[1]Bioethanol Production via Enzymatic Hydrolysis of Cellulosic Biomass

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1 - INTRODUCTION

Energy availability, supply and use play a central role in the way societies organize themselves, from individual welfare to social and industrial development. By extension, energy accessibility and cost is a determining factor for the economical, political and social interrelations among nations. Considering energy sources, human society has dramatically increased the use of fossil fuels in the past 50 years in a way that the most successful economies are large consumers of oil. However, geopolitical factors related to security of oil supply, high oil prices and serious environmental concerns, prompted by global warming - the use of petrol for transportation accounts for one-third of greenhouse gas emissions (Wyman, 1996) - have led to a push towards decreased consumption. Indeed, the world's strongest economies are deeply committed to the development of technologies aiming at the use of renewable sources of energy. Within this agenda, the substitution of liquid fuel gasoline by renewable ethanol is of foremost importance.

Brazil has been a front-runner in the use of renewable fuels. The substitution of gasoline by ethanol started in 1975, when the Brazilian Government launched the “Proálcool Program" (Programa Nacional do Álcool). At the time of the first oil crisis, in the 1970s, the country imported 85% of its oil needs and the potential for ethanol production from sugarcane as a transportation fuel was in good agreement with the Government policy regarding energy supply independence. The Proálcool Program included incentives for distilleries and automobile companies that made ethanol-only cars. Although in the mid-1970s environmental concern was not a major driving force for substituting the use of gasoline, it is worth pointing out the global environmental benefits that have resulted from this policy since then.

Presently, the ethanol industry in Brazil runs without government incentives and the biofuel is distributed by the Brazilian oil company Petrobras. The Brazilian fleet of 20 million cars (the total vehicle fleet including cars, light commercials, trucks and buses is around 24 million) runs on either a gasoline blend containing 22-24% ethanol or on 100% ethanol. Natural gas has also been marginally used. Ethanol consumption is forecast to increase as the number of “flex-fuel" cars, with engines able to run on both gasoline blend or ethanol, is forecast to increase from the present 4 million to 15 million in 2013 (Associação Nacional dos Fabricantes de Veículos Automotores - www.anfavea.com.br).

2 - BRAZILIAN POTENTIAL FOR BIOMASS ETHANOL

Brazil has 851 million hectares from which around 5 million are presently used for sugar cane plantations. The State of São Paulo is the biggest sugar cane producer in Brazil with 68% (3.35 million hectares) of the total sugar cane plantations in Brazil. The country has 365 sugar/ethanol producing units from which 240 produce both sugar and ethanol, 109 produce only ethanol and 15 produce only sugar. It is forecast that 41 new distilleries will be built before 2010 (Carvalho, 2006).

In the units that can produce both sugar and ethanol, the pressed sugar-cane juice can go either to huge fermentation vats to make alcohol from the sugarcane sucrose or be spun in centrifuges to produce sugar (sucrose) and molasses, depending on which product is priced more favourably on any given day. About 70,000 farmers produced 385 million tons of sugar cane in 2006, and refineries made 4 billion gallons of alcohol fuel; enough to replace 460 million barrels of oil. Brazilian alcohol is the cheapest and more sustainable in the world, with a

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production cost of U$ 0.16-0.20 / L. In each sugar and/or alcohol mill the crushed stalk of the cane (bagasse) is burned for the production of steam (heat) and for power/electricity generation. However, the majority of the sugar and alcohol production plants have not yet optimized the processes for electricity generation due to the low price of the electricity that is usually sold to neighbouring agro-industries. Seventy five million tonnes of dry bagasse are produced annually by the ethanol and sugar industry.

Presently, the increase and intensification (more ethanol per sugarcane planted area) of ethanol production are high on the sugarcane agribusiness agenda due to the increasing ethanol demand to supply both the local and the international market. Although, in the near future, the urgent increase in ethanol production will largely rely on the expansion of the sugarcane plantations and on the parallel increase in the number of distilleries, it is expected that the production of ethanol from sugarcane biomass (bagasse and straw) will eventually be of significance. It is reasonable to consider that the deployment and expansion of biomass ethanol technology will move forward, pushed by the increase in sugarcane biomass availability. Indeed, as the traditional practice of burning the sugarcane leaves prior to manual harvesting is being phased out (Brazilian Federal Law 2661 and State of São Paulo Law 11241) and mechanized harvest gradually takes its place, massive quantities of sugarcane leaves, strategically placed, will be available as ethanol feedstocks. Moreover, an increase in bagasse surplus is expected due to the optimization of the boilers efficiency and the electricity generation system. Besides, the deployment of this technology in Brazil is favoured because the biomass ethanol production process can be annexed to the sugar/ethanol units already in place, requiring lower investments, infrastructure, logistics and energy supply. This is a promising scenario as for each 10 million tonnes of dry biomass it is possible to produce 600 million gallons of ethanol, considering solely its cellulosic component.

Other agricultural crop residues such as corn stover, wheat and rice straw, residues generated from citrus processing, coconut biomass, grasses and residues from the pulp and paper industry (paper mill sludge), as well as municipal cellulosic solid wastes, will eventually be also used as raw materials to produce ethanol. Although each source of biomass represents a technological challenge, the diversity of raw materials will allow the decentralization of fuel production with geopolitical, economical and social benefits. Information on the Brazilian sugar-ethanol agro-industry can be found at http://www.ctcanavieira.com.br (Centro de Tecnologia Canavieira); http://www.embrapa.br (Empresa Brasileira de Pesquisa Agropecuária); http://www.cnpae.embrapa.br (Centro Nacional de Pesquisa de Agroenergia) and http://www.portalunica.com.br/ (União da Indústria de Cana-de-Açúcar), among others.

3 – BIOETHANOL PRODUCTION VIA ENZYMATIC HYDROLYSIS OF CELLULOSIC BIOMASS

3.1 Biomass structure and enzymatic degradation

Plant cell walls are the source of lignocellulosic materials, also known as biomass, whose structure is chiefly represented by the physico-chemical interaction of cellulose, a linear glucose polymer, with hemicellulose, a highly branched heteropolymer, and lignin, a very high molecular weight and cross-linked aromatic macromolecule (Himmel *et al*., 2007; Howard *et al*., 2003; Joseleau *et al*., 1992, Meshitsuka and Isogai, 1996; Sakakibara, 1991). Cellulose, the most abundant polysaccharide on earth, is a highly ordered polymer of cellobiose (D-glucopyranosyl-β-1,4-D-glucopyranose), representing over 50% of the wood mass. Approximately 4×10^{10} tons of cellulose is produced annually (Coughlan, 1985). Native cellulose from wood has about 10 000 glycosyl units in the cellulose chain that form fibrils, long bundles of molecules, which are stabilized by numerous strong intermolecular hydrogen bonds between hydroxyl groups of adjacent molecules (Sjöström, 1993). Cellulosic materials present crystalline domains separated by less ordered, amorphous, regions that are potential points for chemical and biochemical attacks. Cellulose is degraded by enzymes know as cellulases that are able to hydrolyse the cellulose polymer to its monomer, the sugar glucose, that is naturally fermented to ethanol by the yeast Saccharomyces cerevisiae. Therefore, this biocatalyst is central for the biomass ethanol technology.

Hemicelluloses (polyoses) are the linking material between cellulose and lignin. Wood hemicelluloses are short (degree of polymerisation within 100 to 200), highly branched heteropolymers of the predominant xylose, plus glucose, mannose, galactose and arabinose, as well as different sorts of uronic acids. Depending on the

predominant sugar type, the hemicelluloses are referred to as mannans, xylans or galactans. The C_5 and C_6 sugars, linked through 1,3, 1,6 and 1,4 glycosidic bonds and often acetylated, form a loose, very hydrophilic structure that acts as a glue between cellulose and lignin.

By contrast, lignin is a three-dimensional polyphenolic network built up of dimethoxylated (syringyl), monomethoxylated (guaiacyl) and non-methoxylated (p-hydroxyphenil) phenylpropanoid units, derived from the corresponding p-hydroxycinnamyl alcohols, which give rise to a variety of sub-units including different ether and C-C bonds. Lignin is hydrophobic and highly resistant towards chemical and biological degradation. It is located in the middle lamella, acting as cement between the plant cells, and in the layers of the cell wall, forming, together with hemicellulose, an amorphous matrix in which the cellulose fibrils are embedded and protected against biodegradation. Lignin content and composition vary among different plant groups. Moreover, the lignin composition varies between the different wood tissues and cell wall layers.

Other non-structural components of plant tissues, including compounds that are extractable with organic solvents such as phenols, tannins, fats and sterols, water-soluble compounds such as sugars and starch, as well as proteins and ashes, usually represent less than 5% of the wood dry weight (Martínez *et al*., 2005). The association between polysaccharide (cellulose and hemicellulose) and non-polysaccharide (lignin) components in the structure of plant cell walls is, in great part, responsible for its mechanical and biological resistance (Joseleau *et al*., 1992, Pérez *et al*., 2002).

In nature, a battery of enzymes (hydrolytic and oxidative) produced by a variety of fungi and bacteria, work in synergy to perform lignocellulose degradation (Pérez *et al*., 2002). Fungi are able to degrade cellulose and hemicellulose and lignin in decaying plants by a complex set of excreted hydrolytic and oxidative enzymes (Gosh and Gosh, 1992) while the filamentous bacteria of the genus *Streptomyces,* among others, are able to degrade lignocellulose found in soil and composts also via the activity of excreted cellulases, hemicellulases and peroxidases. These bacterial enzymes, contrary to the biocatalysts produced by fungi, are more stable towards temperature and are able to perform in an alkaline pH (Macedo *et al*, 1999; Zerbini *et al*, 1999; Gottschalk *et al*, 1999; Bon *et al*, 1999; Bon *et al*, 2002; Bon *et al*, 2003). An extensive review on the biodegradation of lignocellulosic materials by fungi and bacteria can be found elsewhere (Odier *et al*. 1992; Gosh and Gosh, 1992).

3.2 Biomass ethanol

Ethanol is currently produced from the sucrose-derived sugarcane in Brazil and from corn starch in the United States to a total of 10.6 billion gallons (40 billion L). In both cases it is a proven technology and in expansion. Considering however that the use of land for energy crops conflicts with its use for food crops, and the enormous ethanol amounts - 26.5 billion gallons (100 billion L) by 2015 (Licht, 2006) - that will be necessary for the blended fuels used in the world vehicle transportation, it is reasonable to expect that lignocelluloses will be used as ethanol feedstock. Needless to mention, biomass is abundant, renewable and worldwide available at a low cost. It has been historically explored by humans as a favoured source of energy, whereby the polyaromatic lignin structure is of foremost importance as solid fuel.

Although the biomass constituent lignin has represented a major hurdle to the design of relevant industrial processes, such as the pulp and paper industry (Odier and Artaud,1992), the biomass polysaccharide part has been considered a safe source of energy for the production of fuel ethanol (Galbe and Zacchi, 2002; Bon *et al*, 2002; Bon *et al*, 2004).

Two main approaches have been developed in parallel for conversion of lignocellulose to ethanol - “acid based” and “enzyme based” (reviewed by Licht, 2006; Galbe and Zacchi, 2002; Hahn-Hägerdal *et al*., 2006). Biomass hydrolysis, i.e. the depolymerization of the biomass polysaccharides to fermentable sugars, must be performed via environmentally friendly and economically feasible technologies (Lynd *et al*., 2005). The enzyme based application is advantageous over chemical treatments due to its higher conversion efficiency, the absence of substrate loss due to chemical modifications and the use of more moderate and non-corrosive physical-chemical operating conditions, such as lower reaction temperatures, “neutral” pH and the use of biodegradable and non-toxic reagents. Nevertheless, biomass degradation is a highly complex multi-enzymatic process.

The enzyme-based route involves biomass pre-treatment because the lignocellulosic materials are structured for strength and resistance to biological, physical and chemical attack. Pre-treatments such as steam explosion, hydrothermolysis or using catalytic amounts of acid (Dekker and Wallis, 1983; Dekker, 1991; Söderström *et al*., 2003), render the raw cellulose digestible by cellulases as it alters the organization and the chemical interactions amongst the cellulose, the hemicellulose and the macromolecule lignin. Pending on the process conditions there is formation of significant sugars and lignin degradation products that are inhibitory to ethanol fermentation and therefore must be lessened.

Steam explosion or mild acid treatment, performed under adequate temperature and time of incubation, render soluble the biomass hemicellulose part with the formation of oligomers and C_5 sugars that are easily extracted from the biomass. The C_5 sugar stream can be individually fermented to ethanol by microorganisms such as *Pichia stipitis* and *Pachysolen* (Dekker, 1982), that are able to metabolise xylose, or be used as carbon source in a variety of other fermentative processes. The altered cellulose-lignin matrix is then submitted to an enzyme cocktail that is able to deconstruct the remaining sugar–lignin linkages and to hydrolyse the cellulose to glucose (C_6). Subsequently, the sugar syrup is separated from the lignin and fermented by *Saccharomyces cerevisiae* to ethanol. The development of strains with a broad substrate-utilization range, capable of using hexose and pentose sugars simultaneously, would greatly improve the efficiency of ethanol production from lignocellulose derived substrates (Hahn-Hägerdal *et al*., 1994, 2001; Zaldivar *et al*., 2001).

Lignin can be used as fuel or as raw material in the chemical industry. Biomass hydrolysis (saccharification) can be done simultaneously to the ethanol fermentation (the simultaneous saccharification and fermentation (SSF) process) using conventional yeast and bacteria strains or genetically modified organisms. The SSF process has the advantage of avoiding enzyme inhibition by threshold sugar concentrations, allowing higher biomass conversion yields and, by extension, higher ethanol concentrations. Decisions concerning the route to be followed depend on the process yields and productivity parameters as well as on the process robustness and cost.

3.3 Cellulase production and costs

Industrial cellulases and xylanases are well-known commercial products, tailor-made according to the target commercial application. However, the importance and interest in enzymatic biomass hydrolysis has renewed and increased the focus on several aspects of cellulases i.e. the search for high cellulase-producing organisms; the production of hypercellulolytic mutants of organisms suitable for cellulase production; genetic engineering for the construction of high cellulase-producing organisms with high specific activity; and theoretical studies on the mechanism of action of a multi-enzyme system on a complex polymer. Stunning progress in the engineering of enzymes using advanced biotechnology techniques, like directed evolution and rational design studies, continue to open new markets, with enzymes being increasingly tailored for specific applications and higher activities. These improved enzyme preparations must present different characteristics, such as higher catalytic efficiency, increased stability at elevated temperatures and at a certain pH, and higher tolerance to end-product inhibition (Zhang *et al*., 2006). Protein engineering of lignocelluloses-degrading enzymes, including mutagenesis of potential active centre residues with subsequent kinetic analysis, has been used as a tool in the study of the catalytic mechanism and improvement of some properties of industrial enzymes (Schülein, 2000). The construction and analysis of site-directed variants for structural and mechanistic analysis have been successfully carried out in cellulase engineering. In addition, analysis of the determinants of substrate specificity has led to the engineering of enzymes with modified functions (Schülein, 2000).

Presently, due to the importance of biomass ethanol, there is a significant potential market for biomass-degrading enzymes. Nevertheless, there is a need to decrease enzyme production costs and to produce new biocatalysts showing higher temperature stability. Driven by commercial demands for enzymes that can operate under certain process conditions, a number of microorganisms have been selected, in particular those from mesophilic (with an optimum growth temperature in the range of 30 to 37ºC) and thermophilic (optimum growth temperature in the range of 40 to 50ºC) origin. Enzymes can be produced by solid-state and submerged fermentation, where the microorganisms are cultivated on the surface of a solid substrate or in a liquid medium respectively. Solid-state fermentation has some advantages over liquid-state cultivation (Considine and Coughlan, 1989; Tuohy *et al*.,

1989; Techapun *et al*., 2003): it is less equipment-orientated, and hence more applicable in less-developed or less-sophisticated situations; aeration requires lower pressure than that needed for liquid cultivation; and vigorous agitation is not required. Other advantages are lower cost, improved enzyme stability, generation of a protein-enriched by-product for use as animal feed and easier downstream processing. However, most of the enzyme production procedures are carried out via submerged cultivation, where it is easier to control the environmental factors required for cell growth and enzyme production. For both solid- and liquid-state cultivation, the control of parameters such as moisture content, depth of culture, O_2 and CO_2 transfer, temperature and pH under optimum limits is necessary to achieve a maximum yield of enzyme (Considine and Coughlan, 1989; Tuohy *et al*., 1989).

Most of the commercial enzyme preparations are blends of biomass-degrading enzymes whose composition can be coupled to the suitable choice of the microorganism and the production conditions (Linko *et al*., 1989). The best-known producers of lignocellulose-degrading enzymes are strains of *Trichoderma* and *Aspergillus* species. Other relevant microorganisms include strains of *Humicola*, *Talaromyces*, *Acrophialophora*, *Thermoascus*, *Bacillus* and *Penicillium* species.

The relationship between fungal growth conditions and cellulase production has been discussed. From theoretical assumptions it has been suggested that to be commercially successful it is necessary to produce 1100 filter paper units L^{-1} h^{-1}. This amount of enzyme requires a 70 g L^{-1} h^{-1} growth rate of the culture. Under most favourable conditions fungal cultures produce 35 g L^{-1} h^{-1} of cell mass. This gap could be closed if the chosen microorganism would show any one or both of the following properties: (i) a high-enhanced capacity for cellulase production; (ii) the ability to produce enzymes with a high specific activity. These desirable properties may be achieved by either new strain selection and/or strain improvement (Gosh and Gosh, 1992).

It is well recognized that the economic viability of biomass ethanol depends on the enzyme cost contribution. Among the recent investments aiming to lower the biocatalyst costs, it is worth mentioning the US project "Improved low cost cellulase for biomass conversion", developed in the period 2000–2003, which aimed to make biomass enzymatic hydrolysis economically viable, by reducing enzyme costs 10 fold. This project was developed through a contract between the National Renewable Energy Laboratory and the Department of Energy with the companies Genencor and Novozymes (US$ 15 million for each company). It is reported that the project succeeded in lowering the enzymes cost contribution to US$0.30–0.40 per gallon (US$ 0.08-0.10/L). It is however agreed that a further decrease is still necessary to US$ 0.10/gallon (or 0.026/L). In disagreement with these numbers, data from the industry indicate that the present industrial enzymes cost US$ 2.24/gallon (US$ 0.59/L). Either scenario poses a main challenge to the enzyme industry as enzyme costs would have to decrease about 4 fold (considering the more optimistic scenario) to around 20 fold (less favourable scenario). Another approach to decrease the enzyme cost is "in house" enzyme production, which has been chosen by the Canadian company IOGEN and the US Company Verenium Corporation. In situ cellulase production is gaining ground among the companies to reduce costs and by extension to deploy the biomass ethanol technology.

3.4 Cellulase Market

Most of the industrial enzymes (60%) are produced in Europe, whereas the remaining 40% come from the United States and Japan. Presently, the world enzyme market is estimated to be worth US$ 4 billion, whereof approximately 60% are attributed to industrial enzymes, with a rising tendency of 5.7% per year (Costa *et al*., 2007). On the other hand, the world demand for enzymes is expected to rise 6.5% per year to nearly US$ 5.1 billion in 2009 (market researcher Freedonia). In this context, hydrolases represent 75% of the industrial enzymes and carbohydrases are the second largest group of industrial enzymes. Cellulases contribute to 8% of the worldwide industrial enzyme demands, their major application being in animal feed. Historically, enzyme demand has been concentrated on the more developed economies due to the high value-added nature of enzymes, and the significant technical resources needed for their development, production and application. However, the field of industrial enzymes is now experiencing major research and development initiatives, resulting in both the development of a number of new products and the improvement and optimisation of the industrial process. Countries such as China, India, South Korea and Taiwan, which have recently emerged as industrialised manufacturing centres with strong national research and development programs, will play a much larger role in the world market. In Latin America, the enzyme demand is concentrated mainly in Brazil, Chile and Argentina

(Costa *et al*., 2007). Brazil's external enzyme market was around US$ 147.2 million in 2005 (US$ 126.6 million import and US$ 20.6 export) corresponding to 3.7% of the international market (Costa *et al*., 2007).

The cellulase market has been estimated in the United States to be as high as US$ 400 million per year (van Beilen and Li, 2002; Wolfson, 2005; Zhang *et al*., 2006). In the period 2004-2014 an increase of approximately 100% in the use of cellulase as a speciality enzyme is expected (Costa *et al*., 2007).

4 - BRAZILIAN RESEARCH ON BIOMASS ETHANOL

Parallel to the implementation of the Proálcool Program in the 1970s, several research institutions and companies carried out research on the use of biomass for the production of ethanol, primarily using sugarcane bagasse. In addition, eucalyptus, several types of grass including elephant grass, corncob, babacu coconut, manioc stalks, pinus and municipal cellulosic solid wastes have also been studied. Research was done using different pre-treatment processes and enzymatic hydrolysis. Acid hydrolysis using concentrated or diluted hydrochloric or sulphuric acid (HCl or H_2SO_4) was also studied. The first pilot-scale facility was built in 1981 at Fundação de Tecnologia Industrial (FTI), Lorena, to run using the Scholler-Madison process based on concentrated sulphuric acid to hydrolyze the biomass. It was built to produce 500 L ethanol per day using *Eucalyptus panicutata* as the source of biomass. This same process was used to build, also in the 1980s, an industrial-scale plant with the capacity to produce 30 000 L ethanol per day, by the company Coque e Álcool da Madeira S/A (COALBRA) in Uberlândia. In both cases the low sugar and ethanol yields, among several technical issues, hindered the operation of the plants. At the end, the COALBRA project was used to produce furfural (Carvalho Neto, 1987).

Also in the 1980s a consortium formed by the Companhia de Desenvolvimento Tecnológico, the steel company Aços Villares and the Fundação Universidade de Campinas, Campinas, developed the HIDROCON Project to study, on a pilot scale, the production of biomass ethanol. Here, low saccharification yields, around 60%, were also observed (Carvalho Neto, 1987) and the project was also discontinued.

The Brazilian company Dedini, in Piracicaba, began in 1987 the development of a biomass-to-ethanol production technology (called Dedini hidrolise rapida (DHR), portuguese for Dedine rapid hydrolysis), in partnership with Copersucar (presently the Centro de Tecnologia Canavieira) and the State of São Paulo Research Supporting Foundation (FAPESP), with funding from the World Bank. The DHR process uses the "organosolv" hydrolysis process to convert sugarcane bagasse into sugars to be fermented and distilled into ethanol via conventional ethanol plant processes. The single-stage process employs both a very dilute sulphuric acid for hydrolysis of cellulose and hemicellulose to sugars and an organic solvent for lignin extraction. DHR's main unique feature is the reduced hydrolysis reaction time (only a few minutes) in a continuous high-throughput process, with quick cooling of the hydrolysate. Patents for the DHR process have been issued (beginning in 1996) in Brazil, the United States, Canada, the European Union, and Russia, and applied for in Japan and other countries. Following initial laboratory-scale testing, Dedini developed a 100 liters-per-day pilot plant at the Copersucar Technology Center in Piracicaba, which has undergone 345 test runs over 2,100 hours with the DHR process. Since 1992, Dedini and its partners have also operated a "semi-industrial" demonstration plant with the DHR technology, located at the Sao Luiz Sugar and Ethanol Plant in Pirassununga, Sao Paulo State. The DHR demonstration plant is coupled with the conventional sugarcane-to-ethanol plant, sharing various utility and support systems and using the conventional plant's fermentation/distillation systems for the finished ethanol production steps. For feedstock, the DHR demonstration plant uses a sidestream of the same sugarcane bagasse supply normally used to fuel the adjacent sugarcane-to-ethanol plant's boilers. The demonstration plant has the capacity to process about 2 tonnes of bagasse per hour and produce about 5,000 L (1,300 gallons) of ethanol per day. One of Dedini's primary areas of specialization is equipment for sugar and ethanol production plants as well as complete turn-key plants and over 80% of the ethanol produced in Brazil reportedly uses Dedini's equipment.

In all the aforementioned, independent projects, that studied acid hydrolysis using chiefly H_2SO_4, they observed low sugar yields; the formation of inhibitors of the subsequent ethanol fermentation step; and corrosion of the equipments. Collectively they hindered the full deployment of the biomass ethanol acid-based technology. Besides, the acidic pH of the sugar hydrolysate calls for a neutralization step prior to its fermentation, that is usually done with hydrated lime (calcium hydroxide - $Ca(OH)_2$) that results on the formation of $CaSO_4$ (gypsum)

as a wet fluffy precipitate. Disadvantages of this necessary neutralization step include the reduction in the hydrolysate sugar content and, by extension, the ethanol yield; the need to include a separation step to remove the gypsum; and the accumulation of this solid by-product.

Considering the detrimental aspects of acid hydrolysis, other studies focused on the enzymatic saccharification. For that, sugar cane bagasse was pre-treated via steam explosion using a 1.6 L reactor (FTI, Lorena) (Ferrara *et al*, 1983; Ferrara and Kling, 1985, 1987; Ferrara *et al*., 1984; Ferrara *et al*., 1987; Kling *et al*., 1987). These studies paved the way for the development of the sugar cane bagasse steam explosion process used to date in Brazil for the production of cattle feed. Mechanical pre-treatment was also carried out using ball mill and/or roller mill (Fundação Universidade Estadual de Maringá (FUEM), Maringá; Instituto de Pesquisas Tecnológicas (IPT), São Paulo and the company BIOFERM/BIOBRAS, Montes Claros). Chemical pre-treatments were also performed on sugar cane bagasse using ethylenediamine and sodium hydroxide, in the presence or absence of ethanol (FUEM). Sugarcane bagasse and elephant grass were also hydrolysed via the organosolv process in a reactor able to process 100 kg biomass/h (Universidade Federal do Ceará (UFC), Fortaleza). Alkaline pre-treatment was evaluated using sodium hydroxide (NaOH) and ethanol/NaOH (IPT and BIOFERM/BIOBRAS) and photochemistry pre-treatment at the State University of Campinas.

The production of cellulases, at an industrial scale, was developed by BIOFERM/BIOBRAS. Enzymatic hydrolysis was studied at BIOFERM/BIOBRAS, UFC, IPT and the Instituto Nacional de Tecnologia, Rio de Janeiro. UEM carried out enzymatic hydrolysis using a fluidized bed reactor with soluble cellulases and immobilized beta glycosidase. The immobilization of beta glycosidase was also studied at the Chemistry Institute of the Federal University of Rio de Janeiro (Carvalho Neto, 1987).

For fermentation of the biomass-derived sugar syrups, FTI and IPT evaluated the simultaneous saccharification and fermentation (SSF) process and FTI evaluated the associated saccharification and fermentation (ASF) process (Carvalho Neto, 1988). Research work carried out at the Universidade de Caxias do Sul focused on isolation of thermophilic yeast strains able to ferment sugar syrups obtained from wheat straw and on isolation of cellulolytic bacteria strains from the guts of termites. The Universidade Estadual do Estado de São Paulo studied fermentation of sugar syrups derived from wood acid hydrolysis, using a co-culture of *Saccharomyces uvarum* or *Zymomonas mobilis* that ferment glucose and *Pachysolen tannophilus* able to ferment pentoses to ethanol. Pentose fermentation was also studied at FTI using *Pichia stipitis* and at the Universidade Federal do Paraná using syrups from cotton tree biomass (Carvalho Neto, 1987, 1988; Kling, 1999).

The majority of the above research work continued until the 1980s at the end of the oil crisis. Presently, a revival of research on biomass ethanol is taking place in Brazil and some representative projects have been carried out. One such initiative is the “BIOETHANOL Project: Bioethanol Production via the Enzymatic Hydrolysis of Sugarcane Biomass”. It aims at the development of technology for the conversion of sugarcane biomass (bagasse and trash) into fuel ethanol. This project, initiated in 2006, is supported by the Brazilian Ministry of Science and Technology – Research and Projects Financing (FINEP) and is carried out by a network of more than 20 institutions, including universities and research institutes. The technology aims at the use of bagasse surplus that is not utilized for the production of steam (heat) and power/electricity generation in the sugar and alcohol mills. This surplus will gradually increase upon the optimization of the cogeneration system and also through the plant thermal integration. This technology will use all already existing facilities in the sugar and alcohol plants including juice/sugars syrup treatment system, fermentation, distillation, cogeneration, wastewater and residues treatment and recycling, laboratories, instrumentation and automatic control systems, management, commercialization. The biomass C_6 sugar syrups will be blended with the molasses or with the sugar-cane juice prior to fermentation that will be carried out by *Saccharomyces cerevisiae*. As a result, lower biomass ethanol production cost is expected compared with plants dedicated to only producing biomass ethanol. For the enzymatic hydrolysis of the pre-treated biomass, the project is developing enzyme blends customized for the sugar-cane biomass that will be produced “in situ”. All the issues related to the production of ethanol from biomass will be addressed by several research groups, from Brazilian universities, research centres and industries, according to their areas of expertise. The project has also established international collaborations. Studies are focused on biomass characterization, pre-treatment, hydrolysis, fermentation and energy optimization. Enzyme development

studies are concomitant to pre-treatment studies. The development of this project will increase the international competitiveness of Brazilian ethanol.

The objective of this project is to develop a viable technology for the conversion of the integral biomass of sugarcane, including its lignocellulosic fraction (bagasse and straw) in fuel ethanol, with the intensification of the production, thus avoiding the expansion of the sugarcane fields. This approach will lead to increased production in the most competitive locations, reducing economic and environmental risks and increasing the availability of cost competitive ethanol in the market. It is expected that Brazilian sugar mills will be gradually converted into biorefineries, able to eventually process all the fractions of the sugarcane biomass.

5 - LITERATURE

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