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TRM:

HYDROLYSIS COMPONENT

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INTRODUCTION

Brazil has the world largest and most successful program to replace fossil fuels by ethanol from sugarcane which has made great progress over the last 30 years. The expansion of renewable sources from alternative raw materials and production processes are environmentally friendly requirement for the growing demand for ethanol as a fuel, to reduce emissions of greenhouse gases.

The new world scenario leads to the advancement of technology for biofuels of which ethanol using agricultural biomass is a prime example. In this context, transforming the bagasse and cane straw (trash), processing by-products into raw sugar industry, is an alternative technology route that promises significant productivity gains in the fuel ethanol industry. Needless to say, the global environmental impacts of fossil fuels is one the major causes of pollution. Thus, the change in this matrix with the global adoption of biofuels is, in the sort term, the main way to reduce the environmental impact of fossil fuels.

Therefore, the expansion of this sector in Brazil is expected to occur not only by the conventional technology of using sugarcane juice (sucrose), but also by taking advantage of the sugars (C5 and C6) from lignocellulosic biomass (bagasse and straw). The main challenge is to develop a process that can deconstruct this biomass from the separation of its main fractions of cellulose, hemicellulose and lignin, to the use of the main sugar constituents of cellulose and hemicellulose fractions represented by glucose (C6) and xylose (C5) respectively. The use of straw, and sugarcane bagasse, for the production of ethanol will allow to use 2/3 of the energy potential of biomass that is currently under-used or wasted. That is, the juice, the main raw material in the current processes, represents only 1/3 of the energy potential of the plant. The lignin can be used for the generation of electricity or other compounds such as surfactants, adhesives etc. It is also important to consider within this context the production alternatives of other compounds from the pulp such as buthanol, 1-3 propanediol, bioplastics, acids like acetic, lactic and succinic and also from hemicellulose as specialty sugar xylitol.

Currently, the main problem for the production of ethanol from lignocellulosic materials is the lack of technology for efficient conversion of biomass into liquid fuels. The process requires several steps, basically pretreatment, hydrolysis (cellulose and hemicellulose), fermentation of C5 and C6 sugars and distillation.

In this context the hydrolysis step is a critical factor because it is dependent on the type of raw material and the nature of the plant material and its processing, which at the same time, directly affect the next stage of fermentation. It is well known that products from hydrolysis containt not only sugars but also compounds inhibitory of the enzymatic activities. The type and concentrations depend not only on the nature of raw material, but mainly on the type/conditions of hydrolysis, which can be physical, chemical or biological, as well as the combination of these. Although the enzymatic hydrolysis is most appropriate when compared to other procedures, such as acid hydrolysis to obtain cellulosic ethanol, this alone does not allow the depolymerization of cellulose that requires also the pretreatment of the raw material by physical methods, chemical or biological. This crucial step prior to hydrolysis decreases the crystallinity of cellulose assisting the action of cellulolytic enzymes by solubilization of hemicellulose, by facilitating the accessibility of the cellulose structure. Moreover, the pretreatment may also contribute to the formation and/or release of inhibitory compounds in enzymatic activities and furan compound derivatives (furfural and hydroxymethylfurfural) formed by partial degradation of pentoses and hexoses respectively, as well as phenolic compounds derived from degradation of lignin; and also aliphatic acids such as acetic, formic and levulinic.

At the same time the only viable way, so far, to expand the production capacity of ethanol plants, with no consequent increase in planted area, would be to increase the productivity of these plants. For this task, the urgency to develop technologies to the use bagasse and trash from hydrolysis methods, environmentally friendly, efficient and at low cost to the deconstruction of biomass, without loss of sugars and formation of enzyme inhibitors. However, research so far has been unable to come up with a technical and economic feasible process to produce ethanol from lignocellulosic materials at the laboratory level and less so a larger scale. Within this context, this roadmapping approach seeks some important aspects to be considered in regard to the hydrolysis component.

TECHNOLOGY NEEDS AND CAPABILITIES

Products or technologies targeted

The purpose of this section is to identify the products or technologies that are the focus of the specific component within the Hydrolysis Technology Roadmapping of the sugar and ethanol industry.

Hydrolysis of sugarcane bagasse and straw is shown as an alternative strategic to increase the supply of ethanol without requiring increased plantation area. However, the development of this technology, the object of study in several countries (USA, Canada, European Union), while evolving positively, it not mature to become commercially feasible. The enzymatic catalysis route, however, has so far achieved a performance capable of making this process technically and economically feasible.

We conducted a survey of the most likely route for the enzymatic hydrolysis to identify the steps that are holding back the consolidation of this technology. This technology route, the focus of this study, is one of the elements of the Technology Roadmapping's sugar and ethanol industry.

Figure 1 shows a simplified diagram of the process, considering the hydrolysis bagasse and subsequent conversion to ethanol.

It is shown in dotted lines the critical steps assumed to be those that are preventing to achieve a hydrolysis feasible process from a technical standpoint, and economic feasibility.

Steps are in order of importance:

1. Biosynthesis of cellulase enzyme complex.
2. Enzymatic hydrolysis of pretreated bagasse.
3. Pretreatment physical chemical bagasse.
4. Fermentation of pentoses to ethanol.

The identification of these steps shows that there is need for basic in-depth studies in order to diagnose the problems and possible solutions; and the necessary expertise and material resources to address these bottlenecks. This means involving basic research to address these technology barriers.

Critical system requirements (CSR)

Having defined the products or technologies that are the targets of roadmapping, the purpose of this section is to identify the critical qualities that they must have, called critical system requirements – CSR. Here are identified the functional requirements and performance, featuring high-level dimensions to which the hydrolysis is related, as well as their goals over time to meet a set of strategic goals in terms of:

- productivity;
- cost reduction;
- consumption of natural resources and energy balance ratio (EE/EU and GHG emissions).

Regarding the *productivity* of the industry as a whole, the hydrolysis component is considered

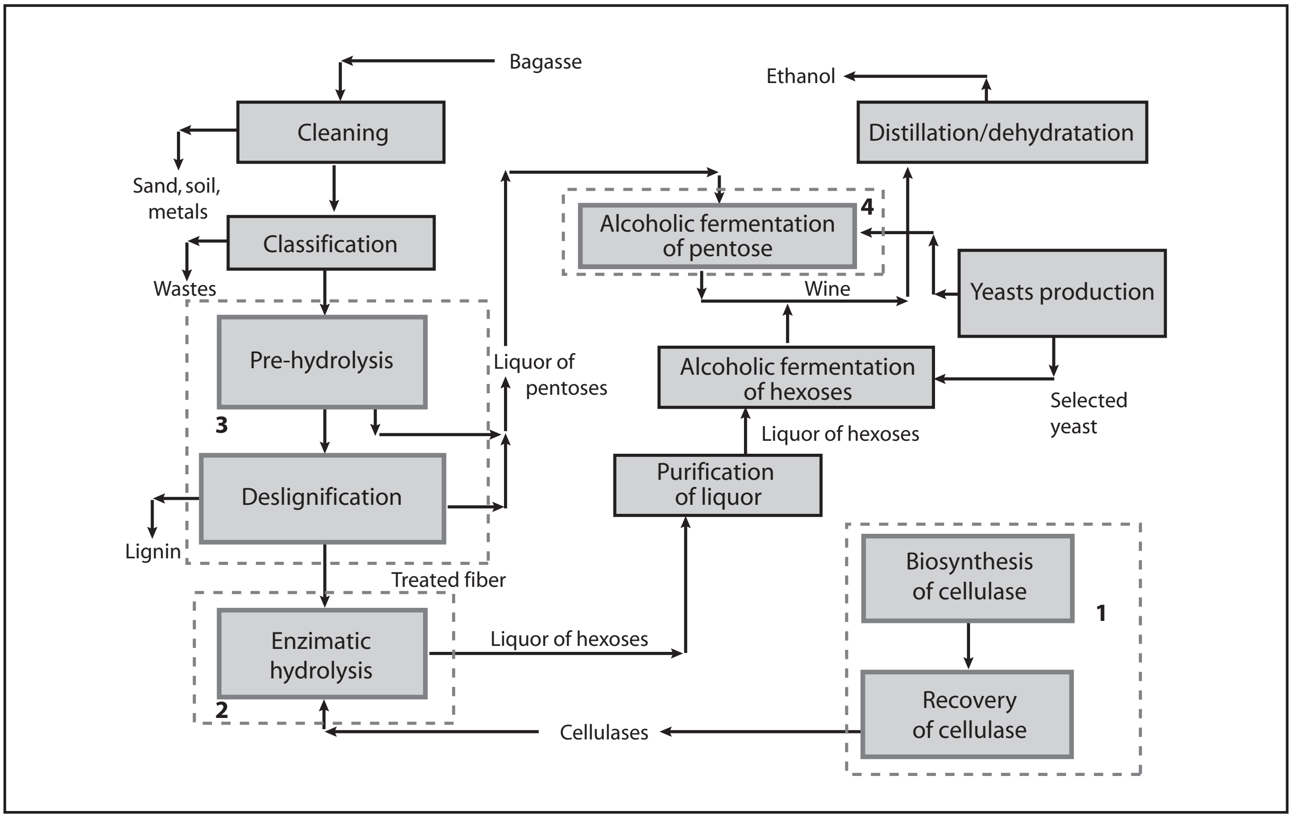

FIGURE 1 Pre-hydrolysis and enzymatic hydrolysis with prior delignification.

here in terms of its contribution to increase ethanol production, starting with a condition zero to reach 2.5 liters per ha per year in 20 years.

For the requirement of *cost*, the cost of ethanol obtained by enzymatic hydrolysis of bagasse is expected to reach the goal of US$ 0.50 per liter.

Since the environmental targets to be achieved by the hydrolysis component, in terms of a qualitative specific use of natural resources (water, bagasse and straw), and pollution, whereas in the short term (5 years), this condition will be high and will move to a low condition in 20 years.

Major technology areas

While the two previous sections have identified products and technologies focused on this work and their system requirements, the objective of this third section is to identify the key technology areas to be explored so that these attributes are achieved.

As mentioned in section Products or technologies targeted, the scope of this work comes down to four steps identified in Figure 1. Such steps are considered here with the major technology areas which demand scientific and technology developments. Afterwards each area is commented.

Biosynthesis of cellulase enzyme complex

Biosynthesis of enzymes that make up the enzyme complex responsible for the catalysis of cellulose into sugars is a major step that is preventing the consolidation of hydrolysis technology

This step is extremely strategic, considering that:

- The current cost of production of an IU (international unit of cellulase) is the major factor that adversely affects and prevents cost reduction of ethanol from bagasse for enzymatic hydrolysis.
- The enzyme activity (IU per ml of enzyme preparation) of the enzymatic complexes obtained is low, compared, for example, to the amylase activity responsible for industrial saccharification of starch.

- Research efforts that Brazil dedicated to the understanding of the biosynthesis of cellulase are not very significant compared to the research programs, public and private development, in USA, Canada and the EEC.
- There is a risk of falling into a high technology dependence through licensing procedures for enzyme production or selected strains of fungi and even producers through the sale of the enzymes by the holders of the technology.

A brief diagnosis of the problem that hampers the development of enzymatic conversion efficiency and high productivity indicates the following facts:

The enzymatic complex is composed of three main fractions: *endo*1,4β – *glucanase*, *exo*1,4β – *glucanase* and β – *glucosidades,* and their relative ratio must be balanced in order to promove an efficient saccharification.

The chemical structure of enzymes, their active centers and their role in catalysis need to be clarified these chemical structures.

The enzymes in the reaction conditions (pH 4 to 5 and temperature 45 to 55 °C) have an average life relatively low, signaling the need to develop techniques to stabilize them.

At present, there is not a complete mastery of the mechanisms of enzymes that form the enzymatic complex and the person responsible for conversion of cellulose into glucose.

We must improve our knowledge on the effects of interaction between the enzymes and lignin attached to cellulose during hydrolysis. Also, the enzymes are sequestered by the fraction of cellulose and this mechanism is not well known either.

The few groups participating in the bioethanol project, which involves several educational and research institutions have very limited amount of resources available to them. This project aims to develop technology using enzymatic hydrolysis for the conversion of sugarcane biomass into alcohol fuel from fungi selected for the production of cellulases, either submerged or semi-solid. The fermentation studies were performed on a laboratory scale, with no opportunity to develop a pilot-scale fermentation and thereby improve the process, evaluate the performance of different strains of fungi, that produce and the downstream end, develop and stabilize the enzymes, and obtain fermentation protocols that allow technical and economic evaluation of the biosynthesis of the enzyme.[1]

Enzymatic hydrolysis of pretreated bagasse

Concerning the enzymatic hydrolysis, the results still are not favorable. The reaction time was very high and do not reach the desired conversion and liqueurs sugars are low. Again, this is an undeveloped field, where reports of Bioethanol Project showed little activity of researchers. This requires laboratory facilities and pilot plants for development and optimization of enzymatic reactor's with productivity and efficient conversion rate.

There are several constraints in enzyme reactors. There is a limitation of the suspended solids in the reaction of saccharification, especially in the case of bagasse and its fractions derived from the density of this material is very low (150 to 350 kg/m^3). This limit is below 50 kg per 1,000 kg of suspension, leading to low-end liquor concentration of sugars and a high consumption of mechanical energy in the hydrolysis and thermal energy in the later stages of recovery of ethanol. The enzymatic reaction has a kinetic disadvantage. The results of tests on a pilot scale (representative) indicate reaction times of 75 to 100 hours. Part of this is due to the low activity of enzymes available (much less than that of amylase, for example) and the part that displays enzymatic inhibition by the substrate, the intermediates and the final product.

To work around this problem, we have proposed alternative reactors that combine the hydrolysis step with the step of fermentation, citing, for example, simultaneous saccharification and fermentation (enzymatic reaction by cellulase complex and fermentation with selected yeasts) and conversion directly by microorganisms that have activity cellulase and ethanol production (in

[1] Reports, Bioethanol Project – Production of ethanol by enzymatic hydrolysis of biomass from sugarcane, Contract FINEP N. 01-06-004700, Executor: State University of Campinas, Coordinator: Prof. Rogerio Cezar de Cerqueira Leite, 2006-2007.

mono-culture or co-culture). However, although these alternatives are the subject of laboratory studies, it is difficult to obtain practical results. In the case of Brazil, it is difficult to reconcile them with the process of producing ethanol from the first generation. This indicates the importance of focusing on processes of saccharification and fermentation to separate and the need to develop a reactor capable of improve the inhibitions, retain the enzymes and substrates in suspension.

Pretreatment physical chemical residues

The physical-chemical pretreatment of lignocellulosic material is a critical stage, because it depends on the efficiency of the later stages of saccharification. The cellulosic material is highly crystalline structure, hemicellulose and lignin block access of the catalysts union β 1-4 that bind together the molecules of glucose. Pretreatment involves among others:

- Reduction and uniformity of particle size in order to facilitate access of the catalytic hydrolysis of the glycosidic unions.
- Pre-hydrolysis and removal of pentoses to facilitate the subsequent stages.
- Removal of lignin to facilitate catalysis and prevent its interaction with the cellulolytic enzymes.

Although this is one of the fields in which Brazilian researchers have been active, it is appropriate to increase activities in this area, whereas the efficiency of pretreatment determines the success of the later stage of hydrolysis.

The research effort undertaken so far in Brazil has focused mainly on routes:

- steam explosion;
- explosion with steam and acid;
- organosolv delignification;
- pre-hydrolysis with dilute acids;
- delignification with alkali;
- delignification by wet oxidation.

Studies have been conducted in laboratory scale, because of deficiency of material resources. The characterization of raw materials, the current intermediate and final products showed the precarious nature of the disability analytical resources available. Again, this diagnosis leads to the necessity to introduce basic research to monitor the disintegration of the polymer cellulose-hemicellulose-lignin by the pretreatment.

Fermentation of pentoses to ethanol

Unlike the fermentation of hexoses (glucose and others) when a process is fully established, the fermentation of pentoses must be developed. Few microorganisms have the ability to ferment them into ethanol. The transformation of pentoses to ethanol is critical to achieve an efficient technology of hydrolysis at a cost of ethanol production from sugarcane. It should be remembered that the conversion of hexoses to ethanol accounts for 34% of the total potential conversion of bagasse into ethanol, and should not be neglected. At this stage, studies are still at laboratory scale, and from a commercial stage. Researchers in Sweden commented in a Latin America – European Union Biofuel Research Workshop, held in Campinas-SP, 23 to 27 April 2007, that favorable results would be obtained only in the middle and long term.

According to the literature, research in progress in this area include:

- procedures for selection and breeding of yeasts that ferment naturally pentoses in ethanol;
- development of recombinant strains of *Saccharomyces cerevisiae*;
- selection of thermophilic bacteria;
- selection of mesophilic bacteria.

Three species of yeasts were identified as high potential for fermentation of pentoses despite limited performance: *Pichia stipitis*, and *Candida shehatae* and *Pachysolen tannophilus*. The metabolism pentoses requires the presence of a minimum level of oxygen, which must be strictly controlled. These strains have a low tolerance to ethanol and aliphatic acids. Research is undergoing on the selection of more resistant mutants and protoplast fusion.

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

- Insertion of bacterial genes that carry out the isomerization of xylose to xylulose (xylose isomerase); latter fermentable by *Saccharomyces*;
- Insert in *Saccharomyces cerevisiae* of genes that allow the assimilation of xylose;
- isomerization of xylose into xylulose via the addition of an isomerase.

At present we have not achieved promising advances. For example, the use of thermophilic bacteria, studies of *Thermoanaerobacter ethanolicus* demonstrate the necessity of use pentoses – very dilluted broths. *Clostridium thermohydrosulfuricum* has been widely studied in cases of direct conversion (CDM). Among the difficulties highlighted, the authors state the following: formation of significant acetates leading to low yield strength, low tolerance to ethanol and vulnerability to contaminants. Thermophilic bacteria, genetically modified, also have been studied in order to avoid the formation of acetate in parallel to the formation of ethanol.

Generally the main problems related to the use of thermophilic bacteria are low tolerance to ethanol, a strong sensitivity to inhibitors, in parallel formation of significant amount of by-products and the need to add growth factors in the medium.

The possibility of use of mesophilic bacteria, some bacteria such as *Zymomonas mobilis* are not able to ferment the pentoses, but are very efficient in the metabolism of glucose to ethanol via the Entner Doudoroff pathway. The introduction of genes of *Escherichia coli* allowed the fermentation of xylose to ethanol.

An important feature of *Zymomonas mobilis*, as one of the microorganisms most promising for fermentation liquor of hydrolysis, is their strong tolerance to ethanol and inhibitors as well as the high productivity of fermentation. It is considered one of the recombinant microorganisms most promising for the successful fermentation of pentoses. Yet it is also necessary to solve the problem related to the instability of the genetically modified organisms. Other mesophilic bacteria capable of metabolizing pentoses in the absence of oxygen are *Escherichia coli* and *Klebsiella* which are being subjected to genetic modifications, and are investigated as alternatives to alcoholic fermentation liquor of hydrolysis. Importantly, the only experience of industrial alcoholic fermentation of sugar based wort, using a strain of *Zymomonas mobilis*, held in Germany 90 years, was not successful and the unit was turned off back to the conventional process with yeast as a fermentation agent. The information we have about that process is that the conditions in which the fermentation proceeded led to rapid contamination which inhibits fermentation.

To perform alcoholic fermentation of a liquor containing pentoses and hexoses possibilities under study requires sequential or simultaneous fermentation of pentoses and hexoses. In two simultaneous fermentation process microorganisms that ferment, respectively, glucose and xylose are grown in coculture. Most of the work in this field used two yeasts: *S. cerevisiae* and *P. stipitis* (pentoses).

The difficulties encountered were:

- the metabolism of xylose proceeds more slowly than glucose, leading to inhibition of the alcoholic microorganism that metabolizes the pentose;
- catabolic repression of glucose on xylose utilization;
- competition between *S. cerevisiae* and the yeast responsible for fermentation of xylose by oxygen present in the medium;
- possible incompatibility between the two strains.

Another possibility has been operating the fermentation in a sequential scheme, fermenting glucose first and then the xylose (or vice versa). Results were obtained with a mutant strain of *Escherichia coli* unable to metabolize glucose was employed *S. cerevisiae* to a second stage of fermentation of glucose. Again, referring to the reports of Bioethanol project and the available literature, it shows an expressive performance of Brazil in the development of microorganisms for conversion of pentoses to ethanol, compared with number of groups and publications abroad. This indicates that there is a need for skills in this area and to encourage groups to develop these activities, as well as provide material resources for carrying out studies.

TECHNOLOGY DRIVERS

At this point, the critical system requirements presented in section Critical system requirements (CSR) are transformed into technology drivers for each of the major technology areas identified in section Major technology areas. These technology drivers will be the critical variables in determining the technology alternatives to be selected later. To that end, here are presented goals for each technology driver, which are referenced to the critical system requirements that the product or technology must possess. The goals of the technology drivers specify how well certain alternative technology should play at some point in time. In other words, the targets are set for the delivery of the final system.

Each of the tables below shows the indicators to be pursued by each technology area in each CSR.

The projections made on the CSR “productivity” were established according to the mark final route of maximum use of the material. Firstly was considered optimal recovery of straw estimating removal of 50% for conversion of biomass or production of electricity. Another pillar of the stage was recasting the production system (distillation), steam generator operating at pressures of 90 bars and temperature of superheat of 590 °C, tube generator with high efficiency, electrification of the joint preparation and extraction of sugar. In this reformulation we obtained large surplus bagasse for use in the process of conversion of biomass to about 50%; and finally taking into account the projected growth in agricultural productivity roadmap put forward by breeding and farm management, also projecting in 20 years a yield of 130 t/ha/year. It was considered for the calculation basis of the impact of the introduction of the enzymatic hydrolysis pretreatment by steam explosion and ethanol production from hexose, only the first 5 years and

TABLE 1 Technolog drivers of the “productivity” CSR.

Hydrolysis productivity (L/ha.year)	Present	5 years	10 years	20 years (Vision)
2nd generation ethanol	0	1,156	1,800	2,500
1st generation ethanol	6,800	8,350	9,450	11,700
Total	**6,800**	**9,481**	**11,250**	**14,200**

TABLE 2 Technology drivers of the “cost” CSR.

		Present	5 years	10 years	20 years (Vision)
CSR	**Hydrolysis Cost (US$/l)**	n.a.	0.75	0.60	0.50
Technology area	**Enzymes**				
Indicator	*Cost (reference = 1)*	1	1/2	1/3	1/4
Technology area	**Reaction**				
Indicator	*Cost (reference = 1)*	1	1/2	1/3	1/4
Technology area	**Physical pretreatment**				
Indicator	*Still lacks an indicator*				
Technology area	**Fermentation of pentoses**				
Indicator	*Cost (reference = 1)*	1	3/4	1/2	1/3

TABLE 3 Technology drivers of the "environment" CSR.

		Present	5 years	10 years	20 years (Vision)
CSR	**Environmental impact of hydrolysis**	n.a.	High	Average	Low
Technology area	**Enzyms**				
Indicator	*Use of water, environment pollution, use of bagasse*	1	1/2	1/3	1/4
Technology area	**Reação**				
Indicator	*Water consumption (l of water/l of ethanol)*	75	50	40	30
	Reduction of effluents	n.a.	1/3	1/2	2/3
	Conversion yield bagasse to sugars	80%	85%	90%	95%
Technology area	**Physical pretreatment**				
Indicator	Still lacks an indicator				
Technology area	**Fermentation of pentoses**				
Indicator	*Water consumption (reference = 1)*	1	80%	60%	40%
	Reduction of effluents	0	20%	40%	60%
	Fermentation yield (theoretical reference = 123 l/t of bagasse)	30%	70%	85%	95%

from then there is the pentoses uses reaching the process optimization from 20 years.

In addition to the technology presented here, there are some prerequisites that must take into account the availability of bagasse and trash next to the productive sector, acquisition of new equipment to meet the current model of crop and equipment accurately and breeding cane to obtain high biomass production and even a less recalcitrant lignocellulosic structure. This is because alternative technology requirements for certain goals affects another component of the TRM; these goals should be explicit and serve as a prerequisite for the other components.

PRESENT SCIENTIFIC AND TECHNOLOGY CAPABILITIES

The State of São Paulo has good scientific and technology capability in the area of bioenergy, as there are a significant number of research organizations from different sectors that have appeared since the Proalcohol. These studies have received funding support from funding agencies, especially the Fapesp. Also CNPq and Finep, participates in this integration effort and in the promotion of training of personnel.

Thus, the thematic projects, involving different segments of the research including the productive sector, are promoting activities for form specialists in this area.

GAPS AND BARRIERS

This section identifies current and future gaps, barriers and drivers of the technology section-Technology drivers. To that end, here we present the skills and knowledge that the workforce of the future industry will have to develop to implement new technologies. This identification allows to indicate what kind of strategic decision on education and academic programs should be taken by the State.

The partial reports 1 and 2 of Bioethanol Project of the Ministry of Science and Technology, bringing together the recent work of the most representative acting on hydrolysis showed that:

- Permanent researchers in Brazil, working on topics selected as critical, is extremely low for a strategic study such as the hydrolysis of bagasse for ethanol. New researchers should be trained immediately to high standards; the same applies for existing researchers.
- The report also shows the physical deficiencies with regard to the infrastructure of research laboratories.
- In the specific case of the biosynthesis of enzymes, the number of groups involved is very low, considering the impact it will have in the introduction on the technology of converting bagasse to ethanol (40% increase in ethanol production for the same area cultivation). Added to this the fact is the current cost of enzymes which frustrates the commercial introduction of this technology.
- According to the results of these reports, the enzymatic hydrolysis has been addressed only through experiments at laboratory scale, and thus it is necessary to scale-up. The studies carried out do not address the complexity of issues such as enzyme kinetics, inhibitions, the stability of the enzyme, and the impact of substrate matter and reactor design. It follows then that will be necessary to organize working groups dedicated to the study of enzyme reaction.
- In the fermentation of pentoses, there are not yet some microorganism, whether natural or induced mutations to genetically modified varieties, capable of transforming the pentoses of bagasse into ethanol on a commercial scale. The preliminary economic assessments indicate for the hydrolytic processes to become economical and sustainable, the pentoses sugars must also be converted into ethanol. Few groups are engaged in this research in Brazil. In other centers (Sweden and USA) although more advanced, the results still fall far short of expectations, with no promising alternatives in the short and middle term. Thus, it is a matter that requires an urgent need to develop human resources.
- The consensus is that the efficiency of conversion of lignocellulose into sugars is dependent upon a physical-chemical primary treatment to modify the structure of complex highly crystalline cellulose-hemicellulose-lignin, in order to make the cellulose accessible to enzymes and thus allow the course of hydrolysis to be productive and with high conversion factors. Although at this stage, the reports on bioethanol project showed a significant contribution of researchers working in pretreatment, faced many ifficulties in achieving their objectives, and this lead us to the conclusion that the current level of dedication of researchers in this area is not enough to achieve positive results, and thus recommend to increase the number of researchers in this area.
- The barriers that have limited the progress of the study in pre-physical chemical treatment are due to the lack of laboratory equipment, pilot plants to develop research; and also lack of equipment for chemical and physical analysis capable of measurement and quantification of changes in chemical structure of lignocellulosic material.

FINAL CONSIDERATIONS

The world faces a great challenge to find alternatives to replace fossil fuels, a sustainable energy source for starting the use of renewable raw materials with a consequent reduction in emissions of greenhouse gases. The ethanol produced from sugarcane in Brazil is currently the best route to overcome this global challenge.

The development of technologies for the complete use of the whole sugarcane (sucrose, bagasse, straw) is the main bottleneck to increase ethanol production without incurring increasing the land area planted.

The hydrolysis of lignocellulosic biomass is still a major constraint to increase productivity gain of ethanol fuel production to take advantage of the by-products generated in the production of sugar and ethanol, such as cane bagasse and straw. The increased availability of these materials is concomitant to increase productivity of ethanol by the application of new technologies in the production process. On the one hand, the replacement of manual harvesting by mechanical harvesting will provide larger amount of sugarcane tops and leaves, and at the same time there will be larger availability of bagasse by improving the efficiency of boilers.

The critical point to reach depolymerization technology of plant biomass lies in the fact that they have not yet established the right conditions for hydrolysis characterized by an efficient and low cost process allowing the availability of sugar constituents of the raw material for fermentation.

As for the full use of sugarcane, is required higher efficiency of the basic steps of the hydrolytic process as pretreatment of biomass, to facilitate the accessibility of cellulolytic enzymes; the choice of these steps should take into account the fact that it does not contribute to the generation of compounds inhibitory of enzymatic activity as those derived from furan, phenolics and acids. This requirement is essential for the efficiency of the fermentation process.

Considering the total use of sugar constituents of biomass for the use of its cellulose and hemicellulose fractions, the hydrolyzate will contain a mixture of sugars (C5 and C6) which necessitates the discovery of novel microorganisms and of our biodiversity and/or obtain genetically engineered strains capable of fermenting C5 sugars which are represented mostly by xylose and in small amounts by arabinose. At the same time, it is necessary to search for techniques to separate these sugars after the hydrolytic process.

It is important to discover microorganisms capable of detoxification of the hydrolysates in order that the biomass subjected to a pretreatment solubilize the hemicellulose, which helps to release together the sugars, compounds inhibiting enzymatic activities, harming the subsequent stages of the production process.

The major challenge in producing ethanol from any cellulosic material, such as the use of whole sugarcane biomass, is not only in the researches for the selection of bacteria that produce effective and inexpensive hydrolytic enzymes, but also in relation to knowledge of the mechanisms involved in the action of these enzymes to the deconstruction of the matrix of plant biomass. Allied to this, there is also a need for research into genetic improvement of strains producing cellulases and other enzymes that may help in the process of hydrolysis, in addition to studies on changes in polysaccharide structure constituent of plants, to facilitate the deconstruction of lignocellulosic biomass, which is hampered by its complex structure which is heterogeneous, recalcitrant and enzimatic hydrolysis resistant.

Finally, it is safe to say that so far for the establishment of a technology for producing fuel ethanol from lignocellulosic materials, the stage of degradation (hydrolysis) of biomass to fermentable sugars is presented as a great technical and economic challenge and its success is closely dependent on enzymes be highly efficient and profitable. With this in mind, a technology could be designed for obtaining second-generation ethanol that increases the production of biofuels in a sustainable manner.

The above findings justify the need for an immediate creation of a team of researchers in basic sciences: physics, chemistry, biochemistry, and biology, to advance the development of hydrolysis technology.