

---

# **Second Generation Ethanol from Residual Biomass: Research and Perspectives in Ecuador**

---

Enrique Javier Carvajal Barriga, Cristina Guamán-Burneo, Patricia Portero Barahona, Edgar Salas, Carolina Tufiño, Bernardo Bastidas

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/51951>

---

## **1. Introduction**

Ecuador is located between 1°N and 5°S on the west coast of South America. Although relatively small in size, mainland Ecuador can be subdivided nevertheless into three different and quite distinctive climatic regions: the Pacific coastal plain, the Andean highlands and the Amazon basin. In addition, Ecuador possesses a fourth region, namely the Galapagos Islands.

Climatically, the Pacific coastal plain is hot all year, with a rainy season between December and May. In the Andean highlands, the climate is markedly cooler, varying according to altitude. In contrast, the Amazon basin is hot, humid and wet all year round, while the Galapagos Islands are dry, with an annual average temperature of 25° C (77° F).

These characteristics provide Ecuador with a huge potential to develop second generation ethanol from industrial biomass, to replace a portion of the gasoline needed and, thus, the reduction of CO<sub>2</sub> emissions. The climatic conditions as well as the photoperiods and rainfall along the year make this country an excellent candidate to develop second generation biofuels technology from biomass.

Tropical cultures such as bananas, oil palm, sugar cane, and others that are produced mainly in the coastal region of the country generates each year enough cellulose to produce almost all the ethanol the country needs. The current situation in terms of the use of these lignocellulosic materials is still in its very beginning and much work is to be developed to establish a market for the lignocellulosic residues.

Additionally, microbial biodiversity and its research is becoming one important issue in terms of the development of innovative technologies based on biotechnology, pointing out

the search for novel genes and metabolic abilities especially in wild yeasts studied in natural environments all around the country. Local researchers are devoted to the metabolic engineering of yeasts to improve the fermentation yields.

In this chapter we report some results from the or Sustainable Resources for Ethanol (RESETA) project, from the quantification and characterization of the most important cultures in Ecuador, its residues and characteristics, to the development of genetic engineered yeasts and the design and construction of a biorefinery at pilot scale.

The above mentioned project involves one of the most important researches the Ecuadorian Government has founded since 2008. The advances and results of this project can be taken as models for other tropical countries in the world.

Finally, we present the economic viability analysis of second generation ethanol projects in large scale in Ecuador, looking forward the industrial production of ethanol, biogas, biofertilizers and renewable chemicals in biorefineries in Ecuador.

## **2. Biomass: A qualitative and quantitative approach to the concept**

The concept of biomass is largely extended among the bioenergy, agricultural, biotechnology and other specialists. When a farmer talks about biomass, he or she is referring to the foliage, fruits, grains, stems or waste materials produced in crops.

In the animal husbandry activities, biomass is referred to the manure and purines excreted by farm animals. On the other hand, talking about cell's biotechnology, biomass is referred to the cell production in a culture: biomass of yeast produced during fermentative processes.

In other terms, biomass constitutes a broad range of biological matter including the vegetal coverage of the planet, the micro and macro organisms living on the planet, including humankind.

Displaying in this manner the concept of biomass, allows the broadening of its quantitative and qualitative applications and uses. This way of thinking on biomass plays with the absolute and relative values of the concept and may certainly boost the current scheme of exploitation of biomass sources.

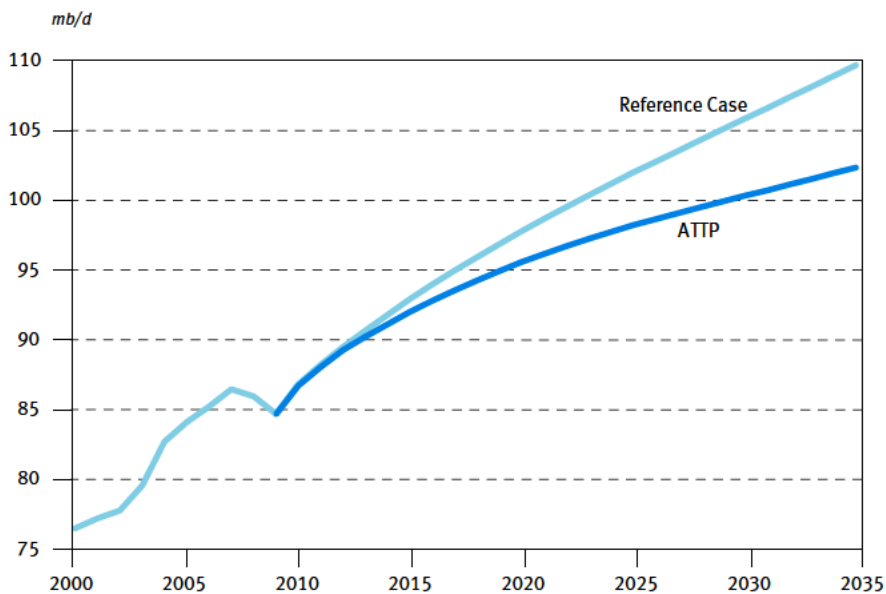
## **3. The cellulosic biomass potential worldwide**

Cellulose is the most abundant carbohydrate polymer in the planet. This remarkable molecule is composed by monomers of glucose linked up by glycosidic ( $\beta$  1–4) boundaries that provides this molecule with the capacity of forming linear, fibrous shapes of straight chains. These simple chains are joined by hydrogen bonds, provoking the formation of resistant and strong fibers which are the main components of plant cell walls.

Cellulose, itself is not only a strong, flexible and resistant fiber, but also insoluble and hard to be decomposed molecule. Nevertheless, fungal and bacterial micro flora which is

abundant in nature has developed certain enzymes groups that allow them to decompose the glycosidic links and use the released glucose contained in cellulose. Thus, cellulose is recycled very efficiently in nature, sustaining the carbon equilibrium in biosphere, which relies fundamentally in anabolic and catabolic cycles of cellulose, where microbes play a main role.

Cellulose is formed during photosynthesis, where  $\text{CO}_2$  and the energy from the sun are taken by plant cells, where this transformation takes place. Cellulose naturally is a structural molecule synthesized by plants to allow them to grow. Glucose is also a fuel molecule in as much as the bonds are in the  $\alpha$  configuration, thus forming fuel reserve molecules such as amylose and amylopectin, both molecules components of starch.



**Figure 1.** The projection for the crude oil needs of the OPEC (Organization of Petroleum Exporters Countries) in the ATTP scenario (Accelerated Transportation Technology and Policy) for 2035. [2]

Cellulose recycling in nature is in the order of  $10^{15}$  kg per year [1]. This number is so high that we could make enough fuel ethanol to supply 100 times the energy requirements of the entire world in a rampant development scenario projection for 2035 [2]. In other words, we may probably need “only” 1 per cent of the cellulose synthesized by plants in one year, so we can have enough liquid fuel to run our vehicles and industries. In this calculation, it is not taken into account the biogas (to run power plants) and biofertilizers (to return minerals to soil) production if all the ethanol from cellulose were produced within biorefineries. If humankind had the technology, cultural and economic conditions to efficiently pick this cellulose up, we may probably reach the sustainable and clean environmental goals for future. Nevertheless, this utopia is not yet possible in current historical conditions, so we

need to focus on much less ambitious objectives, such as the recovery and technological transformation of agricultural feedstocks and industrial leftovers. Figure 1 shows the crude oil we'll need in 2035. The amount is very impressive, that is 110 million barrels crude per day to keep running our industrial and mobilization needs.

Before the worldwide petroleum reserves are depleted, humankind must change the energetic matrix based on oil and look for sustainable and clean sources to produce fuels. An analysis made by Bruce Dale from the Department of Chemical Engineering and Materials Science at Michigan State University (USA), show a clear disadvantage in terms of energy input of gasoline production compared to first and second generation ethanol (Table 1). In this scenario, the issues are not necessarily lying on the economic or technical feasibility of the conversion processes, but in political and ethical issues.

	petroleum	natural gas	coal	other	total	GHG emissions
<b>gasoline</b>	1.10	0.03	0.05	0.01	1.19	94
<b>ethanol today</b>	0.04	0.28	0.41	0.04	0.77	77
<b>cellulosic ethanol</b>	0.08	0.02	0.02 *	0.02	0.10	11

**Table 1.** Energy Inputs of various energy carriers in MJ per MJ of fuel produced and Greenhouse Gas (GHG). Outputs in kg of carbon dioxide equivalents per MJ fuel produced for various fuels. \*Credit for coal not consumed due to process residues being burned to provide heat [3]

The energy input to produce gasoline is 0.19 times higher than the energy harvested; the GHG emission are the highest (94 kg/MJ). The cellulosic ethanol case exhibits a much higher energy output than the energy required to produce it. Moreover, GHG emissions are much lower than gasoline case (11 kg/MJ). In the case of corn ethanol the energy balance is still positive even though the margin remains narrow.

### 3.1. Is there enough biomass to replace oil?

There is a tendency to believe that there is no enough biomass in the world to replace fossil fuels that nowadays fulfill our energetic necessities. Taking into account that in the planet every year about  $10^{15}$  kg of cellulose are naturally recycled, we can do a simple calculation to better understand the real potential of biomass in a hypothetical scenario where we can transform this cellulose material into second generation ethanol.

That huge amount of cellulose is understood that is produced in forests, seas, rivers and crops all around the planet. In this calculation it is not considered the economic feasibility of getting that cellulose; nevertheless it can illuminate a possible future scenario.

In Table 2 it is shown a calculation of the crude necessities for 2035

Talking in terms of volume may allow us to understand the ethanol potential production from cellulose, but it does not explain the energetic issues concerning to both energy carriers when being compared. Ethanol is a fuel that possesses less calorific energy per kilogram than crude oil. If we want to calculate the amount of cellulosic fuel ethanol needed to replace oil it is necessary to take a look at the equivalences.

CRUDE OIL			CELLULOSIC ETHANOL			
Crude necessities mb/d (data OPEC)	L crude/day 2035 (1 bbl. Crude =158,98 liters)	Liters crude oil/year (2035)	Kg cellulose recycled/ year	Enzymatic cellulose hydrolysis (Yield~70%)	Conversion to ethanol (0,45kg ethanol/kg glucose)	Liters ethanol/year (ethanol density 0,789Kg/l)
110	1,75x10 <sup>10</sup>	<b>6,38x10<sup>12</sup></b>	1x10 <sup>15</sup>	7x10 <sup>14</sup>	3,15x10 <sup>14</sup>	<b>3,99x10<sup>14</sup></b>

**Table 2.** The volume of crude oil needs projected to 2035 vs. the potential cellulosic ethanol from cellulose annual production on Earth. Bold numbers show that the cellulosic ethanol potential volume is 100 times more than the oil necessities.

Crude Oil calorific value is about 40,000 MJ/Kg., while fuel ethanol's calorific value is about 28,800 MJ/Kg. Then, we can consider that one Kg. of ethanol is equivalent to 0.72 Kg of crude oil. In other words, crude oil's calorific power is about 1.4 times higher than fuel ethanol.

In Table 2, it is important to notice that the calculation intends to show that in nature there is enough cellulose to fulfill the necessities of energy to replace oil (at least in theory), but not the energy contained in both fuels; the cellulosic ethanol production from such a huge amount of cellulose may overwhelm in two orders of magnitude the figure of the crude oil requirements by 2035.

As a resulting corollary of this analysis, it is possible to infer that the need of cellulose produced naturally on Earth in order to replace crude oil may be only 1% of its total weight. However, it still represents an immense amount of cellulose to be collected and technologically processed in an efficient and financially feasible way (i.e. ~10 billion MT of pure cellulose/year).

In this scenario, the easiest and more efficient way to start producing second generation ethanol is by utilizing the residual lignocellulosic feedstocks such as by-products from agriculture or industrial activities.

In this analysis, we have taken into account only cellulose—a glucose polymer—to be converted into fuel ethanol by alcoholic fermentation. Nonetheless, most of cellulose in nature is associated with hemicellulose and lignin. Hemicellulose is mainly composed by pentoses such as xylose and arabinose, while lignin is composed mainly by aromatic compounds.

#### 4. Availability of residual biomass in Ecuador

Ecuador is a biodiverse country with rich and fertile natural regions. In the coastal zone of Ecuador there is the large scale agriculture of a wide variety of crops which have positioned this country as one of the most important producers of bananas, palmito (palm heart), oil palm and other valuable products in South America. Moreover, Ecuador has unique vegetal species that are being exploited in small scale, presenting novel and potential sources of lignocellulose for the future.

In terms of abundance of lignocellulosic residues, the most conspicuous industries producing leftovers—as a consequence of the harvest or the extraction of valuable commodities—are the bananas farms, and the oil palm and sugar cane mills. There are still other important industries located mainly in the highlands such as flowers and cereals that produce lignocellulosic material potentially usable. Nevertheless, the amounts of these residues are not enough for huge biorefining installations, nor even available in an economical and technical way.

As for the availability of residues, studies carried out by researchers from the Neotropical Center for the Biomass Research at the Pontificia Universidad Católica del Ecuador, reveal that there is a very high potential for lignocellulosic ethanol and biorefineries setting up in Ecuador. Nevertheless, there still exist constraints due to the disperse areas where the agricultural and industrial lignocellulosic materials are disposed; the local roads infrastructure and networks; the lack of development of markets for certain specific residues; the traditional uses and ways of final disposal; the physical and chemical composition of residues; and, the prices per dry ton. There are also social and environmental components to be taken into account when projecting lignocellulosic biorefineries to take the most of the agricultural and industrial byproducts, leftovers or residual material. In our survey we have considered the above-mentioned factors to develop the feasibility study for a biorefinery based on local lignocellulosic residues in the country.

In this survey we have pursued the following general objectives:

1. Evaluate the abundance and the potential of the main crops produced in Ecuador.
2. Determine the utilization, destiny, and availability of the agricultural residues.
3. Estimate the evolution of the agricultural production and residues generation in 5 years (until 2014).

Moreover, we have focused the following specific goals:

1. Determine the main crops in Ecuador, its exact geographical location, and the quantity of biomass residues produced per year.
2. Establish the temporality of crops and harvest.
3. Take current and historical data on volume of waste biomass produced to project future volumes, considering a period of five years. Analyze the succession of crops
4. Determine on the basis of the previous information, the more adequate zones where to install a future biorefinery plant.

In Ecuador there are three crops that worth to be studied with biorefining ends, because of their characteristics in terms of composition, final disposal, abundance and lack of sustainable use. These crops are: bananas, sugar cane and oil palm.

Table 3 shows the complete results of our survey on 13 different crops in Ecuador, the calculation of its dry mass and cellulose average contents as well as the potential for ethanol production. As it is going to be seen, Ecuador potentially could provide at least half of the ethanol needs for replacing gasoline in vehicles if the cellulose contained in agricultural residues were transformed into ethanol.

This suggests that biorefinery plants can be a reasonable and sustainable option for the post oil economy in Ecuador. Moreover, there still exists a huge potential for power generation if biogas from stills and residual lignin are burned in biorefineries.

There still exist other valuable products from biorefining of second generation ethanol such that can be produced from the residual water generated after distillation which, after an anaerobic digestion process, yields biogas, liquid and solid fertilizers (sludge). The non-hydrolyzed fibers as well as yeast biomass obtained after fermentation can be dried and sold as animal feed solid matter. The solid matter that can be recovered from fermenters before distillation is really considerable. Moreover, carbon dioxide from fermentation can be collected and treated to be sold in as much as during the fermentation for ethanol production, almost the same amount of CO<sub>2</sub> is released. Theoretically, the production ratio of ethanol to CO<sub>2</sub> in fermentation is 92:88. The uses for this gas are very wide including food, drink and chemical industries. CO<sub>2</sub> is widely used in soft drinks and beer to carbonation of these beverages. It is also used to fill packs of vegetables and meat to keep it fresh. CO<sub>2</sub> can also be used as raw material for the synthesis of methanol, formic acid, and urea. Other applications of CO<sub>2</sub> include its use as a medium in supercritical CO<sub>2</sub> extraction and in fire extinguishing equipment [4].

<b>POTENTIAL OF SECOND GENERATION ETHANOL PRODUCTION FROM AGRICULTURAL RESIDUES IN ECUADOR</b>					
<b>Residues by Product</b>	<b>Dry weight (MT/year)</b>	<b>Average cellulose content (MT/year)</b>	<b>Theoretical potential ethanol (Gal)</b>	<b>Potentially supplied vehicles/year</b>	<b>Percent of potentially supplied vehicles per year (Total number of cars: 1.4 MM to 2014)</b>
Soy bean	19,873	7,949	1'510,192	3,020	0,2
Palmito	24,285	9,714	1'845,509	3,691	0,3
Flowers	29,259	11,704	2'223,489	4,447	0,3
Potatoes	66,790	26,716	5'075,609	10,151	1
Rice	90,742	36,297	6'895,808	13,792	1
Plantain	138,787	55,515	10'546,915	21,094	2
Soft corn	288,340	115,336	21'911,914	43,824	3
Sugar cane	327,422	130,969	24'881,855	49,764	4
Cocoa	343,249	137,300	26'084,624	52,169	4
Bananas	351,031	140,412	26'675,973	53,352	4
Dry corn	447,365	178,946	33'996,714	67,993	5
Coffee	568,736	227,494	43'220,137	86,440	6
Oil palm	2'071,995	828,798	157'457,762	314,916	22
<b>TOTALS</b>	<b>4'767,873</b>	<b>1'907,149</b>	<b>362'326,502</b>	<b>724,653</b>	<b>51,8</b>

**Table 3.** The hypothetical potential of lignocellulosic biomass in Ecuador to produce cellulosic ethanol

## 5. Second generation ethanol from residual feed stocks generated in industrial activities

### 5.1. Xylose fermenting yeast in natural environments of Ecuador

Lignocellulosic biomass is composed by mainly three different fractions of molecules: cellulose, hemicellulose and lignin. We have already talked about some applications for cellulose and lignin, mainly as ethanol and fuel biomass. Nevertheless, hemicellulose which is mainly composed of xylose, a five carbon sugar, is a very important and abundant source that accounts for 23% to 32% of the dry lignocellulosic biomass weight. This sugar can also be used for the production of ethanol as well as other valuable products.

As part of a survey in biodiversity in Ecuador, the CLQCA or Catholic University Yeast Collection has collected some isolates of yeast that exhibit fermentation skills when xylose and a Nitrogen base are mixed up in culture broths.

Xylose fermenting yeasts have been collected from different provinces of Ecuador, including the Galapagos Islands and the Amazonia. Nevertheless, none of these yeast isolates present high ethanol tolerance nor quick fermentation rates, which make these organisms not suitable for industrial processes. It can probably discourage someone to study natural occurring xylose fermenting yeasts, nonetheless, the genes involved in this physiological processes are still useful for metabolic engineering approaches.

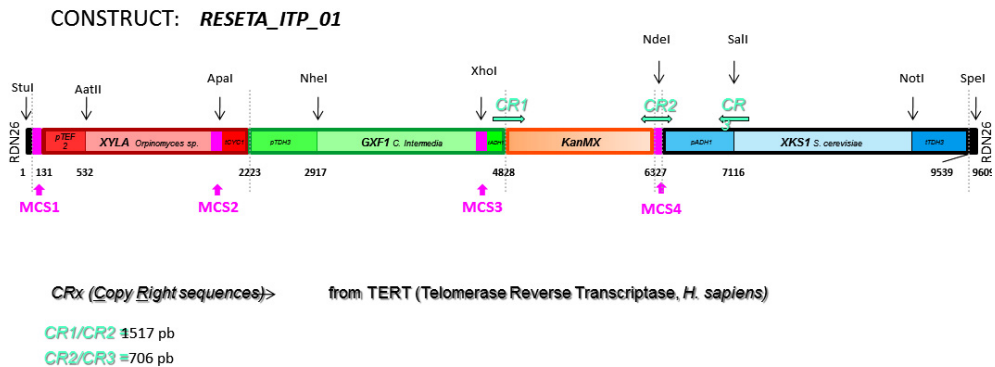
ISOLATE CODE	YEST SPECIES	SUSTRATE	XYLOSE ASSIMILATION	XYLOSE FERMENTATION
CLQCA-24SC-002	<i>Yamadazyma mexicana</i>	<i>Inga Vera</i> (MUCILAGE)	S	W
CLQCA-24SC-016	<i>Yamadazyma mexicana</i>	<i>Bursera graveolens</i> (EXUDE)	W	W
CLQCA-24SC-312	<i>Galactomyces geotrichum</i>	<i>Scalesia</i> sp. (ROTTEN WOOD)	+	W
CLQCA-24SC-320	<i>Scheffersomyces stipitis</i>	<i>Scalesia</i> sp. (ROTTEN WOOD)	+	+
CLQCA-24SC-321	<i>Scheffersomyces stipitis</i>	<i>Scalesia</i> sp. (ROTTEN WOOD)	+	+

**Table 4.** Xylose fermenting yeast isolates collected in Ecuador (Galapagos Islands) and deposited at the Catholic University Yeast Collection (CLQCA). S: slow positive, W: weakly positive, +: positive.

As seen on table 4, only two strains of *Scheffersomyces (Pichia) stipitis* are positive to ferment xylose. This yeast species has been reported to ferment xylose as will be seen further in this chapter.

In terms of ethanol production, there have been a lot of different approaches; in the last times the metabolic engineering of *Saccharomyces cerevisiae* was regarded as a suitable solution to ferment xylose, arabinose and other non-conventional sugars.

One example of this line of research is to be shown in the following scheme, where there are three different genes in charge of the transport, isomerization and phosphorylation in the process to ferment xylose. This construct has been designed at the Neotropical Center for the Biomass Research as part of the RESETA project. This genetic tool is very versatile and has been thought to be used as a genetic platform where to assay a wide variety of genes. The design of this construct was made by Carvajal et al. in 2011.



**Figure 2.** RESETA ITP 01 Construct. Composed by a XYLA gene from *Orpinomyces* sp.; GXF1, from *Candida intermedia*; and, XKS1, from *Saccharomyces cerevisiae*. As a resistant marker it has been used the KanMX4 gene which provides resistance against Geneticin.

This genetic construction is being tested in laboratory conditions, integrated in industrial *Saccharomyces cerevisiae*. This first step forward to the metabolic engineering is expected to give new perspectives to the residual biomass transformation into valuable products in the context of biorefineries in Ecuador and worldwide.

## 5.2. Xylose to Xylitol fermentation

Xylose to ethanol conversion in nature is been done by a few organisms such as yeasts like *Scheffersomyces stipitis*, an ascomycetous yeast that has been extensively investigated for the fermentation of xylose to ethanol, L-lactic acid for its further polymerization into PLA (Poly Lactic Acid) and other products from hemicellulose, the second abundant component of cellulosic biomass [5, 6, 7]. Laboratory strains of *Schef. stipitis*, which are amenable to genetic and physiological manipulation, have been developed by metabolic engineering for xylose utilization [5, 7, 8]. The genome sequence was recently obtained for this yeast and will provide a valuable resource for enhancement of xylose utilization and other industrial attributes by *Schef. stipites* [8]. Genes from *Schef. stipitis* have also been introduced into *Saccharomyces cerevisiae* to enable fermentation of pentoses [5, 7, 8].

### 5.3. Agro-industrial residues for xylitol production

Xylitol is a five-carbon sugar alcohol of high value added as a sweetener for high power, anticariogenic properties and insulin metabolism independent that guarantee its application in food and pharmaceutical industries. The power as a sweetener is similar to sucrose, and higher than ordinary polyols in addition to reduce caloric value, can be tolerated by diabetics. It is an anticariogenic and cariostatic compound that is not metabolized by microorganisms of the oral microbiota; thus, it is used in the manufactures of sweets. Can be used clinically for the prevention of otitis media because it inhibits the growth and adhesion of *Pneumococcus* spp and *Haemophilus influenzae* in nasopharynx cells, and it has skin smoothing properties [9, 10, 11, 12, 13, 14]. Owing to all these characteristics, xylitol is a feedstock of particular interest to the food, odontological and pharmaceutical industries.

Xylose is a sugar widely distributed in nature. Plants and fruits contain relatively low concentration, and extraction from natural sources is usually not profitable [10]. Nowadays, xylitol is derived industrially via a chemical process from hydrolyzates of lignocellulosic wastes by either chemical reduction or microbial fermentation [9]. However, due to a requirement for several chemical purification steps, such a process is very expensive. Therefore, this conversion could be alternatively performed by bacteria, filamentous fungi, yeasts or purified enzymes from these microorganisms which are capable of reducing xylose to xylitol as a first step in D-xylose metabolism [15]. Nevertheless, to make this process exploitable and economical at an industrial level, the bioconversion must be rapid, offer high yield, employ an alternative and cheap culture media and allow for results comparable to those of the present technology.

Lignocelluloses are the most abundant organic mass in the biosphere, which accounts for approximately 50% of the biomass. In nature the annual production of biomass is estimated to 10 to  $50 \times 10^9$  tons [16]. Their major components, cellulose, hemicellulose and lignin, vary with plant species. The pentose fraction, composed of D-xylose (usually not less than 95%) and L-arabinose is much higher in hardwoods (19 to 33%) than in softwoods (10 to 12%) [17].

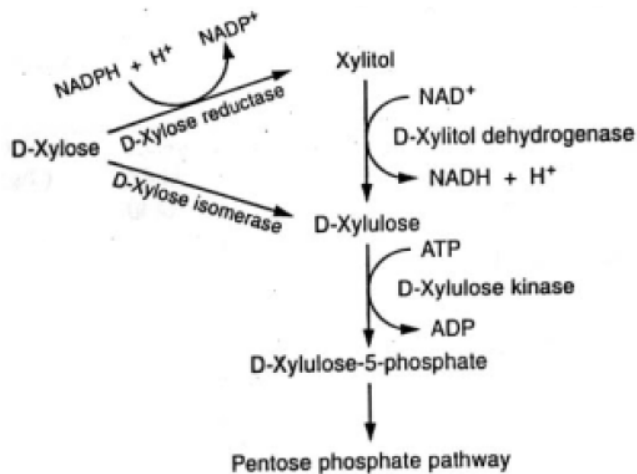
Hemicellulose is a branched polymer, which is composed of both linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid with a high content of xylans, that consist essentially in  $\beta$ -1,4 links with branching variables; due to its heterogeneous structure and low degree of polymerization, it is easily hydrolyzed to xylose [15, 16]. Xylan accounts for 11-35% (dry weight basis) of lignocellulosic materials such as hardwoods and agricultural residues, such as sugarcane bagasse [14, 18], rice straw [19], and soy hulls [20, 21] which are xylan-rich substrates and have been satisfactorily used as alternative media for xylitol production through different treatments [22] and cultivation conditions [23], aimed at increasing process yield and productivity.

D-xylose, also can be converted into a range of substances of industrial interest such as fuels and solvents (ethanol, butanol, 2,3-butanediol, acetone and 2-propanol), alditols (xylitol and glycerol) and organic acids (lactic, acetic and butyric acid). It can also be used as a substrate for production of glucose isomerase [14].

For this process of xylitol production, pure xylose is necessary. The process starts with the production of xylose from xylan after acid-catalysed hydrolysis from hard-wood; however, the chemical process requires several purification steps, because only pure xylose can be used for chemical reduction. Therefore, overall xylitol yield is relatively low (50 – 60 %) from the total xylan content of the wood hemicelluloses [24, 11, 15].

Furthermore, the choice of cultivation and/or conversion system is another crucial point for the success of this bioprocess. Different bench-scale cultivation systems were investigated, utilizing batch, fed-batch and continuous processes [15]. Another important factors which affect the xylitol production is the quantity of inocula, substrate, media, temperature, pH and aeration [17, 25]

On the other hand, the biotechnological procedures are based on the utilization of microorganisms and/or enzymes. These procedures are interesting because they do not require a pure xylose solution as is the case when xylitol is produced by the chemical pathway. The bioconversion process would hold more promises of both hexoses and pentose sugars from lignocellulosic materials. The promising yeast species include the generous *Candida*, *Pichia*, *Debaryomyces* and *Pachysolen* [9, 26, 14, 16] by NADPH-dependent xylose reductase, enzyme which can ferment hemicelluloses hydrolysate from woody plant materials (Figure 3).



**Figure 3.** Pathway for microbial xylose utilization [9]

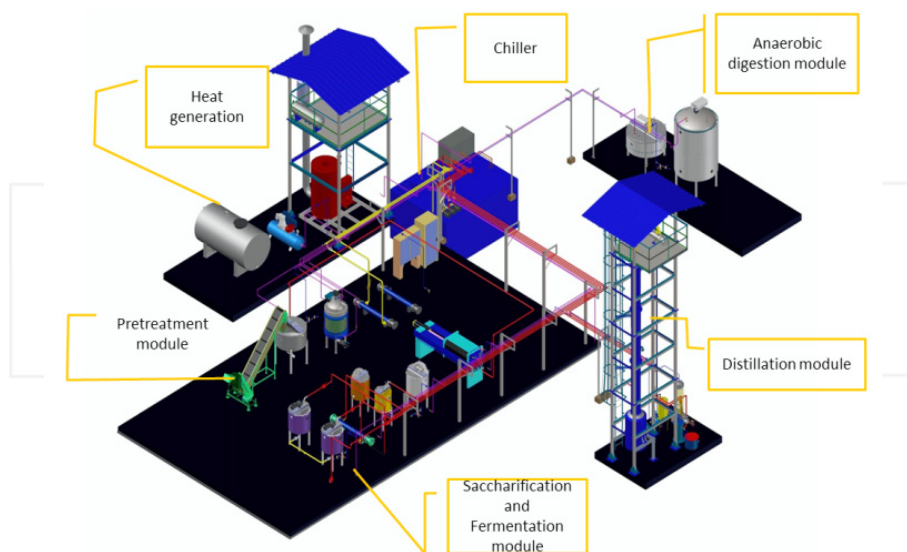
The first step in the metabolism of D-xylose is the transport of the sugar across the cell membrane. Once inside the yeast cell, D-xylose is reduced to xylitol by either NADH- or NADPH-dependent xylose reductase. Xylitol is either secreted from the cell or oxidized to xylulose by NAD- or NADP-dependent xylitol dehydrogenase. The first two reactions are considered to be limiting in D-xylose fermentation. The phosphorylation of xylulose to xylulose 5-phosphate is catalyzed by xylulokinase, which is a prerequisite for its utilization by the central catabolic pathways [23, 17, 16].

In most studies on xylitol production by fermentative processes, xylose of analytical grade is commonly the major substrate. The main problem in the fermentation of these hydrolysates is the presence of toxic compounds released from the lignocellulosic structure during the hydrolytic process, as well as those originated from the sugar degradation, which inhibit the microbial growth and the fermentative activity of the yeasts. In this way, several approaches have been assayed to minimize this effect. According to Silva *et al*, 1998 the maximum xylitol production (54 g/L) occurs when the hydrolysate is first treated with CaO until reaching pH 8.4 and then treated with  $H_3PO_4$  until the pH decreases to 6.0. Thus, pH is an important factor to take into account for the xylitol fermentation. Its effect is related to the acetic acid concentration in the hydrolysate, which concentration, if it is higher than 3.0 g/L, can inhibit the yeasts capability to convert xylose into xylitol [27]. Nonionized acetic acid, which is found in the medium at pH < 7.0, has been found to be the main inhibitor compound in yeast metabolism [28]

The hemmicellulosic hydrolysates from agroforest residues can be efficiently utilized in fermentative processes for xylitol production after an initial treatment designed to remove or reduce the compounds known to be toxic to cell metabolism. This technology is still in its research and development stage, but the results attained points that it may be feasible to

## 6. The biorefinery concept from the perspective of RESETA project

The RESETA project has focused a basic model of biorefinery for producing up to 45,000 liters ethanol/year; 450.000 liters fertilizer. This concept includes the production of ethanol, biogas, biodiesel, fertilizers and animal food (Figure 4). The whole plant is installed in an area of 1,200 m<sup>2</sup>.



**Figure 4.** Isometric plan of the biorefinery built for the RESETA project.

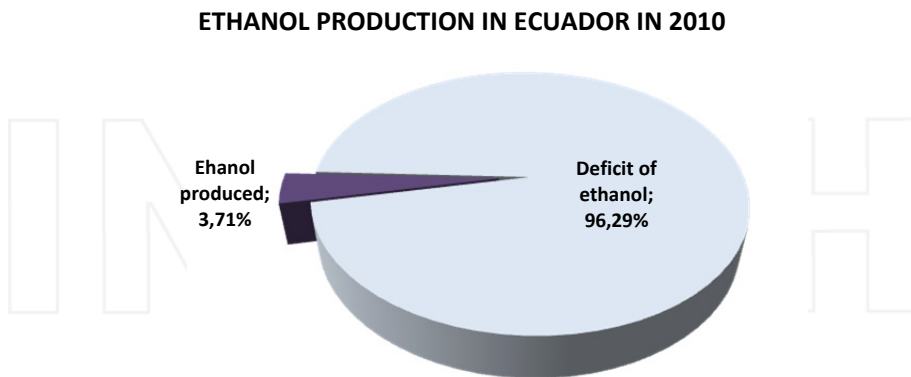
This biorefinery is also able to produce first and second generation ethanol from sugar, starch or cellulose containing feedstocks. From that point of view, the biorefinery concept can be applied not only to large scale, but also in mid and small scale production. In Ecuador, there are communities that produce a wide variety of residues that can be utilized. The biorefinery concept is applicable to promote a sustainable economy in vulnerable and underdeveloped zones.

An important achievement of this R&D project was the development of local technology in order to reduce the dependence of foreign technicians. The RESETA project biorefinery is completely automated which permits the operators scoring historical data of the trials performed during experimentation. Researchers and engineers are working together in the optimization of processes and designs. The resulting ideas and philosophy are being of great value looking forward the future technological independence in these strategic issues for Ecuador.

## 7. Economical and financing analysis on a hypothetical biorefinery in Ecuador

Ecuadorian National Planning and Development Department, has determined a new energetic matrix where it is established the use of 5% ethanol in regular gasoline to be used in vehicles until 2020 in the whole country [29].

To accomplish this endeavor, in 2010 Ecuador's fuel ethanol needs were 32'187,903 gallons; nonetheless, the ethanol production reached was just 1'195,427 gallons [30]. This is, only 3.71% of the national requirements were fulfilled, which demonstrates that there exists a huge unsatisfied demand of fuel ethanol, representing an interesting market for ethanol producers as seen on Figure 5.



**Figure 5.** In Ecuador, only 3.71% of the demand of ethanol was satisfied in 2010 [30]

The price of ethanol per liter has been fixed in 0.76 Dollars by the Ecuadorian Government. This is a very competitive price that the sugar mills are interested in producing this

renewable fuel using molasses as feedstock; molasses are byproducts rich in sugars that represent a valuable source of energy in animal husbandry.

The RESETA Project (the acronym of Sustainable Sources for Ethanol) studies the second generation ethanol production from lignocellulosic materials as well as leftovers and industrial effluents. One of the objectives of such project is the development of a financial model based on a baseline survey on lignocellulosic materials in Ecuador [31]. By means of the data obtained in such survey, it was possible to determine that there are three candidate raw materials taken into account its amount, localization and availability. The sources are banana stems, sugar cane bagasse and empty fruit bunches from oil palm.

Laboratory analysis demonstrated that the high level of moisture in banana stems, as well as the final disposition of the biomass, which is very dispersed in the field, makes its use an unfeasible task in terms of economy. On the other hand, sugar cane bagasse is already been used by sugar cane mills to generate power, thus, just a few biomass of this kind is really available. Finally, empty fruit bunch from oil palms present good characteristics for being used as feedstock in an industrial scale biorefinery. Empty fruit bunches account for about 45% cellulose dry weigh; which is enough to provide the raw material to run about 22% gasoline cars in Ecuador (Table 1).

One of the key factors influencing the feasibility studies to install a biorefinery is the price of the raw materials. Currently, the lignocellulosic residues represent a big environmental problem in Ecuador as well as an unsolved trouble to farmers and industries. One of the most common fates for lignocellulosics is the composting or decomposition on soil to provide organic matter for cultures and as mulch in a number of crops. The uncontrolled decomposition generates lixivates, as well as greenhouse gases that finally are disposed in the subsoil and the atmosphere [31]. It is necessary to provoke a change in the current situation that makes a real shift in terms of the use of the lignocellulosics. One strategy proposed by the RESETA project is pushing to the creation of a market for the lignocellulosics in sustainable and fair conditions for the owners of the biomass as well as for the biorefinery project to be successful. The establishment of companies where the owners of the biomass can participate as shareholders is one of the strategies proposed by our study.

In these terms, we have designed a financial analysis based on the data obtained by laboratory and field surveys. The factors taken into account were:

- a. The implementation zone would be in La Concordia town (Latitude -0.000017°; Longitude - 79,383563°), which is located in a strategic point for its big oil palm production and oil mill concentration.
- b. The raw material in terms of empty fruit bunch is about 640 MT per day.
- c. Enzymes, chemicals, yeasts, are calculated in 0.62 Dollars/Gal of ethanol.
- d. Other costs are about 0.45 Dollars/Gal, as it is detailed in Table 5. Additionally, it has been established an income per by products commercialization. These by products are represented by liquid and solid fertilizers.
- e. Another issue to be taken into account is the energy savings by means of the use of power co-generated using biogas from the biorefinery's processes.

<b>COSTS OF RAW MATERIAL</b>	<b>USD/gal.</b>
Empty fruit bunch oil palm	0.28
Enzymes	0.55
Reactive	0.04
Yeast	0.03
<b>TOTAL COSTS PER RAW MATERIAL</b>	<b>0,9</b>
<b>OTHER COSTS</b>	<b>USD/gal.</b>
Transport	0.11
Electricity	0.05
Fuel	0.11
Waste management	0.02
Water	0.03
Chemicals	0.02
Research & Development	0.05
Administration costs	0.04
Others	0.02
<b>TOTAL OTHER COSTS</b>	<b>0.45</b>
<b>TOTAL COST PER GALLON</b>	<b>1.35</b>

**Table 5.** Detailed costs of raw material and other inputs for ethanol production from empty fruit bunch palm in Ecuador.

A similar European project (i.e. PERESEO project from Spain) establishes that the cost for the construction of a biorefinery plant for 500 MT of biomass per day is about 12 to 15 million Euros. In terms of the RESETA project, we may need to process about 640 MT/day. The criteria for building up a biorefinery are to consider a future growth, so the capacity must be overestimated in about 30% [32]. Then, the investments may overcome in about 1.6 times those of the European PERSEO project. In terms of Dollars, the total investment is among 24.96 to 31.2 million Dollars. We will use the highest number for our calculations.

It has been determined the cost per liter ethanol from lignocellulosics from empty fruit bunch of oil palm in 0,49 Dollars, which is shown in Table 6.

<b>PRODUCTION COSTS USD/L etanol</b>	
DEPRECIATION	\$ 0,10
MAINTENANCE	\$ 0,01
OTHER FIXED COSTS	\$ 0,12
<b>TOTAL FIXED COSTS</b>	<b>\$ 0,23</b>
RAW MATERIAL	\$ 0,24
DIRECT LABOR	\$ 0,02
<b>TOTAL VARIABLE COSTS</b>	<b>\$ 0,26</b>
<b>TOTAL PRODUCTION COSTS</b>	<b>\$ 0,49</b>

**Table 6.** Production costs of ethanol from empty fruit bunch palm in Ecuador.

In this calculation it has been taken into account a 1% growth in costs and incomes with the aim of using the whole installed capacity at the end of the project. Following in Table 7, it is shown the cash flow of the project, where the initial investment (31,2 million Dollars), the investment recovery period; the IRR and NPV are shown. Table 8 is a resume of such values.

CASH FLOW									
Year	Investment	Cash Inflows (Income)	Cash Outflows (Expenses)	Gross profit	Utilities employees	Income Tax	Depreciation	Net Income	Accumulated Cash
0	(31.200.000,00)							(31.200.000,00)	(31.200.000,00)
1		12.480.156,38	7.748.346,30	4.731.810,08	709.771,51	1.005.509,64	1.560.000,00	4.576.528,93	(26.623.471,07)
2		12.604.957,94	7.825.829,76	4.779.128,18	716.869,23	1.015.564,74	1.560.000,00	4.606.694,22	(22.016.776,86)
3		12.731.007,52	7.904.088,06	4.826.919,46	724.037,92	1.025.720,39	1.560.000,00	4.637.161,16	(17.379.615,70)
4		12.858.317,60	7.983.128,94	4.875.188,66	731.278,30	1.035.977,59	1.560.000,00	4.667.932,77	(12.711.682,93)
5		12.986.900,77	8.062.960,23	4.923.940,54	738.591,08	1.046.337,37	1.560.000,00	4.699.012,10	(8.012.670,84)
6		13.116.769,78	8.143.589,83	4.973.179,95	745.976,99	1.056.800,74	1.560.000,00	4.730.402,22	(3.282.268,62)
7		13.247.937,48	8.225.025,73	5.022.911,75	753.436,76	1.067.368,75	1.560.000,00	4.762.106,24	1.479.837,62
8		13.380.416,85	8.307.275,99	5.073.140,87	760.971,13	1.078.042,43	1.560.000,00	4.794.127,30	6.273.964,92
9		13.514.221,02	8.390.348,75	5.123.872,27	768.580,84	1.088.822,86	1.560.000,00	4.826.468,58	11.100.433,50
10		13.649.363,23	8.474.252,23	5.175.111,00	776.266,65	1.099.711,09	1.560.000,00	4.859.133,26	15.959.566,76
11		13.785.856,86	8.558.994,76	5.226.862,11	784.029,32	1.110.708,20	1.560.000,00	4.892.124,59	20.851.691,35
12		13.923.715,43	8.644.584,70	5.279.130,73	791.869,61	1.121.815,28	1.560.000,00	4.925.445,84	25.777.137,19
13		14.062.952,59	8.731.030,55	5.331.922,04	799.788,31	1.133.033,43	1.560.000,00	4.959.100,30	30.736.237,49
14		14.203.582,11	8.818.340,86	5.385.241,26	807.786,19	1.144.363,77	1.560.000,00	4.993.091,30	35.729.328,79
15		14.345.617,93	8.906.524,26	5.439.093,67	815.864,05	1.155.807,40	1.560.000,00	5.027.422,21	40.756.751,00
16		14.489.074,11	8.995.589,51	5.493.484,61	824.022,69	1.167.365,48	1.560.000,00	5.062.096,44	45.818.847,44
17		14.633.964,85	9.085.545,40	5.548.419,45	832.262,92	1.179.039,13	1.560.000,00	5.097.117,40	50.915.964,84
18		14.780.304,50	9.176.400,86	5.603.903,65	840.585,55	1.190.829,52	1.560.000,00	5.132.488,57	56.048.453,41
19		14.928.107,55	9.268.164,86	5.659.942,68	848.991,40	1.202.737,82	1.560.000,00	5.168.213,46	61.216.666,87
20		15.077.388,62	9.360.846,51	5.716.542,11	857.481,32	1.214.765,20	1.560.000,00	5.204.295,59	66.420.962,47

**Table 7.** Cash flow of the project

With such calculations and with a discount rate of 13.76%, calculated in basis to the established formula shown next, we have attained an IRR biggest than the discount rate.

$$\text{Discount Rate} = R_f + \beta(R_m - R_f) + R_p,$$

$R_f$ : Risk of the treasure funds bonus in the USA.

$\beta$ : Sensitivity or risk of the Project in the market

$R_m$ : Stock market index.

$R_p$ : Country-risk

<b>DISCOUNT RATE</b>	13,96%
<b>IRR</b>	14,15%
<b>NPV</b>	\$ 307.655,43
<b>INVESTMENT RECOVERY PERIOD</b>	6 years

**Table 8.** Resume of the main financial indicators of the project

This Project exhibits a positive NPV, combined with a reasonable IRR value; both values can be interpreted as indicators of a feasible project. This financial study doesn't consider the externalities and the social benefits a biorefinery can give the surrounding populations and environment.

## 8. Perspectives and conclusions

Ecuador is a very rich country in terms of biomass, produced by agricultural activities as well as biomass from natural forests. Nevertheless, this biomass is now misused and underestimated, because of the lack of mature technologies to take the best from it.

Ecuador has a high potential for the production of lignocellulosic ethanol, if we take a look of the amount of lignocellulosic waste materials that are being produced every year in crops such as bananas, palm oil, sugar cane, etc. Nevertheless it is to be understood that social and economic costumes are deeply rooted so it's going to be a big task changing the current uses of several waste materials. The case of empty fruit bunches is a typical example where it is possible to analyze the behaviors and preferences of the farmers and industry people.

It is necessary to establish rules and laws to regulate the raising biomass markets, moreover if the biomass is going to be transformed in valuable and useful products such as xylitol, ethanol, foods and renewable chemicals.

The technologies are being improved to get better yields and lower production costs. A demonstrative scale biorefinery has recently sat up in Ecuador at the Neotropical Center for the Biomass Research, under the Pontificia Universidad Católica del Ecuador. This biorefinery was completely designed and constructed in Ecuador and is the very first in its genus in the country as well as one of the few in South America.

## Author details

Enrique Javier Carvajal Barriga, Patricia Portero Barahona, Edgar Salas, Carolina Tufiño and Bernardo Bastidas

*Pontificia Universidad Católica del Ecuador, Centro Neotropical para la Investigación de la Biomasa, Quito-Ecuador*

Cristina Guamán-Burneo

*Departamento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

## Acknowledgement

The authors want to acknowledge the Pontificia Universidad Católica del Ecuador and the SENESCYT (Secretariat of Science and Technology of Ecuador) for financing the RESETA project and scientific activities at the Neotropical Center for the Biomass Research.

## 9. References

- [1] Berg, Jeremy Mark, Tymoczko, Jhon L. and Lubert, Stryer. *Biochemistry*. New York: Freedman and Company, 2007.
- [2] OPEC. *World Oil Outlook*. Vienna, Austria: Secretariat OPEC, 2011.

- [3] DALE, BRUCE. Biofuels: Thinking Clearly about the Issues. *Journal of Agricultural and Food Chemistry*. 56, 2008, 3885–3891.
- [4] Cheng, J.J., et al., et al. *Biomass to Renewable Energy Processes*. New Jersey: Taylor & Francis Group, 2010.
- [5] *High activity of xylose reductase and xylitol dehydrogenase improves xylose fermentation by recombinant Saccharomyces cerevisiae*. Karhumaa, K., et al., et al. 2007, *Appl. Microbiol. Biotechnol.*, pp. 73, 1039–1046.
- [6] *Efficient production of L-lactic acid from xylose by Pichia stipitis*. Ilmén, M., et al., et al. 2007, *Appl. Environ. Microbiol.*, pp. 73, 117–123.
- [7] *Engineering yeasts for xylose metabolism*. Jeffries, T.W. 2006., *Curr. Opin. Biotechnol.*, pp. 17, 320–326.
- [8] *Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeas*. Jeffries, T.W., et al., et al. 2007, *Nat. Biotechnol.*, pp. 25, 319–326.
- [9] Affleck, R.P. *Recovery of Xylitol from Fermentation of Model Hemicellulose Hydrolysates Using Membrane Technology*. Virginia, USA: Dissertation for the degree of Master of Science in Biological Systems Engineering at Virginia Polytechnic Institute and State University, 2000.
- [10] Sampaio, F. *Seleção de Microrganismos para a Conversão de Xilose em Xilitol*. Brasília: Dissertação apresentada na Universidade de Brasília para obtenção do título do Mestre em Microbiologia Agrícola., 2001.
- [11] Granström, T. *Biotechnological production of xylitol with Candida yeasts*. Espoo, Finland: Dissertation for the degree of Doctor in Chemical Technology at Helsinki University of Technology, 7th of June, 2002.
- [12] *Xilitol: Edulcorante com efeitos benéficos para a saúde humana*. Mussatto, S. I. and Roberto, I. C. 2002: s.n., 2002, *Revista Brasileira de Ciências Farmacêuticas.*, pp. 38:401–413.
- [13] Lima, L. H. *Estudos moleculares dos genes XYL1 e XYL2 de Candida tropicalis visando a produção de xilitol*. Brasília: Dissertação apresentada a Universidade de Brasília para obtenção do título do Doutor em Ciências Biológicas- Biologia Molecular, 2006.
- [14] *Crescimento e consumo de Xilose de Candida guilliermondii na fermentação submersa utilizando-se bagaço de cana-de-açúcar*. Jucoski, M. C., et al., et al. 2007, *Evidência*, Joaçaba, pp. V. 7, N.2, P. 119–130.
- [15] *Bioconversion of D-xylose to xylitol by Debaryomyces hansenii UFV-170: Product formation versus growth*. Sampaio, F. C., et al., et al. 2005, *Process Biochemistry.*, pp. 40, 3600–3606.
- [16] *Review, Bioconversion of pentose sugars into ethanol: A review and future directions*. Chandel, A. K., et al., et al. 2010, *Biotechnology and Molecular Biology Review*, Vol. 6(1), pp. 008–020.
- [17] *Microbial conversion of D-Xylose to Xylitol*. Winkelhausen, E. and Kuzmanova, S. 1998, *Journal of Fermentation and Bioengineering.*, pp. 86: 1–14.
- [18] *Evaluation of Sugarcane bagasse acid hydrolizate treatments for xylitol production*. Gurgel, P. V., et al., et al. 1998, *Brazilian Journal of Chemical Engineering* vol.15, n.3, pp. 309–312.

- [19] *Improvement of xylitol production by Candida guilliermondii FTI 20037 previously adapted to rice straw hemicellulosic hydrolysate.* Silva, C.J.S. and Roberto, I.C. 2001, Letters in Applied Microbiology 2001, pp. 32, 248-252.
- [20] Schmirmer, A.C. *Produção biotecnológica de xilitol e etanol a partir de hidrolisado de casca de soja.* Rio Grande do Sul: Dissertação apresentada na Universidade Federal de Rio Grande do Sul para obtenção do mestre em Ciência e Tecnologia de Alimentos., 2007.
- [21] Ribeiro, A. *Otimização da hidrólise da casca de soja (Glycine max) e avaliação da capacidade de produção de xilitol e etanol por microrganismos sobre este hidrolisado.* Rio Grande do Sul: Dissertação apresentada na Universidade Federal de Rio Grande do Sul para obtenção do mestre em Ciencia e Tecnologia de Alimentos, 2010.
- [22] *Pretreatment of sugarcane bagasse hemicellulose hydrolysate for xylitol production by Candida guilliermondii.* Alves, L. A., et al., et al. 1998, Applied Biochemical Biotechnology, pp. 70-72: 89-97.
- [23] *Factors that affect the biosynthesis of Xylitol by Xylose-Fermenting Yeasts.* Silva, S.S., Felipe, M.G.A. and Mancilha, I.M. 1998, Applied Biochemistry and Biotechnology. São Paulo., pp. vol. 70-72.
- [24] *Biotechnological production of xylitol. Part 3: Operation in culture media made from lignocellulose hydrolysates.* Parajó, J. C., Domínguez, H. and Domínguez, J. M. 1998b, Bioresource Technology., pp. 66: 25-40.
- [25] *Chemostat study of xylitol production by Candida guilliermondii.* Granström, T., Ojamo, H. and Leisola, M. 2001, Applied Microbiology Biotechnology, pp. 55: 36-42.
- [26] *Reclassification of the Candida guilliermondii FTI20037 as Candida tropicalis based on molecular phylogenetic analysis.* Lima, L. H., Felipe, M. G.F. and Gonçalves, F. A. 2003, Brazilian Journal of Microbiology., pp. 34 (Suppl.1) 96-98.
- [27] *Effect of acetic acid on xylose fermentation to xylitol by Candida guilliermondii.* Felipe, M. G. A., et al., et al. 1995, Journal of Basic Microbiology, pp. vol 35: 171-177.
- [28] *Biotechnological Production of Xylitol from Agroindustrial Residues. Evaluation of Bioprocesses.* Rodrigues, D. C., et al., et al. 1998, Applied Biochemistry and BiotechnologyY., pp. Vol. 70-72.
- [29] Erazo, Paúl Mena.  
[http://www.bbc.co.uk/mundo/ciencia\\_tecnologia/2010/01/100112\\_0245\\_ecuador\\_gasolina\\_verde\\_jrg.shtml](http://www.bbc.co.uk/mundo/ciencia_tecnologia/2010/01/100112_0245_ecuador_gasolina_verde_jrg.shtml). Ecuador arranca venta de biocombustible. enero 13, 2010, p. digital.
- [30] PETROECUADOR. *INFORME CIFRAS PETROLERAS, PERIODO ENERO - DICIEMBRE 2010.* Quito: EP PETROECUADOR, 2010.
- [31] SmartResearch. *Estudio desechos agricolas en el Ecuador, Proyecto RESETA: Recursos Sustentables para Etanol.* Quito: PUCE, 2009.
- [32] Cedeño, N. M., León, J. E. and Cedeño, J. C. *Modelo de Estimación de Precio para un Combustible de Composición 95% Gasolina Extra y 5% Etanol, que Ayude a Disminuir la Contaminación Ambiental en la ciudad de Guayaquil.* Guayaquil: ESCUELA SUPERIOR POLITECNICA DEL LITORAL, 2010.

- [33] *Biotechnological production of xylitol. Part 1: Interest of xylitol and fundamentals of its biosynthesis.* Parajó, J. C., Domínguez, H. and Domínguez, J. M. 1998a, *Bioresource Technology.*, pp. 65, 191-2111.
- [34] *High capacity xylose transport in Candida intermedia PYCC 4715.* Gárdonyi, M., et al., et al. 2003, *FEMS Yeast*, pp. Res. 3, 45–52.

INTECH

INTECH