### DEVELOPING AN INTEGRATED AGRO-INDUSTRIAL MODEL FOR THE SUSTAINABLE PRODUCTION AND CONVERSION OF BIOMASS INTO BIOFUELS AND ADDED VALUE PRODUCTS

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## 1. Perspectives of a world-wide increased demand for automotive ethanol, possible consequences and perspectives

Ethanol used in flex-fuel cars or simply as an additive for gasoline constitutes one of the most promising possibilities of displacing fossil fuel resources. It is thus expected a great increase in international demand for this product and, in fact, several countries passed bills mandating an increasing production and consumption of ethanol for transportation fuel. World ethanol production experienced a three-fold increase between 2000 and 2007, from 17 billion to more than 52 billion liters. From 2007 to 2008, the share of ethanol in global gasoline type fuel use increased from 3.7% to 5.4%. In 2010 worldwide ethanol fuel production reached 76.7 billion liters, United States as the first producer with 21.5 billion liters or 26% of world market share. However, there has been serious debate on how effective bioethanol will be in replacing gasoline. Concerns about its production and use relate to increased food prices due to the large amount of arable land required for crops, as well as the energy and pollution balance of the whole cycle of ethanol production, especially from corn. Recent studies show that corn-ethanol produced in USA has a positive energy balance of approximately 4:3 (4 units of renewable energy require 3 units of fossil energy), while sugar-cane-ethanol produced in Brazil has an energy balance of about 9:1.

Within this context 2<sup>nd</sup> generation biofuels produced from lingo-cellulosic fibers, a major and universal component in plant cells walls, constitutes an attractive and promising alternative to increase ethanol production because it can coexist with food crops without competing with them. For instance, a maize producer can sell grains to an animal feed industry and use leafs, stalk, and cobs to produce cellulosic ethanol. A great variety of agricultural feedstock could also be used to produce it, such as sugarcane bagasse, miscanthus, switchgrass, eucalyptus etc., as well as many types of industrial wastes such as saw-dust, textile, paper, cardboard remains, etc. To better picture the potential of this idea, consider a typical Brazilian sugarcane conversion plant described by the following numbers:

- Plantation area
- Sugarcane processing rate
- Ethanol production (no sugar is produced)
- Sugar production (no ethanol is produced)
- Bagasse production
- Bioelectricity surplus (from bagasse)

30 kha 500 tsc/h (tons of sugarcane/hour) 45 m<sup>3</sup>/h (1<sup>st</sup> generation) 65 t/h 150 t/h of dry bagasse 50 MW

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Obviously these numbers may vary considerably, particularly the electricity surplus depending on the boiler's operating pressure and temperature. Instead of being transformed into electricity, the exceeding bagasse could be converted into  $2^{nd}$  generation ethanol at a maximum theoretical rate of approximately  $42 \text{ m}^3/\text{h}$ . Thus, it is possible to virtually double the ethanol production without necessity of increasing the total planted area.

Unfortunately, this is only wishful thinking because second-generation ethanol is still not economically viable. Despite the United States 2007 Energy Independence and Security Act mandating a total ethanol consumption of 136 billion liters by 2022, with an increasing share of cellulosic ethanol (1.9 billion liters by 2012 and 60.5 billion liters by 2022), the production of cellulosic ethanol is irrelevant in this country or in any other country as well. Such conversion route is restricted to research laboratories and small demonstration plants, the main roadblock to industrial-scale production being cost. There is a consensus among scientist, engineers and economists that the underlying reason for this is a generalized lack of scientific understanding, which hurdles the development of robust 2<sup>nd</sup> generation biofuels technologies which would be the base of cost competitive industrial processes. But these problems are also great opportunities, as it will be elaborated in the following.

#### 2. First and second generation ethanol production processes

The industrial production of ethanol is based almost exclusively on fermentation. This type of fermentation is a biological process, mediated by yeasts, that converts simple sugars into cellular energy, carbon dioxide. Its chemical reaction can be expressed by  $C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH +$  heat. Fermentation is a process that takes place only in anaerobic conditions and the energy contained in ethanol is still quite large, so it is normally associated with single-celled organisms that do not need much to survive. In aerobic conditions, organisms usually oxidize sugar (respiration) because this process releases much more energy, whose surplus is stored as fat. This metabolic pathway is frequently used by more complex and energy demanding organisms such as plants that store excess energy by building longer chains of simple sugars (polysaccharides) joined by glycosidic bonds, as shown in the **Figure 1**. The various types of starch constitute an excellent example because they are humanity's the main source of food.



Figure 1 – Formation of a disaccharide (lactose) through a glycosidic bond between two sugar monomers

The important point here is that recovery of energy through metabolization of a polysaccharide becomes progressively more difficult as its sugar chain increases in complexity. Therefore, to enable fermentation, it is necessary to release simple sugars from these complex molecules by

breaking down glycosidic bonds through some external action. In nature, several living organisms have developed strategies to use of this energy source, including the direct production of enzymes by fungi and bacteria, or a combination of acids and mechanical action in ruminants. That's why we cannot directly be fed with raw grains such as wheat, rice and beans, without first submitting them to any cooking process. In prehistory, human control of fire allowed a great diversity in our food sources and this is one of the main reasons for our success as a species.

Unlike starches, cellulose is a polysaccharide formed by the polymerization of tens of thousands of sugar monomers. It is mostly synthesized by green plants and totalizes to about 33% of the total plant biomass of the planet, reaching 90% in cotton and 50% in woody species.

Cellulose is the main component of plant cell walls giving them the ability to withstand external mechanical stresses (its own weight, wind, etc..) and osmotic pressure which acts as a selective barrier to help keeping water inside the cell. Its structure splits into layers and forming networks of cellulose microfibers entangled to hemicellulose and pectin filaments. The empty spaces are filled with an amorphous polymeric substance called lignin, which acts as a binder and provides resistance to compression. Such elaborate arrangement is highly optimized, it actually resembles quite closely reinforced concrete, and is most commonly found in woody plants that emerged more recently in nature (higher plants). This is why only a few microorganisms can feed from lignin: because they have not had time to develop specific enzymes for its degradation. Actually this is a very successful survival strategy of higher plants (they cannot run away from an attacking organism) and should be credited to the mechanisms of evolution and natural selection. All these characteristics are major obstacles to access simpler sugars for fermentation, a property known as recalcitrance. Therefore, the use of cellulose for ethanol production can only occur if its recalcitrance is overcome, that is, if its sugar monomers have been previously liberated for the fermentation process.

In general, untangling simple sugars for fermentation is better accomplished in a two stages process: 1) rupture of ligno-cellulosic macro-structures (pre-treatment) and, subsequently, 2) breaking of glycosidic bonds (hydrolysis). A research work conducted at the National Renewable Energy Laboratory by Lynd et al. (1996) [1] demonstrated that hydrolysis of the ligno-cellulosic material is inferior to 20%, while if preceded by one of the main pre-processing techniques it is possible to reach more than 90% of the theoretical limit. Several pre-treating techniques have been developed, particularly for materials such as wheat straw, corncobs, and various grasses. Traditional techniques include acid treatment and steam explosion with or without ammonia or organic solvents. More innovative techniques were developed based on the use of supercritical fluids, microwave irradiation, biological delignification, alkaline and ozone pre-treatment. All these techniques involve advantages and disadvantages which depend on operating conditions and the type of pretreated biomass. However, none of them is feasible for the Brazilian sugarcane industry in which, typically, hundreds of tons of biomass must be processed every hour. A very promising technique, being developed in some research centers in the world, including the Renewable Energy and Environment Pole at University of São Paulo - Brazil, is based on pressurized hot liquid water soaking followed by explosive depressurization.

Once the ligno-cellulosic macro-structures have been disrupted it is still necessary to depolymerize cellulose and hemicellulose into simple sugars that can be efficiently fermented. The breaking of the glycosidic bonds is achieved through a hydrolysis reaction, i.e., by reaction with water molecules. In the case of cellulose this reaction is called cellulolysis ( $[C_6H_{10}O_5]_n + nH_2O \rightarrow nC_6H_{12}O_6$ ) which, to occur efficiently, must be catalyzed by the action of some chemical adjuvant, usually an acid or an enzyme cocktail. The acid cellulolysis (or simply "acid hydrolysis" as it is known in industry's jargon) is a very traditional method and date of mid 1800 when the first techniques for producing ethanol were developed from waste wood. The Achilles heel of acid

hydrolysis is the need to neutralize and separate the acid catalyst and by-products, resulting in inevitable losses of fermentable sugars, as well as contamination in the fermentation process by inhibiting compounds. Another significant problem is the need for corrosion resistant materials such as stainless steel or special ceramic coatings, which increases greatly the cost of the required processing equipment. Although acid hydrolysis has a broad technical background acquired during a long history of successful industrial applications, applying it to the production of cellulosic ethanol suffers from the difficulty of incorporating technological advances that lead to increases in energy efficiency and cost, while minimizing or eliminating the generation of pollutant wastes.

Besides the use of acidic solutions, the rupture of glycosidic bonds can also be catalyzed by enzymes. Enzymatic hydrolysis is a natural process used by several species in the quest for energy. Ruminants and some types of termites can saccharify cellulose due to a symbiotic association with bacteria capable of producing cellulolytic enzymes, also known as cellulases. Similarly some types of fungi are capable of producing highly effective cellulolytic enzymes. For example, the fungus *Trichoderma reesei* produces a series of cellulases capable of hydrolyzing cellulose to a large amount of simple sugars. The fermentation of saccharified biomass by cellulolytic enzymes is greatly facilitated, especially because it is a sequence of reactions usually associated with the metabolism of living organisms. It is for this reason that the same mild temperature and pH conditions are suitable for enzyme production and for its effective action on cellulose, thus avoiding the formation of inhibitory compounds to cellulolysis or to fermentation. Consequently, the process equipment tends to be simpler and cheaper (no need for protection against corrosion and to withstand high temperatures) and also the production of environmentally harmful waste is minimal or easily treated.

However, in the current technological status, enzymatic cellulolysis is not capable of producing high concentrations of fermentable sugars in industrial-scale processes, thus being unsuited for commercial applications. Moreover, given the typical conditions of a sugarcane processing plant in Brazil, the cellulolysis of hundreds of tons of biomass per hour implies a rate of enzyme production far beyond the capacity of current bioreactors. However, these problems should be seen as two major areas of research and development, therefore, as strategic business opportunities to be explored. Although known for a long time, the industrial-scale application of enzymatic cellulolysis is quite new (just over fifteen years). For example, not only the enzymatic action on cellulose is poorly understood, but also effective enzyme cocktails tailor made for specific biomasses such as bagasse or miscanthus have not yet been developed. This can be achieved through the search for new enzymes as well as through genetic modification of known cellulases producing fungi. Molecular biology is thus a very promising research area, as well as the development of new generation bioreactors incorporating innovative technologies, particularly those based on Computational Fluid Dynamics and Artificial Intelligence Algorithms used to optimize design and operation of biological conversion processes.

#### 3. Sugarcane cultivation and its industrial processing in Brazil today

As already mentioned, production of 1<sup>st</sup> generation ethanol is a mature industrial activity in Brazil. Sugarcane occupies approximately 7 million hectares, or about 2% of all arable land in the country, and is concentrated on some areas of the Southeast and the Northeast, as shown in **Figure 2**. Current production meets national demand both in terms of ethanol and sugar. The sugarcane sector in Brazil can be better pictured by the following numbers (source: UNICA, www.unica.com.br):

- Annual gross earnings:
- Foreign revenue:
- Direct investments:
- Industrial processing units:
- Sugarcane growers:
- People directly employed:
- Participation in Brazilian energy matrix:
- Sugarcane production:
- Sugar production:
- Ethanol production:
- Bioelectricity generation:
- Bioelectricity generation potential:
- Avoided CO<sub>2</sub> emissions:

US\$ 23 billion US\$ 7.9 billion (2008) > US\$ 20 billion (2006-2009) 432 plants nationwide 70,000 845,000 16.4% (ahead of hydroelectricity) 562 million tsc (tons of sugarcane) 31.2 million tons 20.3 billion liters 2.1 GW (~3% energy matrix) 7 GW (Itaipu hydroelectric dam = 14 GW) 45 million tons since 2003



**Figure 2** – Sugarcane cultivation areas indicated in red corresponding to 0.9% of the territory (source: NIPE / Unicamp and IBGE)

Industrial processing of sugarcane in Brazil is made in small to medium industrial processing plants (compared with a petroleum refinery for example) spread along the cultivation areas. This is so because the cost of manipulating and transporting the crop from the fields to the processing plant is proportional to the cube of the average traveled distance (productivity  $\times$  area  $\times$  distance) and, since the revenue is proportional to the square of the distance (productivity  $\times$  area ), beyond a maximum size the economical viability of the overall process is compromised. Thus, investing in research and development of more efficient technologies in mechanization and transportation has a strategic importance to push further this maximum limit in order to enable benefits from economies of scale. It is also of crucial importance to invest on the development of innovative equipment and biomass

conversion routes, particularly aiming at obtaining new added value products such as bio-polymers and other green chemicals, in addition to contributing to environmental and social sustainability. To be able to elaborate on these topics it is necessary to describe in greater details the industrial conversion processes of sugarcane into sugar, ethanol and bioelectricity, as it is currently practiced in Brazil.

Once the sugarcane has been harvested and transported to the industrial processing plant, usually by semi-trailer trucks, it passes a quality control to assess the sucrose content and is washed, chopped and shredded through a series of mechanical pre-processing equipments. These operations enhance the subsequent extraction of sucrose by a set of mills (old but still widespread technology) or by a diffuser (more efficient and low cost operation). The efficiency of the extraction process is measured by the sucrose content of the collected juice, usually around 10-15%, and also by bagasse moisture content. A high moisture content compromises combustion efficiency because greater amounts of energy are absorbed to evaporate water contained in the bagasse, and, consequently, less heat is available to produce steam to the electricity generating turbines. After extraction, the sugarcane juice is chemically conditioned, filtered and pasteurized, depending on whether it will be used to produce sugar or ethanol.

The process of making sugar starts by concentrating the juice by evaporation of water in a multiple effect process. The resulting syrup is pumped into cooking vats. The product of that cooking is a mixture of sucrose crystals dispersed in syrup also called "honey". The separation of sucrose crystals is made by centrifugation which produces sugar and molasses. From this point, the sugar refining process continues by steam washing and drying to produce different grades of sugar. Molasses can be destined for consumption due to its high nutritional value, or, because it is still fermentable, it can be used to produce ethanol, usually by mixing it with untreated sugarcane juice.

The first step in the production of ethanol is pre-conditioning the juice, which involves heating it up to  $105^{\circ}$ C without addition of chemicals. After decantation, the clarified juice is reduced at  $115^{\circ}$ C to increase the sucrose concentration to around  $20^{\circ}$ Brix, and also to sterilize wild bacteria and yeasts that otherwise would compete with the proper fermentation yeast. The hot must is then cooled to  $30^{\circ}$ C and sent to fermentation, which can be accomplished in batches or as a in-line process through continuous fermenters. Carbon dioxide and energy are released in the process of transforming sugars into ethanol. Condensers are used to recover ethanol contained in the CO<sub>2</sub> flux and heat exchangers are used to control the process temperature, generally between 28 to  $30^{\circ}$ C, to maintain ideal fermentation conditions. The overall conversion process may take 6 to 8 hours. The fermented must (wine), containing approximately 9.5% ethanol, is centrifuged to recover the fermentation yeasts and then distilled to obtain hydrous ethanol, i.e. containing about 5% of water, which can be used as automotive fuel. A byproduct of distillation is vinasse, mostly composed of water, organic matter and nutrients, thus being adequate for irrigation for some time.

Several other products can be obtained from sugarcane, such as bioplastics (PHB, polyethylene, PVC), biodiesel and biogasoline (butanol), although the production of these compounds are still more expensive than the corresponding ones obtained from fossil resources. Sugar, ethanol and bioelectricity remain the most important and cost competitive products.

# 4. An evolved agro-industrial model for sugarcane conversion: integrating 1<sup>st</sup> and 2<sup>nd</sup> generation ethanol and enhancing sustainability

The average size of a sugarcane cultivation area is approximately 30 kha, which is determined basically by soil manipulation, harvesting and transportation costs as already mentioned. In fact, 70% of the plantations vary between 20 to 40 kha as it can be seen in the histogram shown in **Figure 3**.



**Figure 3** – Frequency distribution of sugarcane cultivation areas in the state of São Paulo – Brazil; average size = 27,34 kha (source: UNICA, www.unica.com.br)

The average productivity in the state of São Paulo, Brazil, is of 85 tsc/ha. Thus, adopting a harvesting period of 210 days, the resulting round-the-clock biomass processing flux will be about 500 tsc/h. The equipment necessary to process this biomass flux, according to the operations described above, will cost close to US\$ 150 million and will be able to produce approximately 65 t/h of refined sugar or 43  $m^3$ /h of hydrous ethanol during the harvesting period. In the process, after separation of straw and extraction of sucrose, 130 t/h of bagasse will be generated (50% moisture content) from which electricity can be produced at the rate of 50 MW, although this number can vary significantly depending on the moisture content and on boiler pressure. More specifically, bioelectricity generation is based on cogeneration steam cycles at pressures around 2.2 MPa, from which it is possible to attend internal energy demands and still produce small amounts of bagasse (5-10% of biomass) and electricity surpluses (0-10kWh/tsc). Recently, more elaborated engineering design techniques, new materials and more skilled fabrication technology enabled the development of more efficient and better integrated processes equipped with highpressure steam systems (e.g. 6.5 MPa @ 480°C; some units with 12.0 MPa @ 540°C) capable of generating over 100 kWh/tsc (Seabra and Macedo, 2011). Residues are also generated in the process, the most important ones being 1 t/h of filter cake from filtration of sugarcane juice, 2 t/h of  $CO_2$  from fermentation and 500 m<sup>3</sup>/h of vinasse. These numbers characterize a typical sugarcane industrial processing plant in São Paulo - Brazil, and will be used as a reference agro-industrial production and conversion model.

A very important sustainability issue regarding this consolidated model is the amount of vinasse generated in the process. Actually, any large scale production of biofuel based on fermentation followed by distillation will experience similar problems. Characteristics of sugarcane vinasse are strongly influenced by the must which depends on the proportion between molasses and juice before fermentation. Its composition for industrial plant producing exclusively ethanol is 97% of water, 2.3% of organic matter and nutrients, N = 0.028%, P<sub>2</sub>O<sub>5</sub> = 0.020% and K<sub>2</sub>O = 0.147%, on mass basis. Thus, the generation of 500 m<sup>3</sup>/h of vinasse implies the removal of approximately 1000 kg/h of NPK from the fields and it is quite clear that the fertility of the soil will be seriously compromised if nutrients are not replaced somehow. The common practice in Brazil is to pump or to transport it back to the fields and apply it to the soil with the help of trailed spraying systems. There are several problems related to this operation. First, it is a highly uneconomical operation since to apply 1 ton of NPK to the soil it is necessary to transport and distribute nearly 500 tons of vinasse containing 485 tons of water. Second, its high organic matter content implies a high biochemical oxygen demand which can strongly impact the soil, groundwater, or nearby watercourses or lakes. Third, it may be necessary to correct vinasse acidity (pH ranges from 3.7 to

4.4) and/or to biodigest it to decrease the organic matter content in order to reduce impacts on soil microorganisms. Fourth, application of vinasse has a significant effect on soil mechanical properties, particularly on permeability favoring soil compaction. Altogether, due to the fact that there are many types of vinasse and myriads of different soils, research results are inconclusive or refer to particular situations and application of vinasse as a procedure of fertilization/irrigation is still a debatable practice.

Other important sustainability issue is related to the overall energy balance. Although sugarcane ethanol production in Brazil is very well placed when compared with ethanol production from other feedstock, it is our opinion that there is still a lot to be done because 9:1 (9 units of renewable energy requiring 1 unit of fossil energy) is simply not enough in the long term. Opportunities for enhancing the energy balance are related to soil manipulation, crop harvesting and transportation operations in the fields as shown by Seabra and Macedo (2010) [5], mostly because the efficiency of Otto cycle engines decreases as its size increases and these operations have to be based on Diesel cycle engines. Small amounts of biodiesel can be produced from oilseeds used in sugarcane rotation which can be done after 3-6 crops. A promising approach to improving the overall energy balance is to produce microalgae biodiesel from vinasse (rich in nutrients) and CO<sub>2</sub> emissions, with additional obvious advantages.

Microalgae can be viewed as photosynthetic microorganisms that use solar energy to convert carbon dioxide and nutrients into biomass. Depending on cultivation conditions, microalgae oil contents range between 40 to 70% by weight of dry mass, some exceed 80% (Metting, 1996, [2]; Spolaore et al., 2006, [8]), and biomass doubling time during exponential growth are commonly short as 3.5 h. These numbers can be put into perspective by comparison with the productivity obtained from vegetable oil crops on a "per hectare" basis. Considering experimentally demonstrated algae productivity in photobioreactors (small scale), common oil yields range from 58,000 L/ha to 136,900 L/ha which is two or three orders of magnitude higher then palm oil (5950 L/ha) and soybean (446 L/ha) respectively.

In fact, extracting algal oil and converting it into biodiesel is not a new idea and several research programs were brought about in the world, dating back to mid 70's. One of particular interest is the U.S. Department of Energy's Office of Fuels Development program to renewable transportation fuels from algae, which was active from 1978 to 1996, and resulted in significant advances (Sheehan, 1994, [7]). However, from our perspective, algae's ability to produce oil is less important than their ability to recycle nutrients because soil depletion caused by intensive farming for energy production purposes is a major sustainability concern.

The catch is that large scale industrial microalgae biomass production is not economically viable due to technological limitations. Additionally, the vast majority of research and development activities were focused on obtaining oil and not on recycling nutrients. The few attempts to develop an industrial scale process failed to meet expectations and were abandoned (Miron *et al.*, 1999, [3]) and the main reason for this is that easily controllable problems at small scale become critical at large scale. Some of these problems are: inadequate irradiance levels and light cycles, deficient photosynthetic  $O_2$  removal, depletion of  $CO_2$ , inadequate temperature control and contamination by wild microalgae and protozoan species. All these factors combined limit the maximum algal concentration to less than 1 kg/m<sup>3</sup>, which is insufficient for a large scale production. This can be illustrated by sizing an open raceway pond (cheap and simple operation) to cultivate *Chlorella vulgaris*, which is a common choice because of its high photosynthetic efficiency (~8%). Constitution and other characterization parameters of *C. vulgaris* are given in **Table 1**.

Parameter	normal	low N
protein (g/kg)	282	67
lipid (g/kg)	175	385
carbohydrates (g/kg)	495	529
lower heating value (MJ/kg)	17.5	22.6
C (g/kg)	480	538
N (g/kg)	46	10.9
P (g/kg)	9.9	2.4
K (g/kg)	8.2	2
Mg (g/kg)	3.8	0.9
S (g/kg)	2.2	0.5
$CO_2$ (kg/kg)	1.8	2.0
grouwth rate (1/day)	0.99	0.77
productivity (g/m <sup>2</sup> /day)	24.75	19.25

Table 1: Characterization parameters of Chlorella vulgaris (Oh-Hama and Miyachi, 1988, [4])

Supplying enough nitrogen, to favor algal biomass production and nutrients recovery in detriment of lipid content, and considering the reference agro-industrial production and conversion model described above, mass and energy balance equations produce the geometric and operating parameters shown in **Table 2**. It is clear that the required areas are excessively large in addition to raising several issues such as prohibitive earthmoving costs, critical contamination and temperature control, etc. The solution to this is to obtain a significant increase in algal concentration by understanding and engineering the interdependence between light propagation/absorption, three phase fluid flow patterns and algal growth in large scale photobioreactors, i.e. by solving the photobio-fluid dynamic problem.

**Table 2**: Geometric and operating parameters of a raceway pond used to cultivate *C. vulgaris* to recycle nutrients from vinasse from a typical sugarcane processing plant.

	vinasse @ 500 m <sup>3</sup> /h			
parameter	Nitrogen (N)	Phosphorus (P <sub>2</sub> O <sub>5</sub> )	Potassium (K <sub>2</sub> O)	
nutrient flow rate	140 kg/h	100 kg/h	735 kg/h	
elemental flow rate	140 kg/h	43.7 kg/h	610 kg/h	
microalgae production	3043 kg/h	4414 kg/h	74378 kg/h	
raceway pond area	300 ha	400 ha	7400 ha	

The purpose of highlighting these problems is not to discourage, but rather to identify opportunities and stimulate research and development activities which will enable the development of innovative and possibly disruptive technologies for advanced biomass production and conversion into biofuels and added value products. A schematic representation of the proposed integrated model, instantiated for a typical sugarcane industrial processing plant in São Paulo – Brazil, is shown in **Figure 4**.



**Figure 4**: A schematic diagram of the proposed integrated model, representing a typical sugarcane industrial processing plant in São Paulo – Brazil, including 2<sup>nd</sup> generation ethanol production and nutrients recovery through microalgae cultivation

#### 5. Conclusions and perspectives

The perspective of a world-wide increased demand for transportation energy puts a lot of pressure on scientists and engineers to develop efficient, robust and competitive industrial processes for the large scale production of biofuels, among which bioethanol is probably the most important one. Major sustainability issues have already been raised and relate mainly to the overall energy balance and soil depletion due to intensive farming, in addition to impacts on food prices. Second generation ethanol produced by saccharifying lingo-cellulosic biomass obtained from specific crops (eucalyptus, miscanthus, etc.) or from agricultural or industrial wastes (corncobs, straw, etc.) constitutes a very promising conversion route since it can literally coexist without competing with food crops. For example, there are interesting research work on intercropping corn with different species of *Brachiaria* with an increased overall photosynthetic efficiency, the first being a source of food starch, the second constituting the primary source of cellulose for conversion into biofuel.

Sugarcane has one of the highest photosynthetic efficiencies in the plant kingdom, being able to convert around 1% of incident solar energy into biomass with a high sucrose content, which is very easily fermented and converted into bioethanol. Its large scale cultivation is a well established agro-industrial business in Brazil, particularly after the development of flex-fuel technologies, which freed the consumers from their captive condition by enabling the choice of any proportion of gasoline/ethanol depending on market prices. Also contributed to this picture a combination of simultaneous favorable conditions, such as a good incidence of sunlight, amenable temperatures, regular and adequate rain pattern, availability of arable land, fertile soils, long term research efforts to develop hybrids adapted to specific conditions, etc. Despite a highly favorable overall energy balance when compared with other biomass feedstock, there is still a lot to be done to improve conversion efficiency, to minimize GHG emissions, to preserve soil fertility, aquatic resources and so forth. It is also necessary to increase processing flow rates beyond 1000 tsc/h to enable benefits from economies of scale. An integrated agro-industrial model for the sustainable production and

conversion of biomass into biofuels and added value products has been proposed. The instantiation of this model to a typical (reference) sugarcane industrial plant reveals several obstacles and bottlenecks to its implementation, mainly due to lack of scientific knowledge and absence of robust and cost-competitive large scale processing technologies related to 2<sup>nd</sup> generation ethanol and high concentrations microalgae cultivation.

To overcome these difficulties a large spectrum of expertise needs to be articulated, going from genetics, biomolecular sciences and cell biology to engineering design and optimization, industrial processing and sustainability assessing. Specific research topics and actions are: 1) understanding the structure and composition of ligno-cellulosic biomass, 2) development of modern plant genomics and genetic transformations to enable advanced studies of photosynthesis and metabolomics aimed at increasing cell conversion efficiency, 3) definition of sustainable agricultural practices with special focus on water and soil preservation, energy and feedstock requirements and logistics, 4) identification of new biochemical conversion routes, which involve enzyme structural biology and protein engineering, with special relevance to biofuels and added value biochemicals, 5) development of innovative industrial scale processes and equipment for costcompetitive biomass transformation and 6) construction of tools for assessing economic viability, ecological footprint and social impacts of these new technologies. To meet these expectations it will be necessary to orchestrate induced and exploratory research, the first being responsible for scientific discoveries which are the basis of disruptive technologies enabling large evolutionary leaps, the second promoting its consolidation through a series of incremental steps resulting from experimentation, refinement, and increasingly realistic testing.

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