Antonio Bonomi. "WORKSHOP ON THE HYDROLYSIS OF LIGNOCELLULOSIC MATERIAL", p.679-696. In Luis Augusto Barbosa Cortez (Coord.). **Sugarcane bioethanol — R&D for Productivity and Sustainability**, São Paulo: Editora Edgard Blücher, 2014.
http://dx.doi.org/10.5151/BlucherOA-Sugarcane-SUGARCANEBIOETHANOL_58

11

WORKSHOP ON THE HYDROLYSIS OF LIGNOCELLULOSIC MATERIAL

Antonio Bonomi

Today the world is discussing the possibility of producing second generation bioethanol. To make this new production alternative feasible for this renewable fuel, new technologies are under development, trying to use the lignocellulosic component of biomass. Such new technologies focus on two major lines: hydrolysis of the lignocellulosic material to produce fermentable sugars (chemical and biological route), and the gasification of this material followed by the synthesis of liquid fuels (thermal route).

In the new "energy cane" paradigm, the whole cane would be harvested (with improvements and mechanization of the harvesting process), in addition to optimize the energy balance of the mill, in order to increase the quantity of surplus biomass. Residual biomass hydrolysis and gasification technologies, once developed, will be able to convert fiber into ethanol or other fuels from the fermentation of the sugar produced (hydrolysis), or from the synthesis of compounds from the gas generated (gasification). With this new paradigm, it is possible to significantly increase ethanol production per hectare/year, stepping from the current 6,000 liters to about 12,000 liters (projected level).

The use of biomass to produce ethanol fuel by chemical and biological route involves, basically, two processes: hydrolysis of polysaccharides contained in the lignocellulosic materials in sugars and the fermentation of these into ethanol or other fuels.

This theme was discussed in detail during two events that took place within the project Guidelines for Public Policies for the Sugarcane Agriculture in the São Paulo State Public Policy Research Program (Diretrizes de Políticas Públicas para a Agroindústria Canavieira do Estado de São Paulo, Programa de Pesquisa em Políticas Públicas – Etanol – PPPP). The first event was the III Technology Workshop on Hydrolysis held at the Technological Research Institute of the State of São Paulo (Instituto de Pesquisas Tecnológicas do Estado de São Paulo – IPT) on December 14th, 2006, and the second one was the meeting with Prof. Guido Zacchi, from the Chemical Engineering Department of Lund University, Sweden, which took place on February 1st, 2007, during his visit to Unicamp.

III TECHNOLOGY WORKSHOP ON HYDROLYSIS

The workshop dealt with the subject of Hydrolysis, focusing four basic themes. These themes, including key questions for each of the development areas and that were used as starters for lectures and debates, are listed below.

1. Lignocellulosic material characterization:
   - Are there significant advantages between trash and bagasse, in terms of their processing to produce ethanol?
   - Are there important interferents in the sugarcane bagasse and trash for the hydrolysis process?

2. Lignocellulosic material pretreatment and hydrolysis:
   - How to define when pretreatment ends and hydrolysis begins?

- How to separate lignin from the pre-hydrolyzed material?
- Is the hydrolysis enzymatic route better than the chemical routes?
- Can enzymes be economically produced in house in the alcohol plant?

3. Fermentation of the hydrolyzed lignocellulosic material
   - Should cellulose hydrolyzed material fermentation be coupled with the hydrolysis stage, or to the saccharose juice fermentation in the alcohol plant?
   - Is it economically advantageous to convert hydrolyzed hemicellulose into ethanol, or should other options for its use be sought?
   - Is it technically feasible, and how long would it take, to introduce in sugar and alcohol mills engineered organisms capable of fermenting simultaneously with hemicellulose and cellulose hydrolyzed materials?

4. Plant energy optimization, including hydrolysis
   - What is the limit of surplus trash and/or bagasse percentage that can be attained in a standard sugar and alcohol mill, within the context of the new "energy cane" paradigm?

The aspects detailed by lecturers are shown below, with the major issues debated throughout the event, organized into the four themes defined for the workshop.

Lignocellulosic material characterization

The alcohol from lignocellulosic materials production process starts with planting and/or harvesting the material. One factor that is critical for success is the availability of this material, its cost, and the condition it arrives to the processing venue. Here are the first questions calling for answers:

- What is the quantity of sugarcane bagasse and trash actually available for conversion into alcohol? Is bagasse and trash the only viable raw materials? Why?
- What are the ideal logistics for transporting trash to the mill? Are this logistics associated to sugarcane or not?
- How will the likely energy scenario in the next few years (mostly the demand of electricity) affect this availability?
- What are the (opportunity) costs of these materials?

After these materials arrive at the processing venue, a second series of questions may be raised upon the next operation, which is their preparation, or pretreatment, intended to optimize hydrolysis (the process deemed most viable for producing monomeric sugars, and then alcohol via fermentation, though there are other thermochemical options):

- In terms of the desired conversion of these materials into alcohol, what is their recalcitrance, i.e. what is the level of enclosure or protection of the polymers that can provide fermentable sugars? How can this recalcitrance be measured? What is the economic reduction of the recalcitrance, i.e. how aggressive pretreatment should be as a function of the composition or condition of the raw material?
- What components are present in these materials that must be changed in their structure, or that should be economically removed?
- How to process (i.e. store, transport, mix to other components, increase attack area etc.) large quantities of porous and low-density materials? What changes in the original materials' state or composition would be desirable for optimized pretreatment?

After pretreatment, there is the hydrolysis process for producing fermentable material (monomeric sugars) from polymers present in the material, which can also be affected by raw material quality:

- What is the availability of polymers, or what is the minimum polymer digestibility and concentration the material has to reach in the hydrolysis process, in order

to minimize both successive processes and energy costs? Does the original material composition affect the pretreated material composition?
- What components originally present in the material determine hydrolysis efficiency (and therefore its cost)? Do inputs (catalysts) and more or less severe hydrolysis conditions depend on raw material composition?
- How can hydrolysis performance be measured and linked to material components? Is any component originally present in the material converted into a hydrolysis inhibitor?

After hydrolysis, the monomers it generates are converted into ethanol, in general biologically in a fermentation process, preferably associated to the current process, already optimized to convert soluble sugars from sugarcane. The following questions come up:
- Does the original material composition affect the presence of non-fermentable or fermentation inhibiting material (or yet undesirable material, such as e.g. solids in suspension)?
- Do non-fiber impurities reach fermentation? What is the cost to prevent them from getting there?
- Considering material composition and minimum pretreatment and hydrolysis process costs, is it possible to discuss the convenience, or lack of it, to couple current fermentation together with the fermentation of monomers obtained from the material?

There are some other questions across the processing phases that may also be related to material characteristics, such as:
- How will SMS conditions be affected by processing large quantities of low density fibers, the presence of small inhalable particles, self-ignition below a certain moisture level, among other factors?
- Considering that it will be necessary to buy new equipment and inputs, and upon the need to get return from such investments, will it be viable to operate them during harvest time only, as it is done today (max. 200-250 days)? How can lignocellulosic material be stored for long periods of time?
- What kinds of new residues will be generated, and what is the need for post-treatment before disposal? How do material characteristics affect both quantity and quality of these residues (mainly stillage)?
- How will energy demand be fulfilled for the process that produces significantly larger quantities of alcohol? How does material quality affect these requirements?
- Does water and other utilities consumption change with any characteristic of the raw material?
- Are there any other clients interested in bagasse as raw material, e.g. electric power plants (for co-generation or even generation)? Will there be sufficient material for all of them? Is there any feature in the material that may influence the decision on its destination?
- Will thermochemical processes, considerably less demanding on the raw material composition or state, be viable? When?

Some additional issues approached in the workshop, referring to the material characteristics theme and that will have to be assessed upon defining the lignocellulosic material hydrolysis technology, were:
- The need to implement standard analysis procedures for the main components of lignocellulosic materials, to aid in mass and energy balances and to allow estimating material degradability. There is no consensus yet on the methods to follow lignin through, nor to assess degradability or enzymatic digestibility.
- The need to carry out studies on fractioning raw materials, bagasse and trash, to check the viability of removing undesirable fractions, and to check whether the most degradable fractions can be used preferentially.
- The need to define pretreatment requirements for each fraction or raw material

option. Economic and technical feasibility studies for alternative pretreatments should be elaborated.
- Need to study other sugarcane options, i.e., "energy cane" from the standpoint of raw material availability for hydrolysis and its composition, and degradability.
- Consider the quantity of pre-existing sugar in raw materials and its potential impact on lignocellulosic material pretreatment.

Lignocellulosic material pretreatment and hydrolysis

As a result from the synergy that exists among the various steps in the lignocellulosic material hydrolysis process, upon attempting to define and describe the material pretreatment and hydrolysis, it was noticed that it is essential to take into account the fermentation system to be employed.

In the case of ethanol production from glucose obtained by acid/enzymatic hydrolysis of sugarcane bagasse, the **preparation step** is defined as: screening, grinding, washing and physicochemical processes intended for the selective removal of hemicelluloses (without converting them later into ethanol) and lignin (e.g. treating with steam, hot pressurized water, moist alkaline oxidation, hydrolysis with diluted acid) and washing of the solid fraction (pulp) with alkalis, acids or ethanol. In this context, the **availability step** is considered as: enzymatic/acid hydrolysis of cellulosic pulp (coming from the preparation step) intended for the production of glucose, for later conversion of this carbohydrate into ethanol. Proceeding to the decontamination of the saccharidic solution (hydrolyte) produced in the availability step, this operation is considered as **hydrolyte preparation** for the next step of conversion into ethanol. However, for the sake of simplification, the terminology described in literature and industrial practice is used, where the operational sequence is considered: raw material preparation – pretreatment – hydrolysis – fermentation for the systems considered.

The major challenge of an economically viable production of ethanol from lignocellulosic biomass resides in determining the best option to make glucose available from cellulose hydrolysis in terms of overall cost, glucose yield, and hydrolyte fermentability. Essentially, carbohydrates availability processes from sugarcane bagasse by cellulose (and hemicellulose) hydrolysis set out the most promising technological options, in view of their adaptability to existing alcohol producing plants, and their relative ease of implementation on an industrial scale. Such processes are intended for the production of pulps with high cellulose content and huge accessibility of the cellulosic matrix to hydrolytic chemical or enzymatic agents, envisaging the production of glucose from cellulose. Striking differences between the structural and physicochemical properties of bagasse cellulose and hemicelluloses (chemical composition, morphology, molecular orientation, chemical and mechanical strength) demand the execution of hydrolytic processes in two stages, being the first to convert (and remove) hemicelluloses from the bagasse and the second to convert cellulose into glucose.

In spite of all the positive features inherent to the use of sugarcane bagasse as raw material to produce ethanol, a major challenge to be overcome lies in reducing operational costs associated to the availability of carbohydrates for biotechnological conversion. The development of eco-efficient processes for bagasse pretreatment and cellulose hydrolysis emerges in this scenario with particular relevance.

The selection among different technological options for the availability of carbohydrates from sugarcane bagasse for ethanol production must consider parameters relative to the eco-efficiency of the hydrolytic process, such as saccharide yield, selectivity, hydrolyte fermentability, effluents outflow, reuse of materials and inputs, water and energy intake, value-adding residues and ancillary operations (e.g. washing), in addition to factors associated to operational costs (inputs, maintenance, labor), capital (building material and equipment setup), as well as aspects associated to the ease and flexibility of operating systems, and the implementation of efficient technologies and processes.

The pretreatment of lignocellulosic biomass is one of the most relevant operating steps in terms

of direct cost, in addition to its significant influence on the costs of the upstream and downstream steps in the process. Basically, pretreatment relates to raw material preparation operations (grinding, impregnation), as well as hydrolysis (acid or enzymatic) of cellulose (load and consumption of enzymes or acids, reaction rates), generation of enzymatic hydrolysis and alcoholic fermentation inhibiting products, saccharidic concentrations of the hydrolytes produced, purification of intermediate products, waste treatment, mechanical agitation and energy generation. In this context, perfect integration among the different operations should be sought. Performance of a pretreatment technique should be assessed on its influence on the costs associated to upstream and downstream steps, as well as operating, raw material and capital costs of pretreatment itself. Thus, the pretreatment alone should be very efficient in terms of efficiency, selectivity, functionality (ensuring accessibility of the cellulose to hydrolytic agents), operational simplicity, industrial safety and hygiene and environmental attributes, at the same time that it should require a low input of chemicals, energy and utilities. Generally, an efficient pretreatment of sugarcane bagasse for ethanol production should simultaneously offer cellulosic pulp with high fiber accessibility and reactivity to acid or enzymatic hydrolytic agents (digestibility), it should ensure adequate pentoses recovery and yet limit the generation of compounds that inhibit both the microorganisms used in fermentation and the enzymes. Additionally, features associated to the use of low-cost catalysts, inputs recycling, and high value-added by-products from lignin characterize eco-efficient pretreatment systems.

Though various pretreatment techniques are potentially applicable to sugarcane bagasse, it is particularly difficult to carry out comparative studies based on data from literature due to differences in research methodologies, to the physical characteristics of the material, as well as to the preparation methods for the raw material. Nevertheless, it is worth emphasizing the importance of improving the knowledge about the differences among the various types of pretreatment, as well as on the impact of each process on the other operations. Such initiative might aid in selecting equipment and operation sequences in an integrated system, in addition to reducing risks associated to industrial scale process implementation, as well as in identifying improvement opportunities along the integrated system, leading to optimized operating efficiency and minimizing the overall costs of ethanol production.

In practice, it may be considered that bagasse is subjected to a physical pretreatment at the time of its production, after the wet crushing of sugarcane. Due to its reduced granulometry, bagasse does not require previous grinding before the physicochemical treatment, which is an advantage in terms of raw material preparation cost. However, low density and low compaction of bagasse represent a problem in terms of the reactor feeding operation, on top of the difficulty in carrying out pretreatment with solid loads above 50%. On the other hand, the presence of impurities from sugarcane grinding (e.g., ashes, silica) applies a buffering capacity to bagasse, resulting in a higher demand of acid to attain a pH adequate to hydrolytic pretreatment processes.

Proper impregnation of the lignocellulosic biomass consists in a very important parameter for the chemical treatment efficiency of any lignocellulosic biomass. Sugarcane bagasse has a high liquid absorption capacity, as well as reduced "hardness". Furthermore, the high moisture content of bagasse coming from mills (45-50%) makes it easier to impregnate this biomass with acid and alkaline solutions. This aspect has fundamental importance regarding the acid hydrolytic pretreatment efficiency, which requires adequate concentration of hydronium ions (H_3O^+), formed from water with the dissociated acid. Insufficient water in the biomass would cause less hydronium ion formation, as well as reduced availability of conveying fluid from it to the inner biomass, and, therefore, loss of efficiency of the hydrolytic capacity of the process.

The challenge, therefore, lies in determining the "*optimum*" quantity of water in bagasse to ensure effective biomass impregnation, while a solid load is obtained in the reactor, especially in "*steam explosion*" processes. Solid loads in the

25%-40% range of dry bagasse weight with 50%-70% moisture may result in selective extractions of xylose (60% to 80% recovered) with reduced glucose loss (3% to 5%), producing pulps with high fiber reactivity (85% to 95%). Pretreatment processes with steam at 200 ºC and 210 ºC (only) evidence a significant increase in delignification and lignin fractioning.

Based on the above, it is recommended to wash the bagasse before loading the reactor, in order to promote densification/compaction of the biomass, as well as to facilitate its impregnation with the chemical agents used in the pretreatment. It is considered that, in the case of the "*Steam Explosion*" system, reactor load with 25% of solids using bagasse with 75% moisture leads to high recovery of hemicelluloses in the hydrolyte, while high fiber reactivity and high global glucose yield are obtained. Pretreatment systems with diluted acid and "*Hot Water*" may benefit from pre-washing bagasse in view of the reduction of buffering capability of biomass (caused by the removal of impurities) associated to higher material compaction. Bagasse washing may be done on the reactor feeding belt conveyor by spraying, so no complex operation is added between bagasse generation and feeding to the reactor.

Sugarcane bagasse has a high content of hemicelluloses (30%), predominantly made up of pentose polymers (xylan and xyloarabans). As a result of pentoses' high reactivity (especially xylose) at temperatures above 140 ºC, selectivity and saccharide yield from the hemicelluloses hydrolysis process may be compromised when bagasse undergoes hydrolytic pretreatments at temperatures above 180 ºC for processing periods from 30 to 60 minutes. The adoption of severe process conditions tends to increase the degradation of xylose into furfural, as well as to promote glucose degradation into hydroxymethylfurfural (HMF), compounds that potentially inhibit alcoholic fermentation. Lignin solubilization and fractioning associated to extremely severe process are potentially detrimental to later stages (enzymatic hydrolysis and fermentation), as a result of lignin buildup on the cellulosic pulp surface, as well as the generation of fermentation-inhibiting compounds, such as phenol derivates and organic acids.

Thus, it becomes difficult to obtain pentose recovery above 90% by means of conventional pretreatment in single-step processes. On the other hand, the two-step process delivers larger hydrolyte volumes with reduced saccharide concentration (10 to 15 g/l), potentially detrimental to later pentoses to ethanol conversion operations. It becomes necessary to do an economic viability assessment regarding the adoption of a two-step process, due to the energy and inputs consumption, as well as overall process productivity, on top of the generation of liquid streams with reduced saccharides concentration.

In summary, pretreatment processes should be carried out under moderate conditions, to simultaneously promote high cellulosic fibers reactivity and high hemicelluloses recovery, with minimum glucose loss in the hydrolyte, in addition to minimized generation of compounds that inhibit the later hydrolysis and fermentation steps. On the other hand, a treatment step (e.g., purification, lignin-carbohydrates separation) of the hemicellulosic hydrolyte produced should be minimized, for the sake of operational simplicity and process economy. For the SSCF (simultaneous fermentation of pentoses and hexoses), it becomes important to use both solid and liquid fractions of the material from pretreatment without any lignin-carbohydrate separation process on the liquid or pulp washing, in order to maximize saccharide recovery and the sugars concentration in the hydrolyte. Steam pretreatment processes, auto-catalyzing or in the presence of a catalyst, hot water and diluted sulfuric acid come out as the most promising technology options for the implementation of hydrolysis units next to sugar-alcohol plants.

Hydrolytic cellulose conversion has been considered one of the major technology bottlenecks of ethanol production from lignocellulosic biomass. Initially, enzymatic conversions seem to be promising alternatives in terms of total cost, from the possibility of attaining glucose efficiency close to theoretical levels, in addition to the possibility to depend on modern microbiology and genetic en-

gineering techniques, aiming at the optimization of the steps in the integrated process.

At first, the enzymatic routes present important advantages over chemical routes, in the context of producing ethanol from sugarcane bagasse. A major challenge is to render the enzymatic process viable. Enzymatic hydrolysis processes should be conceived as a function of the type of substrate produced, the pretreatment used, as well as the fermentation strategy to be used (SHF – separate hydrolysis and fermentation, SSF – simultaneous hydrolysis and fermentation, or SSCF – simultaneous hydrolysis and co-fermentation) This way, solutions should be tailor-made, considering the specific integration features chosen for each production system.

The enzymatic hydrolysis process usually presents advantages associated to yields above 0.85 g glucose / g cellulose, under moderate temperatures (40 ºC to 50 ºC) and atmospheric pressure. However operational aspects related to the long processing time (48 to 72 hours), catalytic deactivation by inhibition of enzymatic activity, as well as high cost of enzymes, have caused uncertainty regarding the economic viability of enzymatic hydrolysis in the ethanol from lignocellulosic biomasses production context.

The enzymatic hydrolysis rate of a bagasse pulp tends to decrease with the concentration of carbohydrates (e.g. xylose, glucose and cellobiose) and ethanol in the reaction medium, as these compounds above certain concentration levels promote an inhibition of enzymatic activity. It has been noticed that glucose has more influence on enzymatic inhibition, if compared to ethanol, as well as there is some synergy between these compounds in this phenomenon. Such evidence points to the potential of using the liquor displacement technique after 24 hours of process during SHF operations, with partial enzymes replacement. It was also noticed that, due to higher enzymes tolerance to alcohol, the use of SSF systems (where glucose is converted into ethanol by microorganisms as glucose is produced) enables increasing enzymatic activity and consequently the increase of the overall hydrolytic yield. However, the need to analyze the various options on a case-by-case basis must be emphasized, as SSF processes are carried out *"off optimal operating conditions"* for both enzymes and yeasts, so that a gain in yield due to reduced enzymatic inhibition may be offset by less enzymatic activity as a result of not so adequate operating conditions. Though it exerts a lesser inhibiting effect than glucose or cellobiose on the hydrolysis rate, the presence of ethanol in pretreated pulp (after washing with ethanol-water) causes a strong decrease in enzymatic activity during the hydrolysis process. Therefore, organosolv acid processes using ethanol tend, by this principle, to produce pulp with enzyme inhibitors.

Washing pretreated pulp is essential in SHF processes, especially when operating with solid loads above 8%, as a result of the inhibiting effect on enzymatic activity exerted by carbohydrates (xylose and glucose), degradation compounds, and lignin derivates built up on the pulp. Pretreated pulp washing with diluted sodium hydroxide or ethanol-water increases the cellulose content due to delignification of the pulp. However, in some cases, solubilized lignin sediments back on the cellulosic matrix form a film and restrict pulp access by hydrolytic agents. As a result, glucose yield in the hydrolysis process is impaired. Furthermore, the presence of ethanol (from the washed pulp) in the reaction medium has an inhibiting effect on enzymatic activity, lowering production yield. Washing the pretreated pulp with diluted nitric acid represents a potentially interesting technological alternative (mostly in pulps pretreated by acid processes) as in addition to not presenting the aforementioned inconveniences, this technique promotes cellulose swelling, increasing its accessibility by the enlargement of pores associated to the reduced crystallinity in cellulose. Additionally, washing the pulp with diluted nitric acid allows sequestering iron cations present in the pulp, which exert an inhibiting effect on enzymatic activity. Finally, nitric acid promotes nitration and partial removal of lignin with minimum fragmentation of it, so that no substantial resetting of lignin on the cellulosic matrix takes place.

Addition of a surfactant in quantities close to 0.005 g/g of pretreated bagasse promotes a

significant increase in the glucose yield in enzymatic hydrolysis processes in both SHF and SSF processes, leading to some 50% lower enzymes intake. Basically, the surfactant promotes changes in the substrate structure, making cellulose more susceptible to attack by enzymes, while it minimizes enzyme denaturation due to the shearing forces in the reaction medium and, finally prevents enzymes deactivation associated to their adsorption by the substrate. A synergy between the use of Tween-90 is evidenced with the adoption of liquor displacement regarding an increment in enzymatic activity (mostly with substrate loads above 2%) and consequently of glucose yield.

Mechanical agitation used in hydrolytic processes tends to increment enzyme activity, as it promotes further enzyme-substrate interaction, in addition to reducing resistance to diffusion in the reaction medium, especially with solid loads around 5%. However, beyond a certain "*critical value*", mechanical agitation tends to increment tangential displacement of the enzymes, as well as to incorporate shearing stresses to the medium, resulting in loss of enzymatic activity, in addition to less contact between enzyme and substrate. The loss of the β-glycosidase activity tends to be more intense in regimes having vigorous mechanical agitation, tending to increment with the increase of the residence time. Particularly SSF systems using substrate loads around 8% to 10% should adopt a different agitation profile, operating with more vigorous agitation (200-300 RPM) during the first hour of the process, aiming to promote better impregnation of the substrate with enzymes. Next, agitation should be slowed down to about 150 RPM, to minimize β-glycosidase deactivation.

The choice of an enzymes formulation exclusively based on enzymatic activity [cellulase (FPU/g) and β-glycosidase (IU/g)] may induce to mistaken conclusions in terms of hydrolytic process efficiency. Practice has demonstrated that different formulations containing the same activity may present different performances in the enzymatic hydrolysis of lignocellulosic pulps under the same process conditions. Therefore "custom made, case-by-case" solutions should be developed and tested, considering the pretreated pulp characteristics. This means that the development of a pretreating system should be integrated with enzymes production, cellulose hydrolysis, and carbohydrates fermentation.

Compared to chemical routes, from a technical standpoint, the enzymatic route appears as a more proper alternative for producing ethanol from bagasse due to better chances of obtaining high glucose yield, around 90%, with enzyme loads of approximately 7.5 FPU/g of cellulose, at the same time that hydrolytes with reduced toxicity to fermentation microorganisms are obtained. However the economic viability of this process depends fundamentally on obtaining enzymes at a landed price around US$ 1.30/kg, about 75% lower than prices currently practiced in the Brazilian market.

Based on the context that was presented, the in-house (i.e. within the sugar-alcohol plant) production of these enzymes, using part of the pretreated bagasse (about 30% – 40%) as a substrate emerges as a potentially attractive technological option. Among the potential advantages, it would be possible to mention the absence of transportation costs, the possibility of using diluted formulations, lower purification and concentration costs, lower product conservation complexity, in addition to being able to use hemicellulosic hydrolytes for cultivating microorganisms. Considering that the integrated system requires tailored solutions, in-house production of enzymes becomes extremely important. Preliminary studies indicate that it is possible to produce enzymes in-house at a total cost of US$ 20/m^3 of ethanol, i.e. about 9% of the cost of ethanol.

Basically acid hydrolysis has important advantages over the enzymatic process due to availability, guaranteed supply, and lower reagent costs, in addition to mature technology and lower restrictions in terms of intellectual property. On the other hand, there are disadvantages concerning the need of acid recovery systems (in processes using concentrated acid) and higher equipment building material costs. Processes using diluted acid pose problems associated to reduced glucose yield (50% to 60%), in addition to the formation of fermentation-inhibiting compounds, resulting from saccharide degradation. In these circum-

stances, there is a need to process to hydrolytes treatment, thus increasing the cost of the overall process. On the other hand, the adoption of a fed-batch fermentation strategy has emerged as an interesting technological alternative, with the purpose of minimizing such inhibiting effects, no longer requiring hydrolyte treatment.

Among the cellulose hydrolysis processes with diluted acid, special attention has been given to the use of continuous reactors in counterflow, where glucose is extracted practically at the same time it is produced. In this manner glucose degradation is minimized, while saccharide yield is maximized. Relatively diluted hydrolytes are produced, which tends to increase operational costs in later operations. The hydrolysis process with diluted acid in counterflow reactors makes it possible to obtain glucose yields around 80% to 85%. Therefore, in spite of this technological option having inconveniences associated to the higher operating complexity, the cellulose hydrolysis process with diluted acid in counterflow may be considered as a potentially interesting option for producing ethanol from sugarcane bagasse by using the hydrolyte mixed with molasses or sugarcane juice. Alternate settings, such as flow reactors with shrinking-bed-flow-through may lead to hydrolysis processes with diluted acids that can actually be competitive with enzymatic hydrolysis processes.

In strategic terms, it seems particularly proper to develop cellulose hydrolysis processes with diluted acid (including the organosolv process) to create backup solutions that allow facing the uncertainties related to the development of really competitive enzymatic processes, as well as to ensure local supply of enzymes at affordable prices.

Fermentation of the lignocellulosic material hydrolyte

The fermentation of the reducing sugars liquor obtained after the hydrolysis of lignocellulosic materials is a critical stage to reach an ethanol production process that ensures a maximum conversion of these sugars, and that is compatible with a viable production cost from both technical and economical standpoints. Yet, the following should be considered: energy consumption associated to fermentation conditions and the grade of ethanol in the final wine obtained.

To assess all possible fermentation routes for the hydrolysis liquor, it is important to take into account previous experiments made in industrial scale, demonstrations, or carried out merely to set the grounds for a demonstration process.

Diluted-acid catalyzed hydrolysis was industrially practiced in Russia until recently. The process practiced in the Tavda unit was an optimized version of the Schoeller process, which employed forest residues, processed in batches and by percolation, attaining 60% conversion of the hexoses. Fermentation was made by combining amylaceous saccharified mashes with the liquor resulting from the hydrolysis. The final wine presented very low alcohol content, 1.3 °GL, and consequently steam intake was 20 kg per liter of ethanol. Pentoses were not used for ethanol fermentation, being diverted for biosynthesis of unicellular protein. This process requires high investment in equipment and operating costs, not being economical.

The Sugarcane Technology Center (Centro de Tecnologia Canavieira) carried out an extensive study on the fermentation of hydrolysis liquor obtained with the DHR process (Dedini process, under development) to demonstrate the alcoholic fermentation stage. The liquor obtained from acid hydrolysis with the use of a solvent was mixed with waste honey and syrup to reduce the impact of inhibitors from hydrolysis, and attempting to keep fermentation in operating conditions close to optimal: process with yeast recycling, final wine with 8.5 °GL and temperature of 34 ºC. Results were positive, establishing the protocol for performing alcoholic fermentation of the liquor obtained from the DHR process.

The Iogen process, in demonstration in Canada, employs pretreatment of lignocellulosic biomass by steam explosion and pre-acidification with sulfuric acid, followed by a stage of enzymatic hydrolysis made by the addition of cellulase preparations. Alcoholic fermentation is done at a later stage, mixing the liquor from hydrolysis to a previously saccharified starch mash. Pentoses are discarded, as their fermentation technology

– which Iogen intends to employ – has not yet reached demonstration stage. No hydrolysis liquor purification treatment is performed; inhibitors present are diluted to the tolerance level in the mix with the amylaceous mash.

During the pretreatment of the lignocellulosic material or in the acid-catalyzed hydrolysis processes, not only the sugars from hydrolysis, cellulose and hemicellulose dissolution are obtained. Due to the high temperatures and acid conditions where these pretreatments occur, several compounds appear, being capable of acting as potential inhibitors to fermentation. The nature and concentration of these compounds depend on the type of raw material (cellulose, hemicellulose and lignin content in percentage), on the pretreatment used, on the process conditions (temperature and reaction time) and the use of acid catalysts or not.

Degradation products, which are potential fermentation inhibitors, are grouped in three categories:

- furane derivates;
- low molecular weight aliphatic acids;
- phenolic derivates.

As a consequence of the high temperatures used in pretreatments, sugars from hydrolysis – mostly the hemicellulosic ones – degrade, generating furane-derived compounds: furfural, formed from pentose (xylose and arabinose) and 5-hydroxymethylfurfural (HMF), formed as a consequence of hexoses (glucose, manose and galactose) degradation.

On their turn, these two compounds may further degrade to other products. Furfural may degrade into formic acid or polymerize. HMF originates equimolecular quantities of formic and levulinic acids. Furthermore, from these two aliphatic acids (formic and levulinic), acetic acid is formed by hydrolysis of the acetyl radicals in hemicellulose.

The content of these inhibitors in the liquor after pretreatment depends on the nature of the lignocellulosic material used. Hydrolytes from materials that contain a comparatively higher percentage of hemicellulose present a greater concentration of furfural and acetic acid.

During pretreatment, part of the lignin also degrades, generating a wide variety of phenolic compounds. This is a quite heterogeneous group of compounds that may be found in the form of monomers, dimers, with a wide variety of replacers. Among them there are acids, aldehydes and aromatic alcohols. Phenols originated in pretreatment vary according to the type of biomass, considering that there are great variations in lignin, depending on the vegetal species it comes from.

One phenolic derivate quite abundant in hydrolytes is the 4-hydroxybenzoic acid, originated from the rupture of the ester links bonding the hydroxyl groups to the cinnamic alcohols of lignin. Other phenolic derivates abundant in hydrolytes are syringaldehyde and syringic acid, resulting from the degradation of syringil propane units in lignin. 4-hydroxybenzaldehyde, gentisic, salicylic, and protocatechuic acids, vanillin and vanillic acid, catechol, guaiacol, hydroquinone, coniferilic aldehyde and homovanillic acid have also been identified in hydrolytes.

One group of compounds (not included in the aforementioned three) released during pretreatment is the extractives. Among them there are different types of resins (fatty acids, terpenoids, sterols and waxes) and phenolic compounds (flavonoids, tannins etc.). Such compounds, though low in concentration, are present in bagasse and may act as inhibitors to the microorganisms used in hydrolyte fermentation.

Among the negative effects of furfural on microorganisms in general and fermentation yeasts for alcoholic fermentation in particular, the following are described:

- reduction of the specific growth rate;
- reduction of the volumetric or specific ethanol productivity;
- reduction of the biomass synthesis.

Negative effects caused by HMF, though less intense – considering that its toxicity to microorganisms is lower than furfural – are the same.

The toxic effect caused by furane compounds seems to be associated to the fact that, being chemically reactive aldehydes, they may react with certain biological molecules, such as lipids,

proteins, and nucleic acids, or cause damage to the cell membrane.

Furthermore, furfural inhibits glycolytic and fermentative enzymes. The inhibition furfural exerts on alcohol-dehydrogenase could explain the acetaldehyde excretion observed during the first hours of fermentation.

Furfural and HMF are metabolized by both bacteria and yeasts. In anaerobic conditions, as a consequence of furfural metabolism, mostly furfurilic alcohol is produced and, in a lesser concentration, furoic acid. The hypothesis that the reduction of furfural to furfurilic alcohol is catalyzed by a NADH-dependent alcohol-dehydrogenase is practically accepted. In anaerobic conditions, during fermentation, glycerol is produced to regenerate the excess NADH generated in biosynthesis and to keep the intracellular redox balanced. In fermentation with furfural present, glycerol formation is not observed, which suggests that the reduction of furfural to furfurilic acid oxidizes the NADH in anaerobic conditions.

Though it is well documented in bibliography that weak aliphatic acids lower ethanol yield and decrease biomass production, the mechanism which causes this inhibition has not been fully clarified.

One of the mechanisms proposed to explain aliphatic acids' inhibiting effect is the uncoupling theory. According to this theory, the toxic effect depends on the acids' pKa and the medium's pH. Only the non-dissociated form of the acids penetrates the cell by diffusion, where, due to higher intracellular pH, it dissociates, causing a lower pH that should be compensated by a membrane ATPase pumping protons out of the cell at the cost of ATP hydrolysis. The lesser ATP quantity available for building the cell biomass would explain the reduced growth when aliphatic acids are present in the medium. When acid concentration is sufficiently high, the proton pumping capacity is surpassed, which causes cytoplasm acidification, and later cell death. Another proposed mechanism to explain this inhibiting effect of acids is the intracellular buildup of anions. According to this theory, while protons are excreted, anions are trapped in the cell, accumulating inside it. Inhibition could be related to anion toxicity. Though the aliphatic acids inhibition mechanism is not known for sure, the toxic effect displayed by these compounds may be due to either the uncoupling effect or the inhibiting effect of anions buildup. Quite likely short-chain aliphatic acids' effect is also due to the direct action of these compounds on membrane integrity. The insertion of aliphatic chains in the membrane may alter its structure and hydrophobicity, increasing its permeability and affecting its function as a selective barrier.

From all inhibitors identified in lignocellulosic material hydrolytes, low molecular mass aromatic compounds have been seen as the most toxic to microorganisms. Though the inhibition mechanism is not completely known, the effect of phenol derivates on prokaryotes like *Klebsiella pneumoniae* and *Escherichia coli* have been studied. The toxic effect of aromatic aldehydes may be related to the interaction with certain hydrophobic cell zones, causing membrane loss of integrity, affecting its ability to act as a selective barrier. The toxic effect of aromatic alcohols is attributed to the damage they cause on the plasmatic membrane. The inhibiting effect presented by aromatic acids may be based on similar mechanisms to the previously described for aliphatic acids. Though several studies were made on the effect of phenolic acids on yeasts, especially on *Saccharomyces*, the inhibition mechanism on eukaryotes has not been fully clarified. Since the plasmatic membrane structure is similar to the prokaryotes', it is said that the inhibiting mechanisms could be similar. Like for furfural and HMF, there is data in literature demonstrating the ability of certain microorganisms – both bacteria such as *K. pneumoniae* and *Z. mobilis* and yeasts of the *Saccharomyces, Pichia, Pachysolen* and *Candida* types – to metabolize aromatic aldehydes. However data available in literature about the role of the alcohol-dehydrogenase of *S. cerevisiae* in converting these compounds is contradictory. Other enzymes that may be acting in aromatic aldehydes' metabolism are vanillin-oxidoreductase, aldose reductase and arialcohol – dehydrogenase.

Having the intent of increasing fermentability of hydrolytes obtained after pretreatment, it is

necessary to lower concentration or to eliminate completely from the medium, the toxic compounds generated in pretreatment and hydrolysis.

Depending on the mechanisms used to eliminate inhibitors, these methods may be grouped as: biological, chemical and physical.

Biological methods

They consist of using microorganisms capable of metabolizing some of the toxic compounds present in hydrolytes. One example of biologic treatment is the detoxification of hydrolytes using *Trichoderma reesei* mycelia. This microorganism is capable of metabolizing pentoses and oligomers present in hydrolytes, without being affected by the toxic products found in it. Treatment with this fungus has eliminated compounds like acetic acid, furfural, and benzoic acid.

Enzymes (lacase and peroxidase) from lignolytic fungi may also be used. Enzymes from *Trametes versicolor* have also been used for complete and selective elimination of phenolic monomers found in hydrolytes. Based on absorption spectra, it seems that the mechanism by which these enzymes reduce the hydrolytes toxic effect is an oxidizing polymerization of low molecular mass phenolic compounds to aromatic compounds with higher molecular mass, however less toxic.

Chemical and physical methods – extraction with solvents

Relatively high volatility – compared to water – organic solvents are efficient in removing aliphatic acids and aldehydes. Low molecular mass esters, from aliphatic acids and alcohols acids, like ethyl acetate present favorable partition coefficients extracting aliphatic acids and aldehydes. Ethyl acetate, for instance, is efficient in removing acetic and formic acids, and furfural.

Chemical and physical methods – treatment with alkaline-terrous hydroxides

Treatment of lignocellulosic hydrolytes with various hydroxides has been one of the most widely used methods for eliminating toxic compounds generated in pretreatment and hydrolysis. By adding calcium hydroxide (others like sodium or magnesium hydroxide) to the medium until a pH of 10 is reached, low solubility calcium salts precipitate is formed, which drags some of the toxic compounds found on the hydrolyte, like furfural, HMF, and acetic acid. This precipitate should be removed from the medium before fermentation. Treatment may be combined with the addition of sulphide, which on its own is an efficient detoxification method. By treating lignocellulosic material hydrolytes with calcium hydroxide, significant increases in ethanol yield and productivity have been achieved.

Chemical and physical methods – removal by evaporation and distillation

This treatment pursues eliminating volatile compounds like furfural, acetic acid, and formic acid. Compounds like levulinic acid, hydroximethylfurfural and phenol derivates are not eliminated. Treatment should be carried out under low pH, as compounds like formic and acetic acid are volatile only in their protoned form. Efficiency is partial, considering that only volatile inhibitors are removed, while HMF and phenolic compounds remain.

Chemical and physical methods – adsorption in active coal and vegetal coal

The use of adsorption by means of active coal or vegetal coal has shown efficiency in the detoxification of hydrolytic liquors.

By applying vegetal coal, prepared from treated wood at temperatures above 600 ºC, it has been possible to increase the fermentability of hydrolytes by the selective elimination of toxic compounds like furfural, HMF and phenolic derivates, without affecting the concentration of fermentable sugars.

Physical and chemical methods – use of ionic exchange resins

Some authors have successfully used cationic resins to detoxify hydrolytes, while others report

negative results, attributing them to negatively-charged sulfonic groups of the cationic resins that cause repulsion effects on the inhibitors present in the hydrolyte. Best results were obtained with strong anion resins at pH 10. With these resins, elimination of phenolic compounds is mostly achieved, due to the formation of strong links with quaternary ammonium groups (positively charged) in the resin with phenols (negatively charged). Upon treating hydrolytes with ionic exchange resins, there is also a reduction in the concentration of furanes (in this case, due to hydrophobic interactions) and aliphatic acids. In spite of the good results achieved in eliminating inhibitors through ionic exchange resins, their high cost, for the time being, renders unviable their industrial application. The loss of sugars in the resins is yet to be determined. The industrial use of ionic exchange resins has often been challenged environmentally, due to the effluents generated in the regeneration phase, and the water volumes required.

Chemical and physical methods – use of residual lignin as adsorbent

A new method proposed for detoxifying lignocellulosic material hydrolytes consists of using lignin, produced as a residue in hydrolysis, as adsorbent in an extraction using its hydrophobic properties. The advantages of using residual lignin as a detoxifying agent, compared to chromatographic resins, are mostly economical, to lower treatment costs. As lignin is a by-product of hydrolysis, after its use in detoxification, it may still be used as primary fuel.

Physical and chemical methods – use of zeolites

The term zeolites encompasses a large number of minerals, both natural and synthetic, composed of a crystalline skeleton formed by the tridimensional combination of tetrahedrons TO4 (T = Si, Al, B, Ga,) linked to each other by plain oxygen atoms. This structure gives zeolites several properties, such as:

- strong ionic exchange capability;
- high specific surface;
- presence of active centers that allow an important catalytic activity.

Though their mechanism of action is unknown, zeolites have been successfully used in many processes. They are used as catalysts in hydrolysis reactions with various disaccharides, such as cellobiose, maltose, lactose etc., in the environmental control of industrial waste for the elimination of toxic metals (chrome, cobalt, nickel). In ethanol production processes from molasses they are used to remove fermentation inhibitors, such as alkaline and alkaline-terrous salts, plus organic inhibitors present in molasses. Experiments made with hydrolytic liquors showed an improvement in fermentation conditions after depuration with zeolites.

Glucose fermentation is a fully determined process. There is no more adequate microorganism than the *Sacharomyces cerervisiae* yeast that, by its intensive use in industrial scale fermentation, has passed through a natural selection process, presenting the best performance in converting glucose into ethanol, productivity, and tolerance to alcohol. As long as the inhibitors' negative impact is under control, fermentation occurs without any major problem.

Regarding fermentation of pentoses, few microorganisms are capable of fermenting them into ethanol. Transforming pentoses into ethanol is essential for achieving an efficient hydrolysis technology. In this item, ongoing researches are following these lines:

- yeast selection and improvement procedures to naturally ferment pentoses into ethanol;
- development of recombinant strains of *Sacharomyces cerevisiae*;
- selection of thermofilic bacteria;
- selection of mesofilic bacteria.

Three yeast species were identified as having the highest potential for alcoholic fermentation of pentoses: *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus.* However, their performance is quite limited. Pentoses metabolism requires the presence of a minimum level of oxygen, which must be strictly controlled. These strains

have low tolerance to ethanol and alifatic acids. As alternatives, the selection of stronger mutants and the fusion of protoplasts have been tried.

Studies to obtain genetically modified strains of *Sacharomyces cerevisiae* to metabolize pentoses were directed toward the following strategies:

- insertion of bacterial genes that perform xylose isomerization into xylulose (xylose isomerase), the latter fermentable by *Sacharomyces*;
- insertion of the genes that allow assimilation of xylose into *Sacharomyces cerevisiae*;
- isomerization of xylose into xylulose, by adding an isomerase.

So far no further progress was accomplished in these studies.

As to the use of thermofilic bacteria, studies have been made with *Thermoanaerobacter ethanolicus*. This organism requires operating with highly diluted pentose medium. *Clostridium thermohydrosulfuricum* has been widely studied in CDM processes (direct conversion of the lignocellulosic material by the microorganism into alcohol). Among the difficulties found, authors mention: significant formation of acetates, which leads to low alcoholic yield, low tolerance to ethanol, and vulnerability to the presence of contaminants. Genetically modified thermofilic bacteria have also been studied, aiming at preventing acetate formation in parallel with ethanol. In conclusion, the major problems related to the use of thermofilic bacteria are: low tolerance to ethanol, strong sensitivity to inhibitors, parallel formation of a significant quantity of by-products and the need to add growth factors to the medium.

Regarding the possibility of using mesofilic bacteria like *Zymomonas mobilis*, they are not capable of fermenting pentoses, however, they are very efficient in the metabolism of glucose through the Entner Doudoroff via. The introduction of *Escherichia coli* genes makes the fermentation of xylose into ethanol possible.

Zymomonas mobilis is one of the most promising microorganisms for fermenting hydrolysis liquor. It has tolerance to both ethanol and inhibitors, as well as high fermentation productivity. It is considered one of the most promising recombinant microorganisms to successfully perform pentose fermentation. Nevertheless, the problems related to the genetically modified microorganism remain unsolved on the short run. Other mesofilic bacteria capable of metabolizing pentoses in the absence of oxygen are: *Escherichia coli* and *Klebsiella.* These, after having been subject to genetic modifications, are being studied as alternatives for the alcoholic fermentation of hydrolysis liquor. It is important to observe that the only industrial experience with sugar-based mash alcoholic fermentation, using a strain of *Zymomonas mobilis,* performed in Germany in the 1990s was not successful, and the unit was phased out, returning to the conventional process using yeasts as fermentation agent. The information available says that under the conditions that fermentation is performed, a quick contamination comes up, inhibiting fermentation.

To perform the alcoholic fermentation of a liquor containing pentoses and hexoses, the possibilities being studied are: simultaneous or sequential fermentation of pentoses and hexoses. In the simultaneous fermentation, two microorganisms that respectively ferment glucose and xylose are co-cultivated. Most of the works in this field use two yeasts: *S. cerevisiae* and *P. stipitis* (pentoses). Difficulties found were:

- the metabolism of xylose is slower than glucose, causing alcoholic inhibition on the microorganism that metabolizes pentoses;
- catabolic repression of glucose on the use of xylose;
- *S. cerevisiae* competes for the oxygen present in the medium with the yeast in charge of fermenting xylose;
- possible incompatibility between the two strains.

Another option is to operate fermentation in a sequential scheme, first fermenting glucose, and later xylose (or vice-versa).

The best results achieved so far used a mutant strain of *Escherichia coli* incapable of metabolizing glucose, followed by a second glucose fermentation stage with *S. cerevisiae*.

The conversion of lignocellulosic materials into ethanol involving hydrolysis of cellulose and hemicellulose into reducing sugars, and their alcoholic fermentation may be performed simultaneously in one single step or sequentially in two steps.

In two step processes, hydrolysis (acid or enzymatic) and fermentation are done separately (HFS). The advantage of this process is that, the hydrolysis and fermentation stages being separate, each can be performed under optimal conditions. In the case of enzymatic catalysis, the hydrolysis step is done at the ideal temperature for the enzyme (around 50 °C), while fermentation is carried out at the optimum temperature for the ethanol-producing microorganism (28 to 32 °C). The main disadvantage of the HFS process is due to glucose and cellobiose being released during enzymatic hydrolysis, as they inhibit the enzymes involved in this process, causing low yield. When acid hydrolysis is used instead of enzymes as hydrolysis catalysts, hydrolytes must be neutralized before fermentation. Furthermore, a more intense generation of degradation products during hydrolysis may affect the microorganism in charge of fermentation.

In single-step processes, hydrolysis and fermentation take place in the same reactor. The key advantage of these processes is the reduced inhibition by the end product which occurs in the two-step operation, as the presence of fermenting microorganisms with cellulolytic enzymes reduces sugar buildup in the fermenter. For this reason, higher hydrolysis rates and conversion percentages are achieved in comparison with separate hydrolysis and fermentation, requiring a smaller quantity of enzymes, obtaining, as a result, increased ethanol yield. The main disadvantage of this process relates to the different optimal conditions in pH and temperature in hydrolysis and fermentation steps, respectively. For this reason, it is necessary to perform the process in one condition compatible with both steps. Considering that the optimum temperature for enzymatic hydrolysis is close to 50 °C, and that conventional ethanol producing yeasts operate around 28 °C to 34 °C, it is recommendable to use temperature tolerant microorganisms to perform both process in one step. Single-step processes may be divided into two groups:

- Processes where the same microorganism produces enzymes and performs fermentation, known as direct conversion by microorganism (DCM).
- Processes with simultaneous saccharification and fermentation (SSF), where cellulases from a cellulolithic microorganism are used (usually a *Trichoderma* fungus), with the presence of an ethanol-producing microorganism.

In DCM processes, monocultures may be used, as one sole microorganism hydrolyses lignocellulosic materials and ferments sugars into ethanol. For this, bacteria of the *Clostridium* type is used, ethanol yield is low due to the formation of by-products, the low tolerance of the microorganism to ethanol, and the limited grown of the microorganism in hydrolytes due to the existence of toxic products. Co-cultures may also be used, where there are two microorganisms. One of them performs cellulose hydrolysis (*Clostridium thermocellum)* and the other microorganism, ethanolgenic by nature, ferments the sugars produced.

Currently SSF process is the one offering the best outlook. Cellulases come from cellulolithic fungi, usually *Trichoderma reese,* and the fermenting microorganism is a yeast. *Kluyveromyces marxianus* and *Kluyveromyces fragilis* seem to be the more appropriate strains to produce ethanol in a thermofilic environment. Studies carried out to assess SSF process performance showed difficulties to perform alcoholic fermentation in a thermofilic environment. Conversion yields were below expectations, and the final wine had low alcohol content due to the strong inhibition by produced ethanol when operating at high temperatures. Authors confirmed the incidence of contamination in the operating conditions for a SSF process. Tests were carried out using *Kluyveromices* strains.

Plant energy optimization including hydrolysis

One of the major issues for inserting the hydrolysis process in ethanol production is that it as-

sumes using with other purposes the material that is the main energy source of the mill: the bagasse.

The possibility of using surplus bagasse in the hydrolysis process for producing ethanol has been repeatedly discussed. For that, it will be necessary to optimize energy use processes in the mills, to reduce bagasse consumption.

Would the equation be so easy?

It shouldn't be neglected that the use of surplus bagasse in the hydrolysis process will increase the use of steam, mostly in the pretreatment stage and in distilling the additional ethanol, as well as electric power intake in the new mill.

The use of lignin (which has considerable heating power) and pentoses, once separated in the hydrolysis process, as a source of energy, opens the possibility to treat larger quantities of bagasse in hydrolysis.

One of the major problems in using fractions of the lignocellulosic material as a source of energy is that the lignin produced will be very moist and, quite likely, pentoses will be obtained in an aqueous solution, mixed to other components. Once again there is a need to engineer the hydrolysis process, i.e. to think technically about viable options, proper equipment, and acceptable costs.

Another possible solution is using trash. Trash is an option that may be either hydrolyzed or used to replace bagasse in boilers.

In either case, some heavy investment in research will be required: laboratory, pilot plant, and equipment development for:

- harvesting trash;
- cleaning and preparation of sugarcane trash;
- possibilities of applying the hydrolysis process to trash;
- burning the trash in boilers; characteristics and problems.

There is yet another possibility: gasification of both bagasse and trash, for better utilization in the simultaneous electric power generation, in gas turbines (poor gas), associated to recovery boilers to produce the steam required for the unit operation.

MEETING WITH PROF. GUIDO ZACCHI, FROM LUND UNIVERSITY, SWEDEN

Date: Feb. 1st 2007, from 2 PM to 5 PM
Venue: FEQ Principal's Office – Unicamp
Attending: C. V. Rossell, J. Finguerut, A. Bonomi, Filomena A. Rodrigues, R. Maciel, T. Franco, M. das Graças A. Felipe, M. Benossi, Elba Bon, Silvia Nebra, Marcelo Poppe, Ester dal Poz, and Luiz A. B. Cortez.

At the outset of the meeting, Prof. Cortez made a brief explanation to Dr. Zacchi on the objectives of the Project Guidelines for Public Policies for the Sugarcane Agriculture in the São Paulo State Public Policy Research Program (Projeto Diretrizes de Políticas Públicas para a Agroindústria Canavieira do Estado de São Paulo, Programa de Pesquisa em Políticas Públicas – Ethanol – PPPP). A report was made on the Hydrolysis Workshop: reference term, main issues, discussions and conclusions from the debates.

A debate was begun on the likely scenario for the hydrolysis technology for using fibers to produce ethanol. One scenario was described, and Dr. Zacchi suggested that other scenarios should be imagined, modeled, and used in simulation work.

Evolution led to the idea of preparing a document defining the three to five more likely or possible scenarios for the new ethanol industry in Brazil, considering from the use of the existing industrial park to more radical scenarios, such as "cane energy", or distilleries wholly dedicated to produce ethanol, without conventional extraction.

It was also reported the difficulty of obtaining data, information for simulations, e.g., regarding the energy balance in mills and distilleries, what is the energy required by the hydrolysis process etc.

These scenarios and their respective models would then be useful to answer what is required to make them viable. Questions would be about costs, kinetics, environment, others.

In a first discussion about the scenarios, these were suggested:

- current mill (conventional ethanol + sugar) with hydrolysis of C6 only, using surplus bagasse;

- distillery wholly-dedicated to ethanol; partly conventional ethanol, partly ethanol from bagasse, others, leaving lignin from bagasse for energy generation;
- same, as the preceding one, using sugarcane energy, without conventional harvesting.

However these were only suggestions. Scenarios assembly would be left to specialists. These scenarios should also consider the polemic issue of viability of acid vs. enzymatic hydrolysis, as well as simultaneous hydrolysis and fermentation vs. two separate steps.

The assembly of a multifunctional committee was suggested to work on each scenario. It should also include one chemical engineer, one agronomist, and one economist or equivalent. Each scenario should be worked on with the same computing tools and methodology, to offer comparable results.

The results of this study, from scenarios and simulations, will allow to define public investment policies in certain technologies able to ensure not only competitiveness, but also to fulfill the requirements of other areas, such as environmental protection or new jobs generation.

Prof. Zacchi suggested, regarding the development of pretreatment options, to spend more time on developing standard assessment systems than in creating many different treatments. Assessment systems should be the most encompassing possible, i.e., take into account all relevant operations (hydrolysis, fermentation etc.) in the most similar conditions to those simulations indicated as the most convenient (e.g., high solids concentration).

Next there was a discussion on the options of producing enzymes on-site versus buying commercial products. In this discussion Prof. Zacchi stated that cellulases will always cost more than amylases, for being enzymatic complexes (various enzymes and proteins with little-known functions). Additionally, the cellulases activities per protein unit are much smaller, which increases the cost of each activity unit. One major difference between commercial and on-site products is that the former have to be purified and stabilized to endure storage and transportation so they can be sold anywhere, while the on-site enzyme doesn't need these features that render the product more expensive.

There was also a comment from Dr. Zacchi that hydrolysis ethanol will never be able to compete with conventional ethanol produced in Brazil. This remark unquestionably reflects reality and Dr. Zacchi's experience, and cannot be withdrawn from this context. Of course factors like: developing a technology nonexistent today, the need Brazil has now to significantly increase its ethanol production and the future increase in land prices that may render unviable opening new areas to grow sugarcane, among others, may revert this statement, by making it compulsory and economically viable to make full use of sugarcane in ethanol production.

AKNOWLEDGEMENTS

The author aknowledges Jaime Finguerut (CTC), Henrique Baudel (UFPE) and Carlos Eduardo Vaz Rossell (CTBE) who, with the documents and presentations in the III Technological Workshop, made great contributions to this chapter.