researches have basically been developed with two approaches. In the first, it is searched for the construction of a recombinant with additional hability to ferment xylose (JEFFRIES & JIM, 2004), inserting genes which codify for xylose transport and metabolim and in the second, it is aimed at increasing the ethanol yield through the Genetic Engineering in microrganisms which already possess the hability to ferment hexoses and pentoses (DIEN *et al.*, 2003).

As the majority of the sugars in the lignocellulosic hydrolysates are constituted of glucose and xylose (with smaller quantities of arabinose, galactose and mannose), the initial efforts for the construction of ethanologenic strains have focalized the co-fermentation of glucose and xylose. In this approach, genes which codify for the xylose catabolism have been inserted into wild strains of the yeast *Saccharomyces cerevisiae* and of the bacterium *Zymomonas mobilis* (DIEN *et al.*, 2003 e JEFFRIES & JIN, 2004). Recombinant strains of *Saccharomyces cerevisiae* with the ability of co-ferment glucose and xylose have been constructed through the addition of genes of *Pichia stipitis* (XYL1 e XYL2), which codifies for NADPH-dependent xylose reductase and NAD^+-dependent xylitol dehydrogenase, as well as through the improvement of the xylulokinase expression (JEFFRIES & JIN, 2004). In this way, xylose is converted to xylulose-5-phosphate, which is a central metabolite of the pentoses-phosphate pathway.

Even though these mutants have been constructed with success and demonstrated satisfactory performance on a laboratorial scale, the anaerobic co-fermentation of glucose and xylose has not yet reached the requirements for the industrial production. This is because the metabolism of xylose with these recombinants presents an imbalance of the cell redox potential in relation to the co-factors, in particular concerning the ratio $NAD^+/NADH.H^+$, which leads the cell to require oxygen, even in low tensions. With the aim at circumventing this problem, other strategies have been utilized, with the insertion of a $NADP^+$ dependent glyceraldehyde-3-phosphate dehydrogenase which aids for the regeneration of the NADPH (VERHO *et al.*, 2003) or through the construction of a mutant which express a xylose reductase with greater affinity for the NADH and, therefore, decreases its consumption for NADPH (JEPPSON *et al.*, 2005).

Additionally, in contrast to the promptly fermentable streams of sucrose and of the starchy hydrolysates, the lignocellulosic hydrolysates tend to have fermentation inhibitors, arisen from the pretreatment (acetic acid, furfurals and aromatics), which need to be removed when their concentration is very high or will require the development of robustness strains which can be resistant to these inhibitors.

Developments in this area have advanced speedily and in the next future recombinant yeasts, which ferment efficiently glucose and xylose from the hydrolylates of lignocellulosic biomass with production rates compatible to the industrial requirements, will be available.

STRATEGIES FOR THE ETHANOL PRODUCTION FROM LIGNOCELLULOSIC MATERIALS

The transformation of lignocelulosic materials for the production of ethanol has been studied under different strategies of processing. Due to the presence of different sugars, very often the multiprocessing is made necessary, in other words, the use of enzymes simultaneously to the action of microorganisms. Or even the use of different microorganisms in successive stages, or of recombinant microrganisms as for utilizing the utmost of the available sugars (substrates). In this sense, four strategies are conceived, each one in a different development stage, which will be described as follows.

SEPARATED HYDROLYSIS AND FERMENTATION (SHF): It is the most ancient conception, in which the cellulose hydrolysis, after the pretreatment of the feedstock for hemicellulose solubilization and hydrolysis, occurs in a separated fermentation stage. The fluxogram shown in Figure 15 depicts a process which utilizes diluted acid for hemicellulose hydrolysis. Next, the cellulose is hydrolyzed enzymatically, before the alcoholic fermentation stage. This strategy has been left behind, due to the low efficiency of the enzymatic hydrolysis of the cellulose, when it occurs separately from the glucose fermentation.

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF): As the name implies the cellulose enzymatic hydrolysis and the fermentation occur in the same stage. The hemicellulose fraction is hydrolized and fermented in a separate stage, as well as the enzyme production (Figure 16). Contrary to what occurs with the hemicellulose, from which sugars can be obtained through its hydrolysis, when it is aimed at hydrolyzing cellulose enzymatically, this should be associated to a transformation process. This comes from the fact that (even they present high catalytic activities) the enzymes of the cellulolytic complex are inhibited by their own hydrolysis final products, particularly glucose. Thus, the alternative to sort out the inhibition problems consists in moving the equilibrium of the hydrolysis reaction, through the "glucose removal" from the reactive medium. To achieve this goal, the adopted strategy is to couple the enzymatic reaction to a fermentative process, which should occur simultaneously, while glucose is being formed. This process is called in the literature by *"Simultaneous Saccharification and Fermentation"* (SSF).

On one hand, this process offers the advantage of minimizing the inhibition problems, on the other, the optimum operational conditions for an efficient enzymatic hydrolysis are not necessarily the same of those from the fermentation. In relation to this aspect, efforts have been made in the sense of producing enzymes which act in temperatures e pH values close to the optimum of the fermentation process.

SIMULTANEOUS SACCHARIFICATION AND CO-FERMENTATION (SSCF): This process involves three stages, of which the hydrolysis of the hemicellulose and the production of celulases take place separately, as illustrated in Figure 17. In accordance to this conception, the liquid stream, rich in pentoses, obtained after the pretreatment remains in the bioreactor to which cellulases are added, followed by inoculation with a recombinant strain (capable of fermenting pentoses and hexoses). The main advantage of this strategy resides in the fact that only one reactor is used for ethanol production.

CONSOLIDATED BIOPROCESS (CBP): It is the most advanced process conception, in which all, or at least three of the stages, can be carried out in the same equipment. In the CBP, ethanol and all required enzymes are produced in the same bioreactor. With the modern tools of the Molecular Biology there exist possibilities to get expressed several activities in only one microorganism, be it associated to the producing capacity of the enzymes of the xylanase and cellulase complexes, as well as to the efficient fermentation capacity of pentoses or hexoses. Figure 18 displays this conception of the process, which seems to be the final logical point of the evolution of the lignocellulosic biomass conversion

technologies, being a middle/long-term perspective, where Molecular Biology plays a fundamental role.

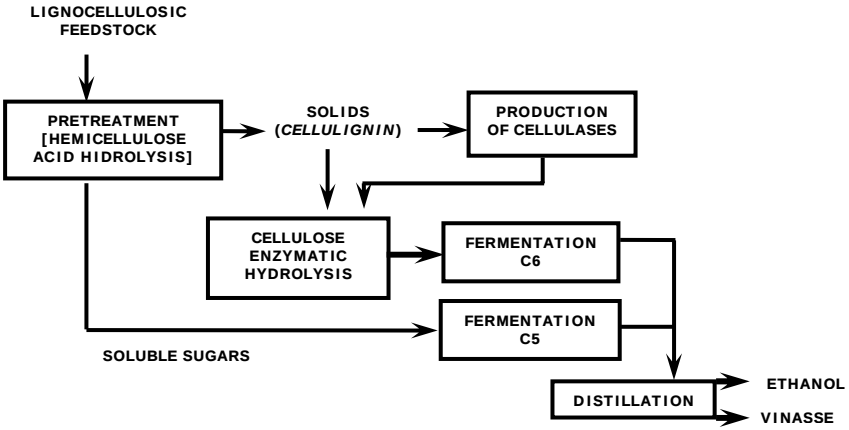

Figure 15: Diagram of the Separated Hydrolysis and Fermentation (SSF) Process. Source: Wingren *et al.* (2003).

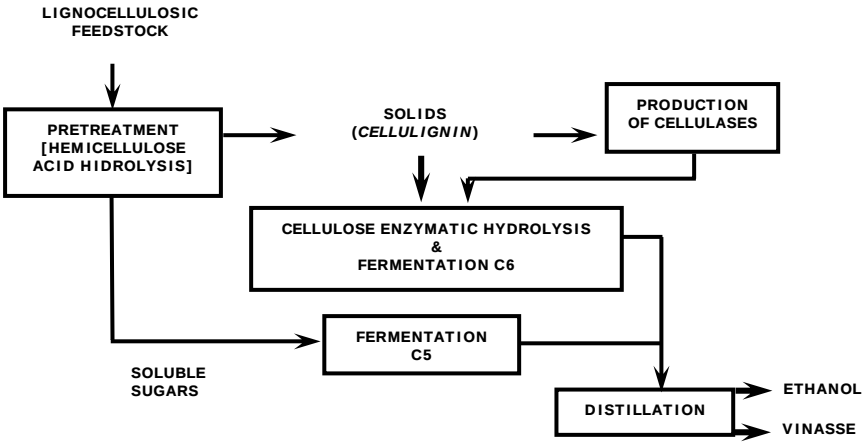

Figure 16: Diagram of the *Simultaneous Saccharification and Fermentation* (SSF) Process. Source: Wingren *et al.* (2003).

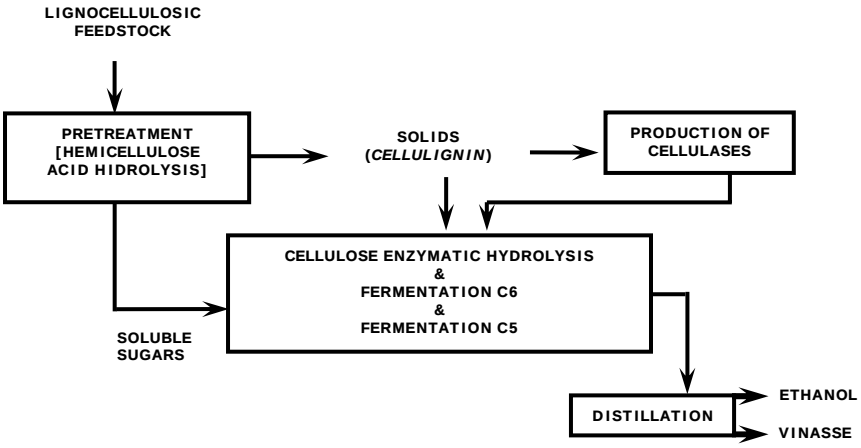

Figure 17: Diagram of the *Simultaneous Saccharification and Co-Fermentation* (SSCF) Process. Source: Wingren *et al.* (2003).

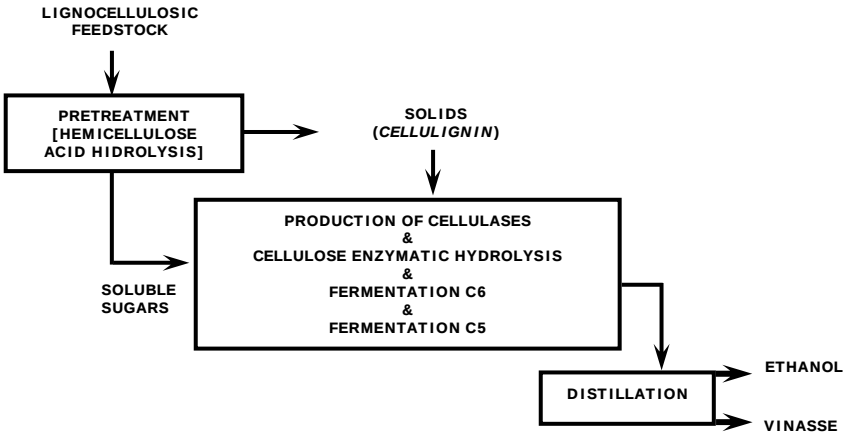

Figure 18: Diagram of the Consolidated BioProcess (CBP). Source: Wingren *et al.* (2003).

TRENDS AND CHALLENGES

The lignocellulosic materials, especially the residues of the agroindustry, have been object of intensive researches all over the world because they are renewable feedstocks of carbon and energy available in great quantities. The integral and rational utilization of these abundant feedstocks can revolutionize a series of industrial segments, such as the liquid fuels, the food/fodder and the chemical supplies, bringing immeasurable benefits for countries with great territorial extensions and with high productivity of biomass, amongst them, Brazil occupies a distinguished position. The sugar cane bagasse is the main Brazilian agro-industrial residue, being produced in approximately 250 kg per ton of sugar cane. In spite of the great potential of this residual biomass of lignocellulosic composition (50-70% carbohydrates) for the production of fuels and chemicals, the majority of it is burnt in sugar mills and alcohol distilleries for energy generation, and a smaller fraction is used for animal feeding, yet there still have surpluses (ZANIN *et al.,* 2000).

The effective utilization of the lignocellulosic materials in biological/fermentative processes faces us two principal challenges: the crystalline structure of the cellulose, highly resistant to the hydrolysis and the lignin-cellulose association, which forms a physical barrier which hinders the enzymatic access to the cellulose fibers. Additionally, the cellulose acid hydrolysis presents the inconvenient of requiring the use of high temperatures and pressures, leading to the destruction of part of the carbohydrates (sugars) and the generation of toxic substances, derived from lignin partial degradation (JACOBSEN & WYMAN, 2000). On the other hand, the enzymatic saccharification requires the use of physical (grinding, heating, and irradiation) or chemical (sulphuric acid, phosporic acid, alkalis) pretreatments, to reach viable yields.

To make possible that the ethanol production technology from lignocellulosic biomass can be implemented industrially, the following aspects should be focused:

(1) Development of pretreatment technologies which should be efficient and do not generate toxic substances that can hinder the alcoholic fermentation, neither should require onerous high pressure equipments;

(2) Combination of cellulose enzymatic conversion with alcoholic fermentation to maintain low levels of sugars, resulting in improvements of the enzymatic conversion rates due to the minimization of enzymes inhibition by their final hydrolysis products (cellobiose and glucose);

(3) Construction of “optimum” microrganisms, through Molecular Biology, for an efficient fermentation of pentoses and hexoses;

(4) Development of cellulase production processes by submerged and solid state fermentations (with natural-occurring or recombinant microorganisms), as well as to develop a deep knowledge about their structures and properties in order to formulate an enzymatic preparation (*product engineering*) for an efficient hydrolysis of cellulose;

(5) The industrial production of cellulases should be *in plant* as for reducing the inherent costs with enzymes in the process;

(6) Incorporation of reduced temperatures for ethanol separation to allow the enzyme recycling without thermal denaturation.

(7) Realization of a detailed study of process integration (mass and energy), including all the streams, be they of the process or utilities, in order to favour the *input/output* ratio of energy;

(8) Realization of a detailed technical-economic evaluation of the process viability for the utilization of agricultural and agroindustrial residues, including the logistic issues.

CONCLUDING REMARKS

The dependence of petroleum remains as the most important factor which affects the worldwide distribution of wealth, global conflicts and the quality of the environment. The population's growth and the associated demand for fuel and goods have intensified Research and Development for the utilization of renewable feedstocks in substitution to the fossil sources. The advances in this area point out that the utilization of renewable raw materials, including their residues, will revert this dependence.

For countries like Brazil, with a strong agriculture tradition, the fermentation industry of lignocellulosic feedstocks is of great importance to the creation of technologies for the production of a range of useful compounds, within the context of Biorefinery. This concept offers innovative possibilities, since it can bring solutions to supplant technologies which pollute the biosphere or contribute to the depletion of finite sources. Nevertheless, the industry, the scientific community and the government need to work together in order to allow Brazil to reach its industrial/economical/environmental sustainability and to follow its natural vocation for the BIOMASSES. In developed countries, integrated researches and the development of chemical and biological processes from lignocellulosic residues have advanced speedily and commercial plants for the utilization of such materials are turning into reality.

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The Laboratories of Bioprocess Development (LADEBIO) of the School of Chemistry of the Federal University of Rio de Janeiro

The research themes developed in LADEBIO advocate new concepts in the economical activities, particularly of those related to industrial production processes, integrating principles and strategies of total quality with the requisites of environmental preservation.

In this context, the theme in focus proposes to create a new paradigm for the sustainable industrial development, based on the use of lignocellulosic biomass in the form of residues (*Residual Biomass*), minimizing the environmental impact resulted from their generation, and at the same time aggregating value to these residues that are abundantly produced in the agricultural, agro-industrial and pulp & paper sectors.

The researches carried out in LADEBIO, along 30 years of experience, aim at the development of processes with technological potential carried out either by naturally-occurring or recombinant microrganisms or enzymes. Studies involving the screening and strain improvement, medium optimization, modes of bioprocess operation, kinetics, cell/enzyme immobilization, bioproduct characterization and application, as well as evaluation of bioreactor performance are approaches commonly adopted in LADEBIO's research projects. They are subjects of study: the development of processes aiming for the production of biofuels, enzymes, polyalcohols, antibiotics, biosurfactants, fragrances, as well as the development of biological processes for the treatment of industrial residues and effluents.

Due to their technological nature, in all works a compromise is established as for developing bioprocesses that could turn into an industrial reality. Also, studies involving technological management are developed in order to have a boarder view, and identify trends and challenges of different Biotechnology-comprising segments, as for example: prospective studies related to bio-based technology mapping, transgenic, biodiversity, environment and patent. Because of the multidisciplinary character of the Biotechnology area, a great number of works is developed in partnership with other research groups of the University itself and with external teaching and research Institutions, as well as with enterprises.

With distinction, the subject "**Biotechnology of Lignocellulosic Materials**" has been centralizing efforts to fulfill the social and economic demand in front of the problematic of exploration and use of fossil fuels, associated to the search of solutions for utilizing the abundant and cheap agricultural, agro-industrial and forestall residues. The intention is to displace them from the position of residues to the position of valuable feedstocks.


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