Maria das Graças de Almeida Felipe. "RAW MATERIAL QUALITY IN THE PRODUCTION OF SUGARCANE ETHANOL", p.553-560. 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_50

RAW MATERIAL QUALITY IN THE PRODUCTION OF SUGARCANE ETHANOL

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It is urgent to advance the discussion on the guidelines that Brazil should adopt to diversify energy sources in order to reduce dependency and promote the replacement of fossil fuels. This is one of the main ways to reduce the already deleterious negative impact that human activities are causing to the environment. This agenda includes the development of technologies for the expansion of sugarcane bioethanol and its transformation into an internationally competitive commodity, as the only way to ensure the country worldwide market leadership. After all, sugarcane ethanol is at present the only fuel capable of meeting growing demand for renewable energy at low-cost and environmentally friendly. For example, gaseous emissions from burning ethanol are about 60% lower compared to emissions from burning gasoline.

Currently Brazil produces ethanol mostly from sugarcane juice fermentation. The inclusion of quality of raw material as a prerequisite for the proper performance of the alcoholic fermentation is due to the characteristics of sugarcane, including high water activity, high percentage of sugars, and the presence of amino acids and proteins that contribute to their easy deterioration. Thus, changing the harvesting system, in the near future due to phasing out of sugarcane burning practices and increasing mechanical harvesting in appropriate areas, indicates that technological innovations can be seen with growing optimism.

In addition, ethanol production proceses will expand the raw material used today e.g. sugarcane juice, to include also bagasse and trash (straw). A major bottleneck today is the cost of the raw material which represents close to 60% of the final cost of Brazilian ethanol.

This was the scenario that guided the discussions of the workshop on "Ethanol Production: Quality of Raw Materials", held at Lorena Engineering School – EEL/USP, on May 30, 2008. This event also incorporated a number of other activities, for example, "Guidelines for Public Policies for Sugarcane in the State of São Paulo" supported by Fapesp. The main themes included quality of raw material delivered to the mills; impacts of the quality of raw materials on the fermentation process; effect of inhibiting factors in the fermentation and fractionation of bagasse and trash (straw), and new challenges facing the sugarcane sector in order to obtain ethanol.

QUALITY OF THE RAW MATERIAL DELIVERED TO THE MILLS

The sugarcane used as raw material for ethanol production is composed not only of the stalks (81.2%) in which are sugars, but also palm (6.1%), green leaves (5.6%) and dry leaves (7.1%). From a total energy contained in the cane, about 1/3 is in juice and the remainder divided equally between the bagasse and trash (straw).

The environmental and legal requirements for the end of the cane burnings and the replacing of manual by mechanized harvesting lead to discussion of the impact of each of these procedures on the production of ethanol. The type of harvesting method with the correspondent cut system and basic leaf cleaning are a major factor contributing to the levels of dirt (soil) along with the cane. The fire also contributes to the increased pollution leads to weight loss (0.3% to 2.6%) and accelerated biodeterioration by removing the wax acts as a protective film of the plant. On the other hand, mechanical harvesting of sugarcane for processing larger mass of plant, has disadvantages to allow increased rates of impurities (mineral and vegetal) favoring decay.

Still on the interference of the harvesting procedure in the fermentation process have been the fact that the cracks and fissures caused by cutting the stalks provides microbial contamination leading to the formation of acids. This could be avoided if the cane was harvested as whole cane and cutting only at the apex at the same time which would result in an increase of about 20% of the biomass to be processed. Thus, when comparing the quality of raw material, sugarcane versus burnt cane, the first is better. The problem of mechanical harvesting in today's technology that allows the harvest of sugarcane with a large amount of soil which requires further separation of impurities. This could be prevented from picking a technology that allows the removal of the cane without soil avoiding the later stage of separation. The same must be done to the trash (straw).

It is important to consider that the trash (straw) will gain space as an energy resource but still requires technological developments as the removal of soil and trash from the material being processed at the same time its separation at the time of harvest would be recommended. There is no doubt about the use of trash (straw), but there is controversy about the separation because this step would lead to use of fossil fuels. Due to the speed of the process it should work with two fractions: bagasse and trash (straw), the latter would be used to produce thermal energy at once.

Another point highlighted is that burning improves the performance of the harvester in 20% and that not only the ban on burning is necessary but also technology. The burning of sugarcane is only a matter of time and in today's scenario the cane is already harvested mainly with machine. One can not talk in technology with manual harvesting.

IMPACTS OF THE QUALITY OF RAW MATERIALS ON THE FERMENTATION PROCESS

The "plant cane" corresponds from the roots to the flowers and as a living being is subject to action of environmental factors that cause changes in gene regulation of the plant, the photosynthetic efficiency and especially the hormonal balance that influences and affects the development of the plant. In its basic composition as already mentioned in the previous section, the juice is responsible for the largest portion. Its constitution in water (75% to 82%) and soluble solids (10% to 25%) that are represented mostly by sugar (15.5% to 24%) and different compounds such as organic acids and phenolic compounds, may change due to several factors. We highlight the climatic conditions, variety and age of the plant, temperature, rainfall, crop health, harvesting, loading and transportation systems. Other indicators such as the presence of rods in the plant, alcohol, high acidity, amount of dextran, soil, burning time and the drill should be taken into consideration to increase the efficiency of fermentation. Recommended values of these indicators, in practice, are hardly attained and the quality parameters should be expanded.

Special attention should be given to the problems caused in terms of pest attacks, as the drill due to the loss of fermentative efficiency. These attacks disrupt the plant, providing the production of defense phenolic compounds, and an increase in the acidity by its hormonal system, which result in toxicity to fermentative microorganisms. The phenolic compounds are removed by clarification of the broth, but the industry does not compute the loss of viability of yeast occured due to the attack of pests. The pest control as the "froghopper" these act by reducing the size of the stems of the plant further increasing the content of phenolic compounds, is a major challenge to overcome, since the trend is that terminating the practice of burning of sugarcane. This pest is favored with the use of raw cane (cane that is harvested without burning). Besides the toxic effect caused by the attack of pests, the reuse of microorganisms is difficult because the presence of defense compounds such as phenolic compounds changes the morphology of microbes. The deterioration is also a function of temperature as in the case of crystallization of sucrose observed in the field as a function of crack caused by heat depending on burning time.

Another challenge to be overcome is the cane field management, which causes a lot of soil to stick together in the raw material throughout the whole process of fermentation. For every kilogram of soil there are approximately 10^{11} bacteria that contribute to the formation of lactic acid increasing the acidity of the environment and jeopardizing the final yield. One of the mechanisms known about the action of bacterial metabolites is the impermeability of the wall of yeast caused by them with consequent decrease in cell viability. Despite these adverse effects, the land attached to the cane carries minerals which somehow can contribute to the fermentation as the participation of these metabolites on the enzymatic activities, different in the case of sugar production since the presence of minerals is highly undesirable.

The time length in which the raw material is stored after harvest can contribute to its deterioration, as a large storage time enables the development of lactic acid bacteria. In the case of sugarcane "bisada" (which grows during two summers in the field), this is more fibrous and has more sugar, which causes it to be heavier during harvest bringing more impurities to the factory. The application of maturating agents (growth hormone inhibitor) also leads to the accumulation of sugars at the tip of the plant due to the photosynthesis processs and if applied in excess can cause death due to necrosis of the apical bud of the plant which provides development undesirable microorganisms. This technique is stressful for the plant which must be balanced against other factors such as lack of rainfall that is another stress factor. The combined action of these two factors compromises the quality of raw material. However, there is no relation to the application of maturity for the production of dextran and what may influence its production is the inadequate management of matured cane due to the accumulation of sugar on the tip of the plant.

Other relevant aspects are an increase in the harvest period (March to December) that can promote the growth of contaminants due to high soil moisture during this period and productivity (stalks/unit area) in order to obtain higher yields in the final fermentation. Also the growth of the culture of sugarcane in new areas of questionable soil quality (sandy soils), can influence the quality of products and byproducts of the ethanol industry. The mixed cultivation of vegetables and green manure are methods and proposals for the improvement of raw material generated in these new areas for planting sugarcane.

EFFECTS OF INHIBITING FACTORS IN THE FERMENTATION

Here, are presented the main factors interfering in the fermentation and their individual and synergistic effects on the fermentation process. Among these may be cited as the substrate quality, environmental considerations, the by-products formed during metabolism as a function of microbial contamination, the type of process also including the cleaning and disinfecting. Attention should be given to the production of organic acids, mainly lactic acid, because its dissociated form that allows it can enter the cell. Besides this the acetic and formic acids also inhibit the growth of yeast but a lower concentration of lactic acid by the fact that these are more soluble in lipids. Among the effects caused by organic acids are interference in cellular maintenance energy and absorption of nutrients combined with the effects caused by the pH and the inhibition of enzymes and cell death in conditions of strong acidity. The inhibitory effect caused by the acids may be enhanced due to the synergistic action exerted by these and the temperature which causes the accumulation of trehalose with its increase. For the reduction of acidification of the must during alcoholic fermentation requires the presence of a single decanter in the production line of alcohol. With the clarification of molasses is also a possible reduction of up to 90% of acid.

The presence of sulfide in fermentation medium is another factor that interferes with the process by reducing cell viability and thus decreasing the alcoholic fermentation leading up to exaggerated increase in the acidity of the alcohol produced.

Ethanol is also harmful to the fermentation process because it causes changes in the composition of the lipid layer of the cell membrane. This reduces the metabolic activity as a function of inhibition of glucose transport with consequent water stress, limits the yield, and the process productivity and also reduces the cell tolerance to higher temperatures.

The synergy between the effects of weak acids, pH, ethanol, osmotic pressure of the substract and temperature act all together in an exponential way on the strength of proton motive force. The low pH is the most stressful physiological factor in the case of *Saccharomyces cerevisiae* from the distilleries to produce ethanol in relation to other factors presented which requires attention about the pH of the must. In addition pH the cellular inhibition can be caused by the sulfuric acid added to the inoculum for the cellular deflocculation. However, research has not advanced towards the replacement of this acid. What has been studied is the use of immobilized protease and mannanase for cleavage of the bound responsible for flocculation (monosaccharides, amino acids). Research in nanotechnology have been performed and the cost of this technique is the biggest obstacle to their widespread use.

Improvement of raw material used in the manufacture of bioethanol can be achieved by procedures such as clarification of cane molasses, control the deterioration of sugarcane and asseptic milling. In the case of clarification for its implementation in industrial scale requires a reactor separate of the manufacturing line which is now the biggest obstacle. If the case of controlling the deterioration of the cane, because of its richness on biopolymers and microorganisms, it will occur losses in both the manufacturing process line for both ethanol and sugar. An efficient planning of the agricultural and industrial area will control the deterioration of the cane field. Not only the deterioration control is important, but also the insects control mainly through the use of biological control. It can be included among these control procedures to sterilize the must although there is an increasing cost associated. The gains from the reduction of impurities could be compensated by the practice of this procedure. Alternatively these can be found through the delivery of natural products that were able to inhibit the growth of undesirable bacteria.

FRACTIONATION OF BAGASSE AND STRAW OBJECTING THE NEW CHALLENGES TO OBTAIN ETHANOL

The sugarcane bagasse and straw account for 2/3 of the energy potential of sugarcane and represent a rich potential source of sugars. Research studies have been intensified to the use of these sources of renewable resources in obtaining cellulosic ethanol. The development of this technology route will allow to increase significantly Brazilian ethanol production without expanding the area cultivated. However, for the scope of this technology is a great challenge to be overcome that is the establishment of suitable conditions for hydrolysis of these materials for the release of its sugars (hexose and pentose).

Some things to consider regarding the exploitation of sugarcane bagasse and straw are presented with a focus on the incorporation of these new sources in the industrial production of biofuels as follows:

- new concept of the sugar and alcohol plants with the introduction of the sugarcane straw;
- introduction of the concept of Bio-refinery in the sugar and alcohol plants;
- creation of a research network on pretreatment of these biomasses.

Initially, it is important to note that the bagasse and straw as energy sources have the favorable characteristics to the incorporation of these in the production of ethanol, the large amount of material generated in the ethanol industry. For each ton of cane processed it generates about 140 kg dry of each of these materials. The bagasse is burned to generate energy for the plant and the surplus sold to the grid. Improving the efficiency of co-generation and distribution of this surplus will increase (currently around 10%) while the expansion of the mechanized harvest and emergence of the energy market for the straw will help to increase the availability of biomass to produce biofuel.

It is questionable which is the best option for the use of bagasse and straw due to the various alternatives presented to produce biofuels, electricity, specialty sugars or other chemicals. There are also doubts about the need for separation of straw and if this amount could be removed from the field without damaging the soil, taking into account the productivity criteria and final profitability. It is important that harvesters are appropriate for this type of biomass for production of biofuels. In the case of the production of ethanol from corn as it does in the U.S. waste processing such as the cob is ground by the harvester and left the field. Other options suggested for the use of the straw is the extraction of chemicals with high added value such as drugs since it must contain several compounds of interest to the pharmaceutical industry.

As far as the introduction of the Bio-refinery concept – development chemistry platforms through integrated technologies of biomass, this is a need anticipated for the coming years. Research in this field are essential for the development of new technologies for converting lignocellulosic biomass particularly regarding the efficient separation of its major fractions, cellulose, hemicellulose and lignin. The concept of Bio-refinery is important to the fractionation of biomass to allow the use in each fraction separately. This includes the need for advances in research on thermal, chemical and biological process agents to enable the production of both fuels and chemicals involving the conversion of biomass and integrated equipment.

In this new technological scenario which includes the production of ethanol from bagasse and straw, the discussion on biomass hydrolysis of these is undoubtedly the most prominent. Hydrolysis as a step in the production of cellulosic ethanol is now the main bottleneck to the achievement of this technology so that this biofuel can be competitive with fossil energy.

To understand what happens in the hydrolysis process is important to know the structure of lignocellulosic materilas mainly about the relationship between the physical and chemical associations between lignin and polysaccharide components of plant cell walls together with the crystallinity of cellulose. These structural features make it difficult to release the sugars from polymeric matrices therefore, requires pretreatment of biomass as a step prior to hydrolysis. There are different types of possible pretreatments such as mechanical, organosolve, alkali, hydrothermal, steam explosion and dilute acids. All these have in common an increase in enzyme accessibility to cellulose but have significant differences as a condition of reaction, process efficiency, complexity and impact on the rest of the process.

In the case of research with the bagasse this article mentions the pretreatment by steam explosion and dilute acid. In this first, an additional post-hydrolysis is required to separate the fraction corresponding to the C5 sugars from biomass allowing the fractionation of oligosaccharides into simple sugars. In relation to the use of acids, it is known that certain ions interfere with microbial activity depending on their concentrations. As well certain compounds, from the pretreatment, toxic to microorganisms, such as acetic acid and phenolic compounds also undertake the process requiring an additional step to eliminate or reduce the concentration of these. The corrosion of equipment by the action of acids must be considered. Optionally these pretreatments has been the explosion in steam environment with ammonia (Afex). The fact is that it is necessary to create a national network of researchers to work together so that we can provide the optimum pretreatment to be used in order to facilitate the next step of hydrolysis and consequently favoring the fermentation process. This will be reflected in the next step that is the distillation.

Having established the optimal conditions for pretreatment, there are still doubts as to the optimal procedure of hydrolysis, the enzymatic, chemical or combination of which requires major input of resources in relation to the fractionation of biomass. There will be technological competition, while the chemical process allows a better process control but has little selectivity, the enzyme is more selective, but is very sensitive to environmental conditions such as, pH, temperature, contaminants. At the same time it is important to consider the need for advances in research into the use of C5 sugar constituents of hemicellulose or the bagasse or straw. In this context, it is proposed to evaluate the recirculation through the chemical hydrolysis of what was not possible at this stage by returning to the reactor again.

The use of lignocellulosic wastes such as seed oil cakes extracted in the biodiesel production process should be considered in this new production process of ethanol. It can not be forgotten even in the process of production of biofuel are several devices available in the market such as in the pulp and paper industry that already has the technology of fractionation. These could be exploited, as are already optimized. Finally we will see growing interest by the businesses to replace their raw materials for biomass, however, it is important hat the plants do not just think in "commodity" ethanol but also the concept of biorefineries.

FINAL CONSIDERATIONS

The discussions during the "Workshop Ethanol Production: Quality of Raw Materials"[1], enabled various arguments and proposals on improving the productivity of ethanol of first generation and the inclusion of bagasse and trash or straw (residue left on the field after harvest cane) to produce second-generation ethanol. The main points to consider which can serve as parameters for use of resources in research and development aimed at increasing the country's competitiveness in the production of biofuels, are highlighted below:

- Both the manual harvesting of burned sugarcane or unburned cane mechanized harvesting contributes to the deterioration of the cane, even when comparing the two procedures the harvest unburned cane (raw) is better.
- Doubts exist about the need for separation of straw at the time of harvest and there is also proposal to use of bagasse and straw together.
- Care should be taken during the preparation of the straw and bagasse and remembering that boilers were not designed for the use of straw.
- The development of technology for harvesting machines that meet the new harvesting practices will allow greater availability of straw for use in energy production, ethanol or other chemicals.
- Obtention of appropriate indicators about the collection and the amount of straw that must be left in the soil.
- Fixing quality more accurate indexes of raw material.
- Definition of quality parameters of bagasse and straw for use in ethanol production due to the great heterogeneity of these materials.
- Redefining the current model of the production chain for quality control of raw material with new methods of evaluation in order to reduce the negative effects on the alcoholic fermentation.
- Adoption of efficient planning, covering both the agricultural and the industrial

[1] "Workshop Ethanol Production: Quality of Raw Materials" held at the Engineering School of Lorena – USP on May 30, 2008. This event is part of a series of other activities occurring as the project "Guidelines for Public Policy for the Sugarcane Industry of the State of São Paulo" supported by Fapesp. The main speakers and contributors are listed below:

Quality of the raw material delivered at the mills: Dr. Paulo Graziano Magalhães – Feagri/Unicamp (speaker); *Dr. Oscar A. Braunbeck* – Feagri/Unicamp and *MSc. Humberto Carrara* – Usina São João (panelists).

Impacts of the quality of raw materials on the fermentation process: Dr. Márcia J. Rossini Mutton – Unesp (speaker); *Dr. André C. Vitti* – APTA (panelist).

Effects of inhibiting factors in the fermentation: Dr. Pedro de Oliva Neto – Unesp (speaker); *Dr. Carlos Eduardo Vaz Rossell* – CTBE and *Dr. Eloísa A. Moucheti Kronka* – Unaerp (panelists).

Fractionation of bagasse and straw objecting the new challenges to obtain ethanol: Dr. Adilson Roberto Gonçalves – EEL-USP (speaker); *Dr. Antônio Aprígio da S. Curvelo* – USP – São Carlos and *Dr. George Jackson de Moraes Rocha* – EEL-USP (panelists).

phases in order to prevent the deterioration of the cane.

- Logistics for the transport of straw to the plants.
- Introduction of technological innovation, designed to remove dirt and straw from the material to be processed.
- Evaluation of quality of sugarcane (juice) not only for the content of sugars, but also acids, polysaccharides, microbial contaminants and impurities (mineral and vegetal) by negative interference of the fermentation.
- Expansion, improvement, standardization/validation of the method quality assessment of raw material in distilleries and its implementation are essential to ensure the quality of the final product.
- If plants are equipped with more energy efficient systems, existing commercial-scale surplus bagasse will increase providing greater availability of energy generation, second generation ethanol or other compounds.
- Attention to the problems caused by the attack of pests which lead to the production of plant defense compounds, such as phenolics, which are toxic to yeast fermentation undermine efficiency.
- Cultivation of sugarcane on soils of questionable quality as sand can interfere with the quality of raw materials.
- The effects of stressors in the must, that are responsible for the inhibition of alcoholic fermentation, are due not only to the individual action of each inhibitor but also to a synergistic action between them which can only exacerbate these effects, the presence of organic acids combined with the high temperature of the must.
- The period of storage of the cane after the harvest is one factor that contributes to increase the population of microbial contaminants in raw materials.
- Development of technology to obtain natural products such as the inhibitors of bacterial contaminants of fermentation and biological control of insects.
- Getting thermophilic and osmophilic yeasts.
- Assessment of our microbial biodiversity.
- It is important not only knowledge of the problems related to the deterioration of the raw material for process control of ethanol production, but the adoption of measures to solve them.
- Research to take advantage of the CO_2 generated from fermentation.
- Control of both the raw material and the fermentation process as a preventive approach to minimize the problems of the sector.
- Establishment of efficient and low cost pretreatments is essential for the introduction of bagasse and straw in the production of second generation ethanol.
- Still can not be establish which is the best procedure for the fractionation of biomass bagasse and cane straw for the inclusion of these as raw materials in the production of ethanol, however, there is evidence for the choice of a combined process such as chemical and biological.
- The use of also sugars C5 constituents of the hemicellulose fraction of bagasse and straw must be taken into account for the increased production of ethanol and/or other products.
- It is important to think in the production of second generation ethanol from the integration of a module of hydrolysis of bagasse and/or cane straw with the processes of a traditional mill ethanol production of first generation.
- Required advances in research on the expansion of production scale second generation ethanol and the economic viability of the process.
- Introduction of the concept of biorefineries in the sugar and alcohol industries.
- Implementation of actions to increase financial input in strategic research and

incentives to drive further integration of research in different areas of knowledge.

- Diversification of raw materials and products generated from them will increase the competitiveness of the sector.
- Policies to address biofuels as commodities (futures markets).
- Overcoming natural, technical and economic barriers between the productive sector, academia and government to achieve the development of technologies for producing biofuels.