

ABSTRACT

DE ASSIS, TIAGO. Techno-economic Analysis for Production of Sugarcane Cellulosic Ethanol in Brazil. (Under the direction of Dr Hasan Jameel, Dr Ronalds Gonzalez and Dr Sunkyu Park).

Despite worldwide research, global effort and availability of sugarcane biomass, industrial commercialization of cellulosic ethanol is still very limited in Brazil and in the world. Feedstock costs, feedstock variability, high enzyme costs, high capital investment, low ethanol price and non-reliable technologies are the main barriers for profitable production cellulosic ethanol. In this context, this work will study alternatives to reduce capital and operating costs, maximize ethanol and power production and increase sugar recovery during enzymatic hydrolysis when sugarcane bagasse and straw are used as feedstocks for ethanol production. Therefore, the following goals will be addressed: i) Techno-economic analysis to compare the performance of greenfield and co-located plants when sugarcane bagasse and straw are converted to ethanol or power, ii) Evaluation of improvement in enzymatic hydrolysis conversion and sugar recovery when delignification and mechanical refining are applied to pretreated biomass, iii) Fundamental understanding about what physical properties (morphology, particle size, swellability, pore area and crystallinity) can be used to explain the improvements in enzymatic hydrolysis when mechanical refining is applied to pretreated biomass, iv) Techno-economic analysis to evaluate the impact of mechanical refining, residence time and enzyme dosage on cellulosic ethanol production, v) Deterministic and probabilistic analysis to evaluate the economic impact of volatility and variability associated with the major cost and revenue drivers on cellulosic ethanol production.

The first study has shown that higher sugar yield when bagasse is converted to ethanol, higher power production when straw is burned in biomass boilers and capital savings when a co-located cellulosic ethanol plant operates during the 350 days have presented as the most promising alternatives among the evaluated scenarios. Higher risks associated with large cellulosic ethanol plants decrease attractiveness of greenfield plants.

In the second study, a significant enzymatic hydrolysis improvement (25% to 30%) was achieved when lignin removal resulted in a high carbohydrate to lignin ratio. However, carbohydrate loss (up to 25%) due to peeling reaction and high costs compromise sugar recovery and economics for delignification. On the other hand, mechanical refining improved sugar recovery by 5% to 10%, confirming its potential as a simple and cheap technology to improve carbohydrate digestibility. Moreover, light microscope has showed fiber bundles disintegration, internal delamination and some extent of external fibrillation when mechanical refining was applied to the pretreated biomass. The increase of refining intensity resulted in reduction of fiber length and enhancement of swellability. Porometry has elucidated increase of pore area (pores > 10 nm) for refined samples. However, additional pore area gains with increase of refining intensity were not observed. X-ray diffraction did not show any evidence of changes in biomass crystallinity during refining.

The third work has shown that reduction of enzymatic hydrolysis residence time (96h to 24h) decreased internal rate of return (IRR). Reduction on enzyme dosage (5 FPU/g to 3 FPU/g) did not change the financial performance significantly. The additional ethanol production (~ 9%) when pretreated bagasse was refined compensates for the reduction in the electricity revenue and additional capital, improving the IRR by 0.8%. However, when straw was converted to ethanol, the additional ethanol revenue (~ 5%) obtained with mechanical

refining was not enough to overcome the costs drivers, resulting in no improvement in IRR. According to sensitivity analysis, ethanol price, CAPEX, enzyme costs, carbohydrate content and straw cost are the most significant economic drivers. Although the most promising scenario has presented an IRR of 11.3%, probabilistic analysis has shown that it has only 50% of chances of having net present value greater than zero at a discount rate of 12% due to high volatility and variability of the main cost and revenue drivers. The calculated MESP using deterministic analysis was US\$ 0.469/L, which is one cent of dollar per liter of ethanol more expensive than the market price assumed in this work. However, probabilistic analysis showed that ten cents of dollar per liter of ethanol is necessary for 99.9% of changes of having a positive NPV at 12 % discount rate.

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Techno-economic Analysis for Production of Sugarcane Cellulosic Ethanol in Brazil

by
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DEDICATION

First of all, I dedicate this work to God, in the name of Jesus Christ, for being my solid rock, my fortress and my rescuer. Secondly, I would like to thank my wife, Camilla, for supporting my dream of studying abroad, for being patient during the time we stayed separated by distance, for helping me during difficult moments and for loving me despite all my mistakes and weaknesses. I would like to thank my parents, Alaerte and Cida, for teaching me the true values of family and for providing me the opportunity of having access to good education. Finally, I dedicate this work to my siblings, Alaerte, Andreia and Alex, for raising me with the same love that parents have for their children.

BIOGRAPHY

Tiago de Assis was born and raised in the beautiful city of Guaxupe, in the state of Minas Gerais, Brazil. In 2002, he moved to Lorena-Sao Paulo where he started a chemical engineering program at Escola de Engenharia de Lorena – USP. In 2003, he moved to Sao Carlos-Sao Paulo, where he finished the chemical engineering program at Universidade Federal de Sao Carlos in 2006. From 2007 to 2010, he worked as a process engineers in different companies in Brazil related to lysine manufacturing and hydrogen fuel cells development. In 2010 he started working at CTBE (Brazilian Bioethanol Science and Technology laboratory) as a process engineer.

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1. Introduction and Background

Sugarcane is a well established industry and Brazil is a leading country with many years of industrial experience with production and conversion of sugarcane to sugar, ethanol and electricity. This privileged position is the result of a long term synergy among producers, government and research institutes to reduce costs, improve technology, increase productivity and create supportive policies.

After crushing sugarcane stalks inside a sugarcane mill, the extracted sucrose juice is typically converted to sugar or ethanol, and the remaining fibers (bagasse) are usually burned to produce steam and electricity. However, with the development of modern sugarcane mills and mechanical harvesting, an increasing amount of sugarcane bagasse and straw (tops and leaves) is available, and this lignocellulosic material can be used as feedstock to produce bioenergy, biofuels and biomaterials. The challenge now is to identify cost effective and reliable technologies to convert the available lignocellulosic material into added value products, improving the sustainability and economic performance of sugarcane biorefineries.

Among different options, the production of cellulosic ethanol is one reasonable alternative because cellulosic ethanol plants can be integrated with the existing ethanol industry to cover the demand for transportation fuels. Despite the Brazilian potential, the production of cellulosic ethanol is still very limited. More work is necessary to reduce production costs, reduce capital and improve performance of current technologies.

Therefore, this work will cover technical and economic aspects of sugarcane cellulosic ethanol with focus on alternatives to reduce capital investment and operating costs, maximize ethanol and power production, and increase sugar recovery during enzymatic hydrolysis.

In this chapter, an introduction about the current status of sugarcane, sugar, ethanol and electricity production in Brazil will be presented. An overview about market trends and the future projection for ethanol demand will be described. The configuration of sugarcane mills and the potential availability of lignocellulosic biomass will be discussed. Finally, the actual production of cellulosic ethanol in Brazil and the challenges associated will be presented. Chapter 2 will present the objectives of this work.

Many techno-economic analysis evaluate stand-alone 2G ethanol plants or integrated 1G2G ethanol plants in a greenfield scenario, where sugarcane bagasse and straw are converted as a single biomass. Chapter 3 will compare the financial performance between greenfield and co-located cellulosic ethanol plants. The economic impact of extended operation of 2G ethanol plants after sugarcane season will also be evaluated. Moreover, the use of bagasse and straw as a feedstock for cellulosic ethanol production or as a fuel for steam and power production will be compared.

Enzymatic hydrolysis of carbohydrates is one of the most expensive process steps for the production of cellulosic ethanol due to long residence time, high enzyme costs and considerably low carbohydrate conversion. Many factors influences enzymatic hydrolysis and they can be divided in factors related to enzyme, process and substrate. Lignin content and accessibility of substrate to enzymes are important limiting factors related to substrate. In Chapter 4, delignification and mechanical refining will be used to reduce lignin content and improve enzyme accessibility to substrate, in order to enhance enzymatic hydrolysis yield of pretreated biomass. The total sugar recovery will be used to compare both options. Finally a fundamental study will be performed to evaluate how the modification of physical properties

can explain the improvement of enzymatic hydrolysis when mechanical refining is applied to the pretreated biomass.

In Chapter 5, the economic impact of using mechanical refining as a tool to improve digestibility of biomass after autohydrolysis pretreatment will be addressed. Moreover, different residence times and enzyme dosages will also be economically evaluated. Finally, deterministic and probabilistic techno-economic analysis will be performed to identify the major economic drivers and evaluate the impact of their volatility and variability on internal rate of return, net present value and minimum ethanol selling price.

1.1.Sugar, Ethanol and Bioelectricity Production - Actual Numbers

Brazil is the biggest producer of sugarcane with 39 % of the world production in 2014 (Food and Agriculture Organization of the United Nations - Statistics Division, 2014). According to (Companhia Nacional de Abastecimento, 2016), the production of sugarcane in Brazil during 2015/2016 season was 665.6 million metric ton (70% moisture content) occupying a total area of 8.7 million hectares, where the average productivity was 76.9 metric ton/ha. Although the production of sugarcane has suffered some oscillations, the total area used for sugarcane plantation has steadily increased along the last 10 years (Figure 1.1).

Harvested sugarcane was consumed to produce 33.5 million metric ton of sugar and 30.5 billion liters of ethanol during 2015/2016 season. Figure 1.2 shows the evolution of ethanol and sugar productions in Brazil for the past 10 years (Companhia Nacional de Abastecimento, 2016).

In 2016, the total installed capacity for bioelectricity production from sugarcane bagasse was 10,549 MW (mega watts), which represents 7% of total installed capacity in Brazil and

79.5% of the total capacity among all facilities that use biomass as energy source (Agencia Nacional de Energia Eletrica, 2016).

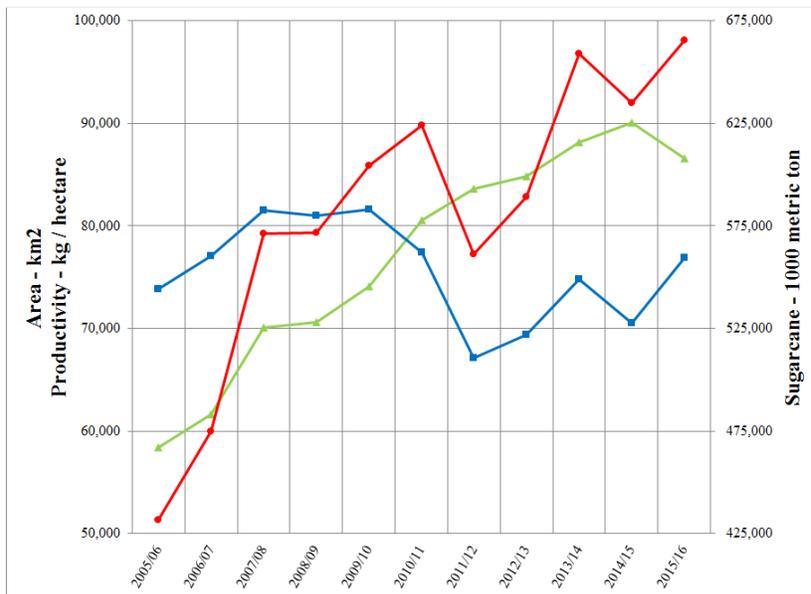


Figure 1.1. Sugarcane Production, Area and Productivity in Brazil. Red circles: Sugarcane Production. Green triangles: Area. Blue squares: Productivity

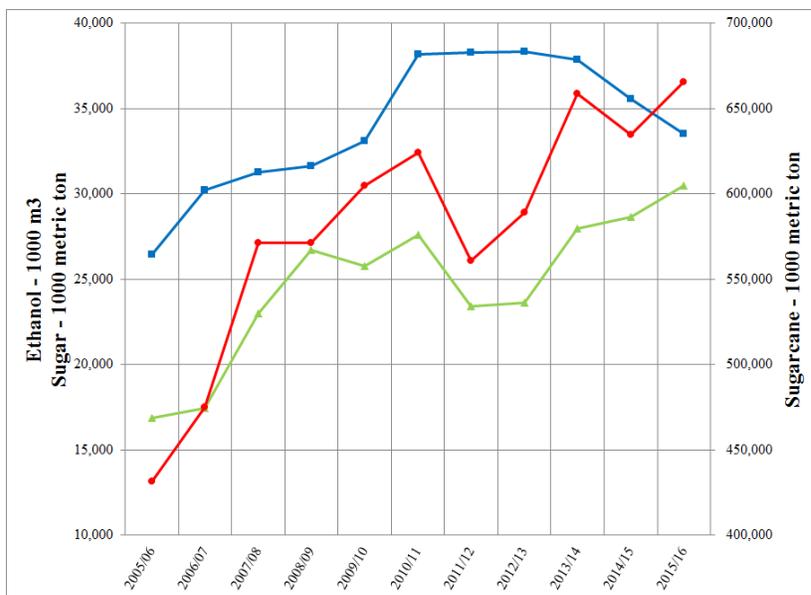


Figure 1.2. Production of Sugarcane, Ethanol and Sugar in Brazil. Red circles: Sugarcane Production. Green triangles: Ethanol. Blue squares: Sugar

According to Figure 1.3, the production of sugarcane in Brazil is divided between two regions: a) Center South: 90.6% of total production (Only São Paulo State (SP) has 53.8%); b) Northeast: 9.4% of total production (Companhia Nacional de Abastecimento, 2015).

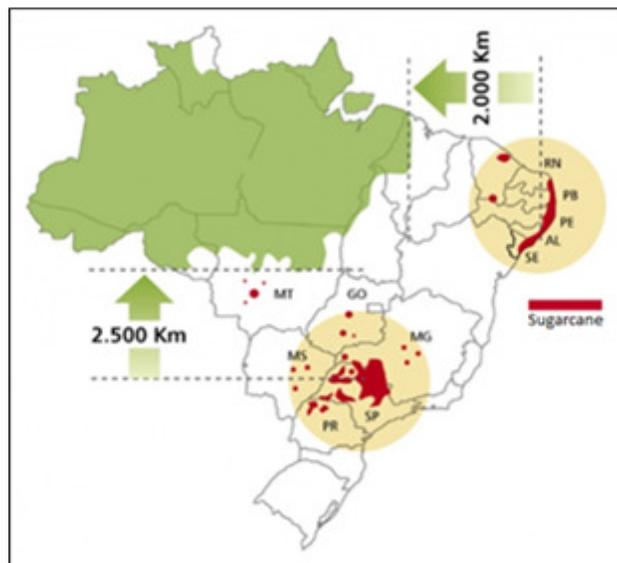


Figure 1.3. Regions for Sugarcane Production in Brazil: Center South and Northeast.

1.2.Sugarcane Industry - Production Trends and Ethanol Market

Last decade was remarked by an increase of sugar demand in the international market and increase of ethanol consumption due to development of flex fuel cars industry in Brazil, especially after 2003. Moreover, there was a perspective to export ethanol to countries and economic blocks (USA, European Union) that have decided to include biofuels in their energetic matrix after 2005. As a response to this scenario, investments in new plants were accelerated and sugarcane production was increased 10.6% annually from 2001 to 2009, year of the world economic recession (Federacao das Industrias do Estado de Sao Paulo, 2013).

After the global crisis, sugarcane production experienced a negative growth and the financial situation of sugarcane industry was difficult: companies stopped investments, credit was strongly reduced, international supply of sugar was increased, climate issues impacted field productivity and gasoline price was controlled by government below international prices. As a result, sugarcane sector experienced many fusions, acquisitions by international companies and a considerable number of mills have bankrupted. The loss of field productivity due to problems related to climate, mechanization and field management started to be overcome during 2013/2014 season (Federacao das Industrias do Estado de Sao Paulo, 2013) and nowadays sugarcane industry is in a recovering process.

1.2.1. Ethanol Production

After the development of flex fuel cars in 2003, the production of ethanol was drastically increased. Between 2000 and 2008, the production of ethanol was increased by 160%. However, with the decrease of sugar production in India (2009/2010) and low profitability of ethanol due to governmental policies to control gasoline price below international market, production of sugar became more attractive. Moreover, the loss of field productivity also contributed to reduce ethanol production (Federacao das Industrias do Estado de Sao Paulo, 2013).

From 2013 to 2016, the production of ethanol was increased to a record of 30 billion of liters per year due to the decrease of sugar price in the international market, increase of ethanol content in gasoline (from 25% to 27%) and elevation of gasoline prices (Empresa de Pesquisa Energetica, 2015).

1.2.2. Ethanol Exportation

With the Energy Policy Act of 2005, the exportation of ethanol to the USA was increased, contributing to a total exportation of 4.7 billion of liters in 2008/2009. However, with the American investments in corn ethanol, the exportation was reduced to 1.9 million of liters in 2010/2011. From 2012 to 2015, the exportation oscillated between 1.4 to 3.1 million of liters per year, reaching 1.9 billion of liters in 2015, although Brazil imports ethanol in lower amounts, especially from USA (0.5 billion of liters in 2015). USA is still the main destination of Brazilian ethanol to meet the American demand for advanced biofuels (Federacao das Industrias do Estado de Sao Paulo, 2013).

1.2.3. Ethanol Consumption

The consumption of ethanol is concentrated in the Southeast of Brazil (69%). From 2000 to 2009, the consumption of ethanol in this region was increased by 147%. However, the governmental control of gasoline price caused a decline on ethanol consumption from 2009 to 2012. After 2013, the favorable price of ethanol has increased its consumption. The expansion of sugarcane industry in the Center West region has increased the ethanol supply and consumption in this area. Moreover, the increase of population income during the last years has contributed for the increase of ethanol consumption in the North and Northeast of Brazil due to the elevation of the number of cars (Federacao das Industrias do Estado de Sao Paulo, 2013).

1.2.4. Ethanol Market - Future Perspective

The market for light cars will increase during the next decade but at a moderate rate. It is estimated that 87% of new light vehicles entering the market will be flex fuel, which will increase the proportion of flex fuel cars in Brazil (Federacao das Industrias do Estado de Sao Paulo, 2013). Currently, the flex fuel technology represents 64% of the total number of light cars in Brazil (Empresa de Pesquisa Energetica, 2015). Moreover, the better price competitiveness of ethanol compared to gasoline will potentially increase the consumption of ethanol (Federacao das Industrias do Estado de Sao Paulo, 2013).

Nowadays, the international market of biofuels is impacted by a decrease of incentives for traditional biofuels, increase of incentives to improve energy efficiency and to develop advanced technologies, modest commercialization of biofuels (among Brazil, USA and European Union). Brazil and USA are the main producers and exporters of ethanol (80% of total production and exportation) (Empresa de Pesquisa Energetica, 2015).

USA is the biggest producer (56 billion of liters in 2015), with a domestic demand of 50 billion of liters consumed as a gasoline blender. This demand has been practically constant during the last years and this tendency will be maintained, at least during a short term. Reasons for this tendency can be highlighted: a) Commitment with the improvement of energy efficiency; b) Challenges to increase the consumption of fuel blends with ethanol content above 10% (modest numbers of flex fuel cars, modest market for fuel blends with high content of ethanol, incompatibility of gasoline-powered vehicles with fuel blends containing more than 10% ethanol, actual low price of gasoline); c) Reduction of target consumption of advanced biofuels, cellulosic ethanol and renewable biofuels established by EISA 2007. As a result, ethanol producers are exporting the surplus production and USA has

become the largest exporter of ethanol (3.2 billion of liters exported in 2015 mainly to Canada and Brazil) (Empresa de Pesquisa Energetica, 2015), (Energy Information Administration, 2016).

European Union still maintains the compromise to replace 20% of its energetic matrix with renewable energy by 2020. Moreover, there is an additional target to reach 10% share of renewable sources in the transportation sector. Actual numbers show that the consumption of renewable energy has reached 15% of the total energy consumption in 2013. However, renewable energy represents only 5.4% of the energy consumption by the transportation sector in 2013. Additionally, the target for renewable energy was raised to at least 27% by 2030 and in order to reach the objective of reducing 80% to 95% the emission of greenhouse gases by 2050, the contribution of renewable sources has to be considerably raised (Empresa de Pesquisa Energetica, 2015), (European Environment Agency, 2015).

Therefore, the growing market of flex fuels cars in Brazil and the international compromise of economic blocks to increase the participation of renewable fuels in their energetic matrix creates a potential driving force to increase ethanol demand in medium/long term horizon.

1.3.Sugarcane Mills Configuration

As reported by (NovaCana.com, 2016), Brazil has around 370 sugarcane mills under operation in 2016. The average milling capacity for a single facility is around 1.8 to 2.0 million metric ton of sugarcane per season and the maximum observed capacity is around 10 million metric ton. The average installed capacity for bioelectricity production in a sugarcane mill is around 25 MW and the maximum observed capacity is around 155 MW.

Sugarcane mills in Brazil can be classified according to their configurations: a) Sugar Mills: produce only sugar and represent 4.5% of total units; b) Autonomous Distilleries: produce only ethanol and represent 31.6% of total units, consuming 28.7% of the total sugarcane produced; c) Annexed Distillery: produce both sugar and ethanol and represent the majority of units (63.9%). They are responsible for processing 67.4% of the total sugarcane produced (Companhia Nacional de Abastecimento, 2013).

1.4.Sugarcane Biomass Availability

According to Figure 1.4, sugarcane plant is composed by stalk, tops and leaves. The production of one tonne of sugarcane stalks generates around 145 kg of dry sugars (mainly sucrose) used to produce sugar and ethanol, 135 kg of dry bagasse (mainly fibers) and 140 kg of dry sugarcane straw or trash (tops and leaves) (Brazilian Bioethanol Science and Technology Laboratory - Technological Assessment Program, 2011).

Sugarcane straw is traditionally burned in the field to facilitate harvesting and transportation in non-mechanized operations. Due to social and environmental issues, Brazil has created a federal law establishing a schedule to end the burning practices until 2018 in areas where mechanical harvesting can be applied. Moreover, in Sao Paulo and Minas Gerais states, responsible for 2/3 of the sugarcane production, a Protocol was signed by sugarcane stakeholders to anticipate the end of burning practices to 2014. Recent numbers show that the level of mechanization in Sao Paulo state was 84.8% during 2013/2014 season. As a result, there is an increasing amount of biomass available on the ground, and this biomass can be partially transported to the sugarcane mill to be used as a fuel for power generation or as a feedstock for biofuels production (Leal et al., 2013).

After crushing sugarcane stalk to extract the sucrose juice, the remaining fibers are called sugarcane bagasse which is traditionally consumed inside sugarcane mills as an energy source. The availability of bagasse is related to the energy efficiency and configuration of the sugarcane mill. About 10% of the total bagasse produced is available for other uses and the remaining bagasse is burned in biomass boilers to meet the demand for steam and power in a sugarcane mill. This is a common situation in the case of annexed distilleries when low pressure boilers are used (21 bar). In modern autonomous distilleries, bagasse savings of 50% approximately can be achieved when high pressure boilers (65 to 90 bar) and high efficiency turbines and generators are used (Centro de Gestao e Estudos Estrategicos, 2009).

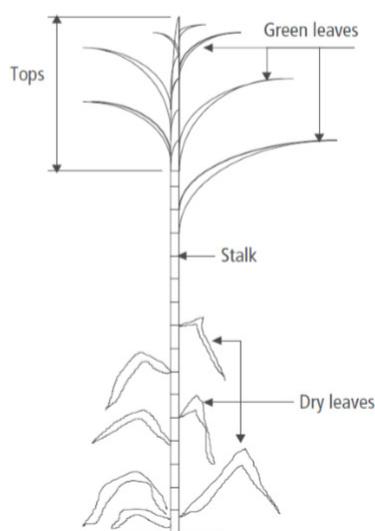


Figure 1.4. Sugarcane Plant.

1.5.Sugarcane Straw Collection, Transportation and Uses

Despite its potential and availability, little research was performed to determine the best method to collect, transport and store sugarcane straw. Literature describes two basic methods and variations of them to collect and transport straw: a) Harvesting machine partially separates sugarcane stalk and straw in the field - chopped stalk is collected and

transported to mill and straw is transported in bulk or in compacted form (baling system); b) Harvesting machine does not separate stalk and straw in the field - chopped stalk and straw are collected and transported to the mill where a cleaning station separates them before cane crushing (integral harvesting) (Leal et al., 2013).

Although some drawbacks of having a mulch of straw on the ground might impact sugarcane production (increase in risk of accidental burning, increase of pests and decrease of ratoon sprouting.) (T.F. Cardoso et al., 2015), the agriculture benefits are clear (protection against soil erosion, protection against water losses, increase in carbon content, weed inhibition, positive impacts on soil macro fauna). However, it is not possible today to define the proper amount of straw that should be left on the ground to take advantage of agricultural and industrial uses due to the presence of many variables (Leal et al., 2013).

According the ultimate and proximate analysis, the components of straw and bagasse have similar compositions, except moisture, chlorine, potassium, calcium and magnesium. This means that straw could potentially be burned in the bagasse boiler. The lower moisture content of straw (less than 35%) is a positive factor for burning purposes. On the other hand, the higher amount of chlorine and potassium in straw can contribute to corrosion in the boilers and deposition on hot surfaces (Leal et al., 2013).

1.6.Second Generation Biorefineries in Brazil

Biorefinery is an integrated industrial facility that converts biomass feedstock into desirable products, such as fuel, chemical and power. Biorefineries are sustainable alternatives to replace the traditional industry of fossil fuels (Fatih Demirbas, 2009). In a biorefinery context, different technologies have to be used simultaneously to convert biomass

to added value products. Those technologies can be divided in mechanical, chemical, biochemical and thermochemical processes (Cherubini, 2010).

Biofuels produced from biomass can be classified as first generation (1G fuels, produced from resources that competes with food industry - sugar, sucrose, starch, vegetal oil, animal fats) and second generation (2G fuels, produced from non-food biomass - lignocellulosic residues from agriculture, forestry and industry) (Cherubini, 2010). Third generation biofuels are produced from algae and fourth generation biofuels comprises the conversion of vegetal oil and biodiesel to biogasoline (Fatih Demirbas, 2009).

As described before, sugarcane industry is a well established biorefinery in Brazil that produces, among other products, 1G ethanol using sugarcane sucrose as raw material. Nowadays the challenge is to develop technologies able to completely utilize sugarcane residues (bagasse and straw) improving the environmental and economic feasibility of sugarcane industry.

Despite the Brazilian potential to supply available cellulosic biomass (bagasse and straw) to biorefineries at a competitive cost, as discussed above, commercial development of second generation biofuels and biomaterials is still limited. A facility to produce commercial lignocellulosic ethanol began operation in 2014 in Northeastern Brazil (Granbio - Bioflex I) to produce 82 million liters of ethanol per year. Other facilities under operation include: i) Raizen - Piraciaba/SP (biochemical conversion of bagasse to bioethanol – 39.7 million liters per year), ii) Cane Technology Center - Sao Manoel/SP (biochemical conversion of bagasse to bioethanol - 3 million liters per year). More work is needed to improve efficiency and decrease costs of the conversion technologies (Scientific Committee on Problems of the Environment, 2015).

2. Objectives

2.1. Chapter 3

The objectives of chapter 3 are:

- a) Compare the financial performance between a greenfield 2G ethanol facility and a co-located 2G ethanol plant where sugarcane bagasse and straw are used as feedstock.
- b) Perform a techno-economic analysis for the sugarcane cellulosic ethanol biorefinery using experimental data for autohydrolysis pretreatment and enzymatic hydrolyses in order to address the recalcitrance of each biomass.
- c) Compare the financial performance when sugarcane bagasse and straw are used as a feedstock for cellulosic ethanol production or as a fuel for power generation.

2.2. Chapter 4

The objectives of chapter 4 are:

- a) Evaluate total sugar recovery when mechanical refining and delignification are applied after pretreatment to improve enzymatic hydrolysis yield.
- b) Evaluate the changes of biomass physical properties (morphology, particle size, swellability, pore area and crystallinity) when mechanical refining is applied on pretreated sugarcane bagasse and straw.
- c) Understand how those physical changes can explain the improvement on enzymatic hydrolysis.

2.3.Chapter 5

The objectives of this chapter are:

- a) Evaluate the financial performance of a cellulosic ethanol facility when mechanical refining is used to increase sugar recovery
- b) Compare the financial performance of a cellulosic ethanol facility when two parameters of enzymatic hydrolysis are changed: enzyme dosage (3FPU/g biomass; 5 FPU/g biomass) and residence time (24h, 48h, 72h, 96h).
- c) Perform deterministic and probabilistic techno-economic analysis to identify the major economic drivers and evaluate the financial impact of their volatility and variability on internal rate of return, net present value and minimum ethanol selling price.

3. Techno-Economic Analysis to Compare Ethanol and Power Production using Bagasse and Straw as Feedstocks in Co-located and Greenfield Plants

3.1.Introduction

The sugarcane industry is a well established biorefinery model in the world and Brazil has a remarkable participation in this market. During the 2014/2015 season, Brazil produced 634.8 million tonnes of sugarcane (40% of the global production) that were used by sugarcane mills to produce 35.6 million tonnes of sugar (21% of the world production) and 28.7 billion liters of ethanol (25% of the world production) (Companhia Nacional de Abastecimento, 2016).

Each tonne of sugarcane or sugarcane stalks (70% moisture content) generates approximately i) 145 kg of dry sugars (mainly sucrose) used to produce sugar and ethanol, ii) around 135 kg of dry bagasse (mainly fibers) which is usually burned in biomass boilers to produce steam and power and iii) around 140 kg of dry straw or trash (tops and leaves) which has been traditionally burned in the sugarcane fields before non-mechanized harvesting (Brazilian Bioethanol Science and Technology Laboratory - Technological Assessment Program, 2011).

Conventionally, about 10% of the total bagasse produced inside a sugarcane mill is available for other uses. The remaining bagasse is usually burned in biomass boilers to meet the energy demand of the process. This is a common practice, for example, in the case of annexed distilleries operating with low pressure boilers (21 bar). Nowadays, with the development of modern sugarcane mills in Brazil (high pressure boilers - 65 to 90 bar, high

efficiency turbogenerators and low process steam consumption ~ 360 kg/tonne of sugarcane) bagasse savings of up to 50% can be achieved (Centro de Gestao e Estudos Estrategicos, 2009). Additionally, due to social and environmental concerns, a federal law to end the burning practices by 2018 was put in place. One of the consequences from this law is the increasing amount of sugarcane straw left on the ground after mechanical harvesting. As a result, more cellulosic biomass is available to be used as a fuel for power generation or as a feedstock for the production of biofuels and biomaterials (Leal et al., 2013).

Despite the intense worldwide research to improve conversion technologies, global effort to ramp up the industrial production and Brazilian potential to provide available sugarcane biomass to biorefineries, the commercial deployment of second generation biofuels in Brazil is still limited. A facility to produce commercial lignocellulosic ethanol began operation in 2014 in Northeastern Brazil (Granbio - Bioflex I) to produce 82 million liters of ethanol per year. Other facilities include: i) Raizen - Piraciaba/SP (biochemical conversion of bagasse to bioethanol – 39.7 million liters per year), ii) Cane Technology Center – Sao Manoel/SP (biochemical conversion of bagasse to bioethanol - 3 million liters per year). Several aspects were identified as barriers for the profitable production of cellulosic ethanol: feedstock costs, availability and variability, high enzyme costs, high capital investment and low market ethanol selling price (Gonzalez, Treasure, Phillips, Jameel, & Saloni, 2011). In order to improve the economic feasibility of cellulosic biorefineries, more work is needed to improve efficiency, decrease capital and decrease costs of the conversion technologies. Improvements in microorganisms, enzymes, pentose fermentation and development of added value co-products (such as lignin) are also needed (Scientific Committee on Problems of the Environment, 2015).

Previous techno-economic studies have used sugarcane bagasse and straw as a feedstock to produce biofuels and power as a tentative to identify potential scenarios, products, technologies and process configurations that could increase the profitability of sugarcane biorefineries.

Many publications compare the financial performance of 1G ethanol sugarcane mills (annexed or autonomous distilleries operating during sugarcane season ~ 6 to 7 months) with 1G2G ethanol integrated plants (usually operating during sugarcane season) and 2G ethanol stand-alone plants in a greenfield scenario. Previous studies have concluded that the economic performance of the 2G ethanol would have a potential to compete with the 1G ethanol when the 2G ethanol process is operated under favorable conditions, such as, high solids loading in pretreatment (30%), low enzyme dosage (2 to 5 FPU/g of biomass), high solids loading in enzymatic hydrolysis (15% to 20%), high cellulose conversion (70% to 85%), high hemicellulose conversion (> 70%), short residence time in enzymatic hydrolysis (48 h), high conversion of C5 sugars to ethanol (80%) (Albarelli, Ensinas, & Silva, 2014), (Wang, Quiceno, Price, Malpas, & Woods, 2014), (Corrêa do Lago, Bonomi, Cavalett, Pereira da Cunha, & Pinheiro Lima, 2012), (Dias et al., 2012), (Dias et al., 2011). Furthermore, sensitivity analysis has shown that ethanol price and capital investment are the major drivers on the economics of 2G ethanol. Prices for enzyme, electricity and straw have lower impact on the financial performance. (Dias et al., 2012), (Seabra & Macedo, 2011). It is clear that any effort to improve the 2G ethanol feasibility has to be focused on the reduction of capital investment and use of water, improvement of sugar recovery (mainly in enzymatic hydrolysis) and fermentation technologies, reduction of costs (enzymes, raw material).

Co-location of a new facility in an existing one is a good alternative to reduce capital investment while provides the opportunity of sharing the existing supply chain of biomass when compared to a greenfield scenario. The co-location of a 2G ethanol plant inside a 1G ethanol facility has the potential to save capital by sharing the existing facilities (land, roads, offices, warehouse, water plant, utilities, process equipment) when possible (Han et al., 2015), (Phillips, Jameel, & Chang, 2013).

Another factor that impacts the capital investment in the case of sugarcane mills is the total number of operating hours. As mentioned before, many authors consider that the integrated 1G2G ethanol facility operates only during the sugarcane season, which considerably increases the installed capacity of 2G ethanol plant when compared to a whole year operation (up to two times bigger for the same annual production), resulting in higher capital investment. However, the whole year operation will require additional investment to store and handle the cellulosic biomass produced during the sugarcane season. Additionally, it is also known that the storage of sugarcane bagasse and straw may result in significant biomass degradation, especially under moist and aerobic conditions (up to 20% to 25% loss) (Purchase, Rosettensten & Bezuidenhoudt, 2013), (J. Lois-Correa, Flores-Vela, Ortega-Grimaldo, & Berman-Delgado, 2010), (Hurter, 1991).

In order to maximize the sugar recovery during pretreatment and enzymatic hydrolysis, avoiding the degradation of carbohydrates and formation of inhibitory byproducts, it is important to match the composition and recalcitrance of the biomass with the type and severity of the pretreatment (Phillips et al., 2013), (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010), (Sun & Cheng, 2002). However, the process parameters usually adopted for

pretreatment and enzymatic hydrolysis in techno-economic studies do not properly address the differences in characteristics and recalcitrance between sugarcane bagasse and straw.

Another point to be highlighted is the comparison between sugarcane bagasse and straw as a fuel for the production of steam and power. It is well known that heating value, moisture content, composition and ash properties of cellulosic biomasses significantly impact the design and operation of combustion systems (Sheng & Azevedo, 2005).

Different chemical compositions will result in different high heating values (HHV). Usually, it is accepted that cellulose has a uniform chemical formula which results in a practically constant HHV of 18.6 MJ/kg. On the other hand, hemicellulose and extractives are more heterogeneous materials and their composition depends on the type of biomass and analysis procedure. Lignin is also composed by various chemical structures and values of HHV from 23.3 to 25.6 MJ/kg are usually adopted (Sheng & Azevedo, 2005).

Higher moisture content will negatively impact the net heating value. The HHV of moisture free bagasse was reported as 19650 kJ/kg of bagasse. However, if 50% is assumed as the usual moisture content of bagasse, the low heating value (LHV) becomes 7929 kJ/kg of wet bagasse (Sosa-Arno, De Oliveira, Corrêa, Silva, & Nebra, 2004). The moisture content of straw is usually lower than bagasse and it will depend on the method and percentage of field collection. Integral harvesting will result in higher moisture content (up to 35%). In balling system, straw is left on the ground before its collection to reduce the moisture content (10 to 15%), which is an advantage for burning purposes (T F Cardoso et al., 2013), (Leal et al., 2013).

According to ultimate and proximate analysis, straw and bagasse have similar compositions, except for chlorine, potassium, calcium and magnesium. This means that straw

could potentially be burned in bagasse boilers. On the other hand, the higher amount of chlorine and potassium in straw can contribute to corrosion in the boilers and deposition on hot surfaces (Leal et al., 2013). Also, if trash is collected by baling system, the amount of mineral impurities incorporated in the biomass will increase with a higher percentage of collection because part of the soil will inevitably be collected with the straw. Higher ash content will decrease the heating value of straw and impact the maintenance of boilers and blowers (T F Cardoso et al., 2013).

Therefore, the objective of this work is to compare the financial performance between a greenfield and a co-located 2G ethanol facility. The impact of extended operation of 2G ethanol plants after sugarcane season will also be assessed. Experimental data for autohydrolysis pretreatment and enzymatic hydrolysis for bagasse and straw will be evaluated in order to address the recalcitrance of each biomass. The use of bagasse and straw as a feedstock for cellulosic ethanol production or as a fuel for steam and power generation will also be evaluated from a financial perspective.

3.2. Materials and Methods:

3.2.1. Feedstock - Sugarcane Bagasse and Straw

Sugarcane bagasse and straw were kindly provided by CTBE (Brazilian Bioethanol Science and Technology Laboratory). Those feedstocks were air dried, characterized (Table 3.6) using a Laboratory Analytical Procedure published by (National Renewable Energy Laboratory, 2011a) and stored at room temperature.

3.2.2. Autohydrolysis Pretreatment

Autohydrolysis pretreatment was executed in a 300 L stainless steel reactor using 15 kg using 1:10 solid:liquid ratio. After pretreatment, the liquid fraction was collected and analyzed to determine sugars, organic acids and degradation products using HPLC (National Renewable Energy Laboratory, 2006). The solid fraction was washed with sufficient tap water to eliminate any dissolved biomass and until neutral pH was achieved. The washed solid was also characterized.

3.2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis was performed in 50 mL tubes using 5 FPU/g (dry basis) of pretreated and washed substrates, 10% consistency, pH 4.8~5.0, 50°C and 96h. Novozymes Cellic CTec 2 supplemented with 1/9 Cellic HTec 2 was used as the enzyme cocktail. Sodium acetate buffer was used for pH control. The incubator (Fine PCR COMBI- D24) was maintained at 50 °C and 15 rpm. After enzymatic hydrolysis, the tubes were centrifuged for 10 min at 4400 rpm using an Eppendorf Centrifuge 5702. The supernatant was used for sugar determination (National Renewable Energy Laboratory, 2006).

3.2.4. Process Simulation - Aspen Plus

A steady state process simulation was built using Aspen Plus 8.2.3. The base case simulation represented by an Autonomous Distillery was kindly provided by CTBE (Brazilian Bioethanol Science and Technology Laboratory). The proposed cases represented by the cellulosic ethanol biorefinery were also built in Aspen according to (National Renewable Energy Laboratory, 2011b). Experimental data for autohydrolysis pretreatment

and enzymatic hydrolysis were used in the process simulation. Detailed information about the process configuration and parameters are presented in the following sections.

3.2.4.1. Base Case - Autonomous Distillery (1G ethanol)

This section presents a short description of the process steps for a Sugarcane Autonomous Distillery (see Figure 3.1). This distillery represents a standard technology used for ethanol production and it will be used as the base case in this study.

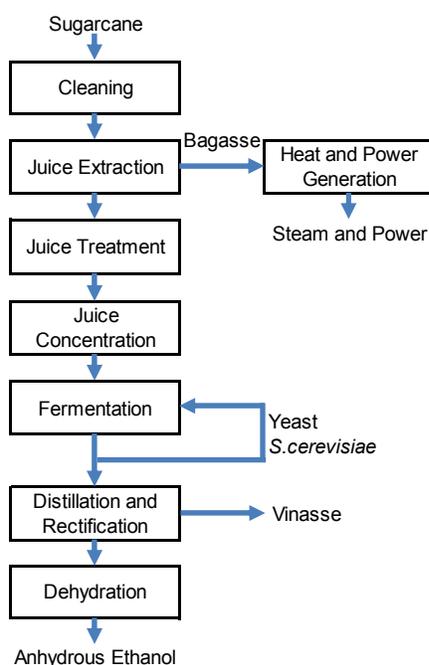


Figure 3.1. Process Diagram - Sugarcane Autonomous Ethanol Distillery.

The base case represents a sugarcane milling capacity of 500 metric ton of sugarcane (TC) per hour and operates 166 days per year during sugarcane harvesting season, resulting in an annual capacity of 2,000,000 TC/y (average milling capacity of sugarcane mills in Brazil). After reception, sugarcane is cleaned to remove sand/soil/organic matter and sent to

a series of preparation equipment (shredders, hammers) used to cut and open sugarcane fibers. Sugarcane juice extraction is done by crushing mills where hot water is used as imbibition in a counter current process. Next process step is called juice treatment and comprehends a chemical treatment to remove soluble impurities (proteins, colloids) and the remaining insoluble material. In this process, the sugarcane juice is heated and receives the addition of phosphoric acid, lime and flocculants. The sugarcane juice is sent to a settler where mud and clarified juice are separated. The obtained mud is then filtered to minimize sugar loss and the cake produced after filtration is used as fertilizer. The clarified juice is sent to a multiple effect evaporator to be concentrated before fermentation. The fermentation process is an anaerobic fed batch fermentation with *Saccharomyces cerevisiae*. During fermentation, sugars are converted into ethanol, carbon dioxide and other byproducts (acetic acid, glycerol, isobutanol, isoamyl alcohol). The final broth obtained at the end of the fermentation is sent to centrifugal separation. After centrifugation, the concentrated yeast stream is recycled and receives an acid treatment to control bacterial contamination and the final wine is sent to distillation. The distillation facility comprehends a set of distillation and rectification columns to produce hydrated ethanol (93% w/w). The obtained vinasse is sent to the sugarcane field to be used for fertirrigation. Fusel oil (higher alcohols as isobutanol, isoamyl alcohol) is removed as a side withdraw in rectification column. Molecular sieves are used to produce anhydrous ethanol with a concentration of 99.6% w/w. Sugarcane bagasse is burned in the Combined Heat and Power (CHP) generation area to produce steam (65 bar boilers) and power. A portion of the power produced is consumed inside the mill and the surplus power is sold to the grid. 2.2% of the available bagasse is used as a filtration medium

in the sucrose juice treatment and 5% of the available bagasse is stored to be used as a reserve for plant start-ups.

Table 3.1 shows the main process parameters adopted in the autonomous distillery giving emphasis to the CHP area, which is used for process integration between 1G and 2G ethanol facilities. Detailed information about process assumptions and parameters can be found in the report published by (Brazilian Bioethanol Science and Technology Laboratory - Technological Assessment Program, 2011).

Table 3.1. Main Process Parameters - Sugarcane Autonomous Distillery.

Process parameter	Unit	Value
Plant Capacity	Metric ton sugarcane/year	2,000,000
Operation	days	167
Ethanol Production	Liters/metric ton sugarcane	83
Power Consumption (with electric drivers)	kWh/metric ton sugarcane	30
Process Steam Consumption	Kg/metric ton sugarcane	351
Boiler Pressure	bar	65
Steam Temperature	°C	485
Boiler Efficiency - LHV basis	%	88
Boiler Outlet Gases Temperature	°C	160
Turbine Isentropic Efficiency - high pressure	%	72
Turbine Isentropic Efficiency - medium pressure	%	81
Condensation Isentropic Efficiency	%	70
Condensing Pressure	bar	0.11
Generator Efficiency	%	98
Deaerator Pressure	bar	1.4
Deaerator Temperature	°C	105
Process Condensate Losses	%	5
Reserve of Bagasse for Boiler Start-Ups	%	5

3.2.4.2. Proposed Case: Cellulosic Ethanol Biorefinery (2G ethanol)

The proposed case comprehends a cellulosic ethanol biorefinery based on the biochemical transformation of sugarcane bagasse and/or straw into ethanol using

autohydrolysis pretreatment, enzymatic hydrolysis and co-fermentation of pentoses and hexoses as conversion technologies (see Figure 3.2). This facility operates 350 days per year.

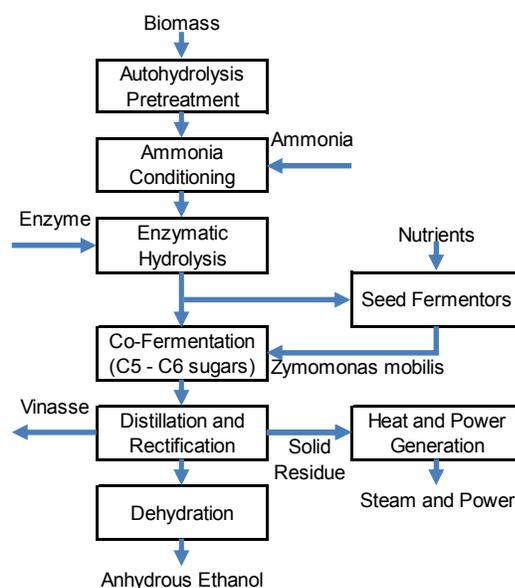


Figure 3.2. Process Diagram - Cellulosic Ethanol Biorefinery

In this process, biomass goes to the autohydrolysis pretreatment where steam is used as the heat source to achieve reaction temperature and pressure. During pretreatment, carbohydrates present in the biomass are partially hydrolyzed (mainly hemicellulose) and lignin is partially solubilized, decreasing biomass recalcitrance. At the end of pretreatment, a flash is used to decrease the system pressure to 1 atmosphere, stopping pretreatment reactions. The pretreatment product (cellulignin slurry) receives the addition of ammonia to neutralize the organic acids produced during the pretreatment (mainly acetic acid) and raise the pH for enzymatic hydrolysis. Following this step, the slurry is cooled to reach the optimum temperature for enzymatic hydrolysis, where an enzyme cocktail hydrolyzes sugar oligomers (mainly hemicellulose) and polymers (mainly cellulose) to fermentable sugars

(mainly glucose and xylose) in batch reactors. After enzymatic hydrolysis, the saccharified slurry is cooled and inoculated with the co-fermenting microorganism *Zymomonas mobilis*. In this process, 10% of the saccharified slurry is split to seed production. After fermentation, the final broth is sent to product purification where ethanol is distilled near to the azeotropic mixture with water and dehydrated to 99.5% w/w using molecular sieves. The stillage removed in the bottom of the beer column will be sent to a solid/liquid separation filter. After separation, the solid cake composed by the unreacted biomass (rich in lignin) and fermentation cell mass will be burned in the CHP area for steam and power production. The liquid vinasse would be used for fertirrigation of the sugarcane fields the same way sugarcane mills do in Brazil with the first generation ethanol stillage. Table 3.2 shows the main process parameters assumed in the simulation. Most of the process assumptions and parameters adopted here were taken from (National Renewable Energy Laboratory, 2011b) and more details can be found in this reference.

Table 3.2. Main Process Parameters - Cellulosic Ethanol Biorefinery

Area	Process parameter	Unit	Value
Autohydrolysis Pretreatment	Consistency	%	20
Enzymatic Hydrolysis	Temperature	°C	50
	Consistency	%	10
Fermentation	Glucose to Ethanol	%	95
	Xylose/Arabinose to Ethanol	%	85
Ethanol Purification	Ethanol Recovery	%	99.5

3.2.5. Studied Cases: Techno-Economic Comparison Between Greenfield and Co-located Sugarcane Cellulosic Ethanol Biorefinery

In this chapter, 6 scenarios will be studied according to Table 3.3. For the co-location alternatives, a cellulosic ethanol biorefinery will be co-located in an existing autonomous

distillery (base case) that crushes 2,000,000 TC/y. For the greenfield cases, sugarcane biomass will be shipped to the cellulosic ethanol biorefinery from nearby sugarcane mills and sugarcane plantations.

Table 3.3. Studied Scenarios for Co-location and Greenfield Allocations

Scenario	Ethanol Plant	Plant Allocation	Operating days	Biomass to 2G Ethanol	Biomass to CHP
0	1G	Greenfield 2MTC/y	167	-	Bagasse
1-CL-BAG-Y	1G2G	Co-location 2 MTC/y	1G = 167 2G = 350	Bagasse	Solid Residue and Straw
2-CL-BAG-S	1G2G	Co-location 2 MTC/y	1G and 2G = 167	Bagasse	Solid Residue and Straw
3-GF 1-BAG-Y	2G	Greenfield 1	350	Bagasse	Solid Residue and Straw
4-GF 2-BAG-Y	2G	Greenfield 2	350	Bagasse	Solid Residue and Straw
5-GF 3-BAG-Y	2G	Greenfield 3	350	Bagasse	Solid Residue and Straw
6-CL-STW-Y	1G2G	Co-location 2 MTC/y	1G = 167 2G = 350	Straw	Solid Residue and Bagasse
7-GF 3-STW-Y	2G	Greenfield 3	350	Straw	Solid Residue and Bagasse

CL = co-location; GF = greenfield; BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant; Y = year operation (350 days); S = season operation (167 days)

Scenario 0 (Figure 3.1) represents an autonomous distillery that crushes 2,000,000 tonne of sugarcane per year and burns the available bagasse to produce steam and power. Scenario 1 (Figure 3.3) considers that all bagasse will be send to cellulosic ethanol plant. Solid residues and straw will be burned in the CHP area to produce steam to meet the demand of both facilities (1G and 2G plants). Excess steam is converted to power and excess power is sold to the grid. Scenario 2 is similar to Scenario 1, however, Scenario 2 considers that both ethanol plants (1G and 2G) operate only during sugarcane season.

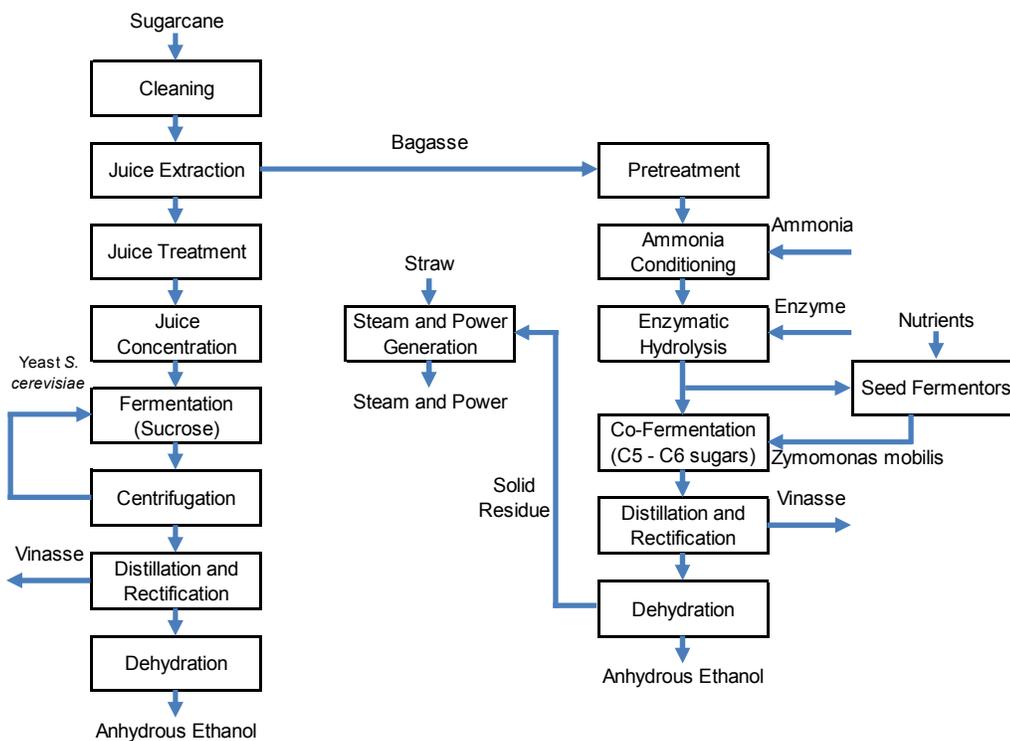


Figure 3.3. Scenario 1 - Bagasse to Cellulosic Ethanol; Straw to CHP

Scenarios 3, 4 and 5 (see Figure 3.4) represents greenfield plants that convert sugarcane bagasse to cellulosic ethanol with 3 different capacities. Sugarcane straw will be collected in the field and burned with solids residues. Scenarios 6 and 7 are similar to Scenario 1 and 5 respectively, however, Scenarios 6 and 7 use straw as feedstock for ethanol production.

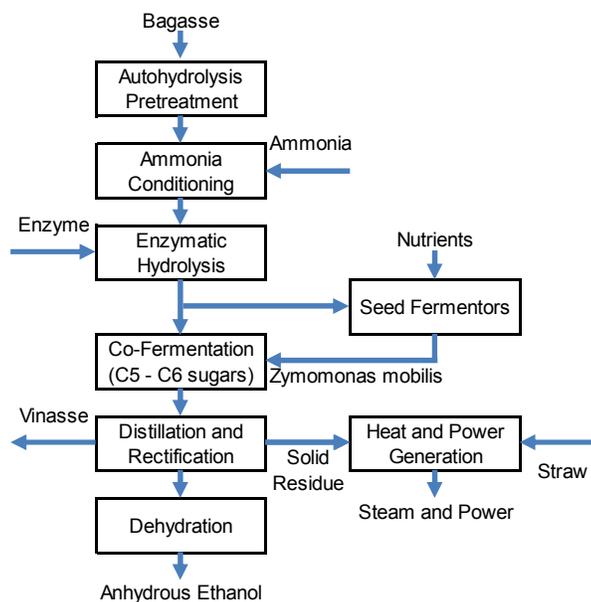


Figure 3.4. Scenarios 2, 3, 4 - Bagasse to Cellulosic Ethanol; Straw to CHP

3.2.6. Economic Analysis and Financial Assumptions

The mass and energy balances obtained from simulations were used as input data for the economic analysis. A traditional cash flow was built using a spreadsheet. The following sections describes the prices and costs of raw materials and products, capital investment and financial assumptions used to calculate operating costs, CAPEX and cash flow. Internal rate of return (IRR), defined as the discount rate that gives a zero net present value (NPV), was used as a metric to evaluate the return on investment. The NPV is defined as the sum of discounted free cash flow at target rate of return for each project year.

3.2.6.1. Biomass Productivity, Transportation Distance, Biomass Availability

The sugarcane productivity adopted in this study is 75 tonne/ha/year, and represents the average productivity for center-south region from 2005 to 2015 (Companhia Nacional de

Abastecimento, 2016). As reported by (Companhia Nacional de Abastecimento, 2013), the average distance to transport sugarcane from the field to sugarcane mills in Brazil is around 25 km. This distance was considered to calculate the costs associated with the transport of bagasse and straw.

As mentioned in the introduction, 1 tonne of sugarcane yields 140 OD kg of straw and the development of mechanized harvesting during the past years has increased straw availability. Although some drawbacks of having a mulch of straw on the ground might impact sugarcane production (increase in risk of accidental burning, increase of pests and decrease of ratoon sprouting.) (T.F. Cardoso et al., 2015), the agriculture benefits are clear (protection against soil erosion, protection against water losses, increase in carbon content, weed inhibition, positive impacts on soil macro fauna). However, it is not possible today to define the proper amount of straw to be left on the ground to take advantage of agricultural and industrial uses due to the presence of many variables (Leal et al., 2013) This work has assumed that 50% of the straw is collected and transported to the biorefineries.

It was also discussed that 1 tonne of sugarcane yields 135 kg of dry bagasse. Its majority is consumed by the sugarcane mills as an energy source to produce steam and electricity, and the availability of bagasse in a specific area depends on the configuration (type of mill, boiler configuration, process configuration) and energy consumption of each particular sugarcane mill. However, as the purpose of this work is to study the economic feasibility of biorefineries with focus on the industrial technology, no restrictions about biomass availability and supply chain were considered.

3.2.6.2. Biomass Cost

The cost of straw depends on the method of collection. There are two basic ways to harvest sugarcane and collect straw: a) Baling System (BS): harvesting machine partially separates sugarcane stalk and straw in the field - chopped stalk is collected and transported to mill. Straw is disposed on the ground, baled and transported; b) Integral System (IS): machine does not separate stalk and straw in the field - chopped stalk and straw are collected and transported to the mill where a cleaning station separates them before sugarcane crushing (Leal et al., 2013). Both options have advantages and disadvantages that need to be considered when the best option for a particular case has to be defined. IS decreases load density in transportation, however, stalk losses are minimized. BS requires more agricultural operations, however, it does not decrease stalk transport efficiency and can be a good option for long distances. Moreover, the decision for one technology has to consider not only the agricultural aspects (transport distance, load density, straw recovery) but also the impact in sugarcane industry (capital for sugarcane dry cleaning, sugar recovery in juice extraction, steam production, boiler operation) (T.F. Cardoso et al., 2015).

The costs for sugarcane straw collection used in this work are based on the work published by (T.F. Cardoso et al., 2015). They performed a statistical study considering tree variables (straw recovery fraction: 30 to 70%, distance: 20 to 50 km, sugarcane productivity: 70 to 100 tonne/ha/year) to determine straw collection costs for BS and IS. They also evaluated the economic impact of those options on a greenfield autonomous distillery (4 Mtonne sugarcane/year) that burns bagasse and straw to produce steam and electricity. Table 3.4 shows that the costs to collect straw using IS were about 40% cheaper for the studied cases when compared to BS due to lower use of machinery and reduction of stalk losses. On

the other hand, the additional capital investment (+1% average) for a sugarcane dry cleaning station, lower ethanol production (-0.9% average) due to higher sugar losses in juice extraction and lower production of electricity (-6.4% average) due to higher moisture content in straw, increases capital investment and reduces revenue in the case of IS when compared to BS. As a result, the economic analysis for the autonomous distillery shows that the internal rate of return is basically the same for both technologies (T.F. Cardoso et al., 2015).

Therefore, the cost of straw collection adopted in this study will assume the baling system to avoid any negative impact in the existing sugarcane mills. Equation 3.1 and Equation 3.2 will be considered to calculate the cost of straw collection, where P is sugarcane productivity (tonne/ha.year), D is distance (Km) and R is straw recovery (%). Additional 10% in the costs is included to account for the payment to the land owner.

The price of sugarcane bagasse usually depends on regional factors, such as, availability (as discussed before) and final application. However, it is known that the majority of the sugarcane bagasse (more than 90%) is burned in biomass boilers to produce steam and power. Therefore, it was assumed an opportunity cost for sugarcane bagasse based on the potential revenue that could be achieved by selling electricity.

$$\text{Straw Cost (US$/OD tonne)} = 27.98873 - 2.02231 * X_1 + 1.17271 * X_2 - 3.19251 * X_3 + 0.89587 * X_3^2$$

Equation 3.1

$$X_1 = \frac{P - 85}{8.9191}; X_2 = \frac{D - 35}{8.9191}; X_3 = \frac{R - 50}{11.8921}$$

Equation 3.2

Table 3.4. Sugarcane Straw and Stalk Costs (T.F. Cardoso et al., 2015)

Variables			Straw Cost (US/OD tonne)		Stalk Cost (US/wet tonne)		IRR (%)*	
Sugarcane Productivity (tonne/ha)	Transport Distance (Km)	Straw Recovery (%)	BS	IS	BS	IS	BS	IS
76.1	26.1	38.1	32.87	13.84	25.58	25.15	19.3	19.9
76.1	26.1	61.9	26.41	13.46	25.58	24.96	20.0	20.4
76.1	43.9	38.1	35.36	21.32	26.95	26.74	17.8	17.9
76.1	43.9	61.9	28.90	21.13	26.95	26.65	18.4	18.2
93.9	26.1	38.1	28.55	11.76	20.98	20.64	24.1	24.3
93.9	26.1	61.9	22.87	11.63	20.98	20.50	24.6	24.7
93.9	43.9	38.1	30.77	18.47	22.19	22.06	22.8	22.8
93.9	43.9	61.9	25.09	18.50	22.19	22.00	23.3	22.9
70	35	50	31.57	18.27	28.43	27.97	16.5	16.9
100	35	50	24.82	14.33	20.38	20.12	24.9	24.8
85	20	50	25.95	10.23	22.60	22.04	22.8	23.2
85	50	50	29.88	22.26	24.75	24.64	20.5	20.1
85	35	30	36.32	16.22	23.67	23.45	21.1	21.4
85	35	70	24.82	17.29	23.67	25.77	22.0	21.9
85	35	50	27.90	16.23	23.67	23.34	21.7	21.7

*IRR for the autonomous distillery

3.2.6.3. Biomass Storage

Deterioration of biomass fibers is caused by the attack of cellulolytic micro-organisms, however, the attack is minimal under dry or anaerobic conditions. In the case of bagasse and straw, the deterioration is rapid because their structure is open and reactive, especially in the case of bagasse, where the presence of free sugars in an open structure, resulted from cane crushing, accelerates the process (Hurter, 1991).

The free sugars and water soluble compounds present in bagasse are attacked by aerobic and anaerobic micro-organisms with release of considerable amount of heat (mainly at aerobic conditions) and production of water, carbon dioxide, alcohol and organic acids. Aerobic and anaerobic cellulolytic micro-organisms will not appreciably deteriorate the fibers if biomass is fully dried. Under aerobic conditions, the deterioration of fibers is rapid when moisture content is over 25%. Under anaerobic conditions, the process is slower but

can be accelerated by thermophilic micro-organisms when temperature is over 40°C and pH is high (Hurter, 1991).

There are different ways to store bagasse or straw, including storage in compacted form (bales) or bulk storage. In the case of bales, it is not possible to have anaerobic conditions and it is important to dry the bales to 15 - 25% moisture as fast as possible. The option for smaller bales stored in a pattern that allows air circulation might contribute to accelerate the drying process and heat dissipation. Baling storage involves fire risk, additional costs with baling but it may reduce transportation costs. (Hurter, 1991), (Purchase et al., 2013). Moist bagasse bales (50% moisture) presented storage losses of up to 20 - 25% over a period of 6 weeks, while dried bales (25% moisture) presented mass losses around 3% after 6 weeks and losses of up to 6.5% after a period of 17 weeks (J. Lois-Correa et al., 2010).

In the case of bulk storage, sugarcane biomass can be storage in moist piles or wet piles. In moist piles, the presence of air, water and absence of air circulation will cause fast deterioration. Fire might occur due to the heat released by fermentation of free sugars. Deterioration can be reduced in compacted and covered piles to avoid water and oxygen ingress. In wet piles, bagasse is saturated with water (> 80% moisture) which causes the displacement of air avoiding aerobic conditions. When temperatures are below 40°C, free sugars will be fermented by yeasts but the attack on fibers by cellulolytic micro-organisms is insignificant. At temperatures above 40°C, the acidification of the pile (organic acids produced by micro-organisms or addition of acid) inhibits the fiber degradation by thermophilic micro-organisms. It is important to maintain the pile wet and compact, and maximize pile size to diminish the impact of surface deterioration. Wet piles are usually used for pulping purposes and high content of water represents a disadvantage for combustion. A

well maintained wet pile can store bagasse with minimal deterioration over a year. Mass losses in wet piles is around 2% to 5% over a period of 2 to 20 weeks (Hurter, 1991), (Purchase et al., 2013). In this work it was assumed a storage loss of 5% for sugarcane straw bales and 10% loss for storage of bagasse in compacted and covered piles.

3.2.6.4. Enzyme Cost

In this work it was assumed that the enzyme will be purchased from an enzyme supplier at US\$ 5.00/kg of protein delivered to the ethanol biorefinery (National Renewable Energy Laboratory, 2011b). An average enzymatic activity of 0.65 FPU/mg of protein was adopted to calculate the costs of enzymes based on the dosage of Cellic CTec 2 and HTec 2

3.2.6.5. Ethanol and Electricity Prices

The adopted ethanol price was collected from (Center for Advanced Studies on Applied Economics, 2016) representing the average price of ethanol in 2015 in Sao Paulo state. Electricity price was calculated as the average price of the sugarcane bioelectricity auctions from 2005 to 2015 escalated for 2015 dollars (Camara de Comercializacao de Energia Eletrica, 2016).

3.2.6.6. Capital Investment

Capital investment was estimated using data reported by (National Renewable Energy Laboratory, 2011b). Equipment cost were scaled using 0.60 as scaling exponent for all areas, except for storage (1.0) and pretreatment (0.85). “Producer Price Index Industry Data” for “Other Heavy Machinery Rental and Leasing” was used to escalate equipment costs to 2015.

Scaled and escalated equipment cost were multiplied by 2.6 to calculate the total installed costs for each process. Biomass Storage includes a concrete floor and trucks to store and transport biomass to CHP area or cellulosic ethanol plant. Bagasse is stored in compacted and covered piles to minimize degradation. Infrastructure (Land, Roads, Offices, Water Plant) is 15% of the installed cost for storage, pretreatment, enzymatic hydrolysis, fermentation and ethanol purification.

3.2.6.7. Financial Assumptions

Table 3.5 brings the financial assumptions adopted in this work.

Table 3.5. Financial Assumptions

Start up year	2015
Terminal Year	2030
1G ethanol Plant Operating Hours per Year	3976
2G ethanol Plant Operating Hours per Year	8400
Capital Spending in year -3 - %	5
Capital Spending in year -2 - %	15
Capital Spending in year -1 - %	30
Capital Spending in year 0 - %	50
Capital Investment in Maintenance and Improvement - % RAV*	2
Depreciation years	10
Nominal Capacity in 2015 - %	60
Nominal Capacity in 2016 - %	90
Working Capital - % Revenue + Direct Costs**	10
Overhead and Administration Costs- % Revenue	3
Maintenance (labor + materials), Operating Materials, Other Costs - % RAV	5
Taxes - %	34
Project Terminal Value - Multiple of EBITDA*** in year 15	5
Internal Rate of Return - %	12
Ethanol Price - US/L	0.46
Electricity Price - US/MWh	60.32
Bagasse Opportunity Delivered Cost - US/OD tonne	48.70
Bagasse Moisture - %	50
Straw Delivered Cost - US/OD tonne	31.84
Straw Moisture - %	15
Enzyme Delivered Cost - US/kg protein	5.00

*Capital investment for continuous improvement and reduction of costs as a percentage of RAV (Replacement Asset Value)

**Direct Costs = Biomass, Chemical, Enzyme and Electricity

***EBITDA = Earnings Before Interests, Taxes and Depreciation

All costs and prices are in 2015 Dollars - US\$1.00 = R\$3.33

3.3. Results and Discussion

3.3.1. Biomass Composition

Table 3.6 brings the chemical composition of sugarcane bagasse and straw used in this study. Results obtained are in the range of chemical composition for sugarcane biomass found in the literature (Rocha, Nascimento, Gonçalves, Silva, & Martín, 2015), (Szczerbowski, Pitarelo, Zandoná Filho, & Ramos, 2014), (Canilha et al., 2012). High content of ash in sugarcane straw is a result of high content of soil collected with the biomass.

Table 3.6. Sugarcane Bagasse and Straw Composition

Biomass Composition	Bagasse - % w/w	Straw - % w/w
Glucan	41.15 ± 0.36	34.32 ± 0.82
Pentosan*	26.30 ± 0.82	25.66 ± 1.45
Acetil Groups	2.87 ± 0.13	1.96 ± 0.02
Lignin	21.91 ± 0.08	23.65 ± 0.72
Extractives	6.45 ± 0.00	9.67 ± 0.41
Ash	1.88 ± 0.00	5.77 ± 0.20
Total	100.56 ± 1.24	101.03 ± 1.35

*Xylan + Arabinan

3.3.2. Sugar Recovery - Pretreatments and Enzymatic Hydrolysis

Sugarcane bagasse and straw were pretreated at 190 °C and 10 min. Pretreatment conditions were taken from (Santucci, Maziero, Rabelo, Curvelo, & Pimenta, 2015). Table

3.7 and Table 3.8 present pretreatment mass yield, pretreatment conversions and enzymatic hydrolysis conversions for each biomass. For simulation purposes, it was assumed that acetyl groups present in the biomass were completely converted to acetic acid during pretreatment and sugar oligomers released during pretreatment will be converted to sugar monomers during enzymatic hydrolysis.

Pretreatment mass yield was 10% higher in the case of bagasse (Table 3.7). This behavior was also observed by (Silva, Arruda, Felipe, Gonçalves, & Rocha, 2011), (Oliveira, Nascimento, Gonçalves, & Rocha, 2014) when both biomasses were pretreated using same reaction system and under same pretreatment conditions. Degradation of glucan was similar for both pretreatments (5 to 6%). However, degradation of pentosan was higher during straw pretreatment (17%) when compared to bagasse pretreatment (4%). Enzymatic hydrolysis of carbohydrates was 10% higher in the case of pretreated straw and similar difference was also observed by (Oliveira et al., 2014) and (Silva et al., 2011). Lower solubilization of biomass components, lower degradation of sugars and lower enzymatic hydrolysis yield suggests that bagasse is more recalcitrant than straw.

Table 3.7. Pretreatment Reactions and Conversions (190°C / 10 min)

Reactions	Reactant	% Converted to Product	
		Bagasse	Straw
$(\text{Glucan})_n + n\text{H}_2\text{O} \rightarrow n\text{Glucose}$	Glucan	0.00	0.26
$(\text{Glucan})_n + n\text{H}_2\text{O} \rightarrow n\text{Glucose Oligomer}$	Glucan	3.52	6.57
$(\text{Glucan})_n \rightarrow n\text{HMF} + 2n\text{H}_2\text{O}$	Glucan	0.12	0.34
$(\text{Glucan})_n \rightarrow \text{Formic Acid} + \text{Levulinic Acid}$	Glucan	0.94	1.74
$(\text{Glucan})_n \rightarrow \text{Unkown}^*$	Glucan	5.65	3.56
$(\text{Pentosan})_n + n\text{H}_2\text{O} \rightarrow n\text{Xylose/Arabinose}$	Pentosan	8.93	7.06
$(\text{Pentosan})_n + n\text{H}_2\text{O}$	Pentosan	51.70	42.75
$(\text{Pentosan})_n \rightarrow n\text{Furfural} + 2n\text{H}_2\text{O}$	Pentosan	3.56	3.48
$(\text{Pentosan})_n \rightarrow \text{Unkown}^*$	Pentosan	0.57	13.96
$(\text{Lignin})_n \rightarrow n\text{Soluble Lignin}$	Lignin	24.40	35.66
Mass Yield**	%	62.62	52.18

*Unkown conversion represents a portion of the biomass converted to soluble products or degradation products that were not determined by HPLC performed with the hydrolysate.

**Mass Yield is defined as the ratio between the dry mass of solids recovered after pretreatment and the initial dry mass of raw biomass before pretreatment.

Table 3.8. Enzymatic Hydrolysis Reactions and Conversions (5FPU/g biomass – 96h)

Reactions	Reactant	% Converted to Product	
		Bagasse	Straw
(Glucan) $n \rightarrow n$ Glucose	Glucan	67.3	78.4
(Pentosan) $n \rightarrow n$ Xylose/Arabinose	Pentosan	61.6	68.6

The total sugar recovery, as defined by equation Equation 3.3, was 71.5% for bagasse and 74.4% for straw. However, as sugar content in bagasse is higher than in straw, the sugar yield (Equation 3.4) was 48.2% for bagasse and 44.6% for straw.

$$\text{sugar recovery (\% w/w)} = \frac{(\text{sugar recovered in pretreatment} + \text{sugar recovered in enzymatic hydrolysis})}{\text{sugar in original biomass}} * 100$$

Equation 3.3

$$\text{sugar yield (\% w/w)} = \frac{(\text{sugar recovered in pretreatment} + \text{sugar recovered in enzymatic hydrolysis})}{\text{original biomass}} * 100$$

Equation 3.4

3.3.3. Simulation Results

Table 3.9 brings the main simulation results. The conversion of bagasse to cellulosic ethanol (297 L ethanol / OD tonne bagasse) produces around 6% more ethanol than the conversion of straw (280 L ethanol / OD tonne straw). Steam and power production is higher when straw and bagasse solid residue are burned in boilers mainly due to two reasons. First, straw has higher heating value due to lower moisture content, as a result, steam production is

5.1 tonne/OD straw and 4.4 tonne/OD bagasse. Second, the conversion of bagasse to cellulosic ethanol generates a higher amount of solid residue to be burned in the CHP. When both ethanol plants (1G and 2G) operate during sugarcane season (Scenario 2), storage of bagasse and straw is minimized and more ethanol and power can be produced.

Table 3.9. Simulation Results

Scenario	0	1 CL-BAG-Y	2 CL-BAG-S	3 GF1-BAG-Y	4 GF2-BAG-Y	5 GF3-BAG-Y	6 CL-STW-Y	7 GF3-STW-Y
Sugarcane to 1G - tonne/y	2,000,000	2,000,000	2,000,000	-	-	-	2,000,000	-
Bagasse to 2G - OD tonne/y	-	226,323	251,470	226,323	452,645	678,968	-	-
Bagasse to CHP - OD tonne/y	251,470	-	-	-	-	-	226,323	678,968
Bagasse Storage Loss - %	-	10	0	10	10	10	10	10
Straw to 2G - OD tonne/y	-	-	-	-	-	-	226,323	678,968
Straw to CHP - OD tonne/y	-	226,323	238,234	226,323	452,645	678,968	-	-
Straw Storage Loss - %	-	5	0	5	5	5	5	5
1G Ethanol Production - m3/y	166,024	166,024	166,024	-	-	-	166,024	-
2G Ethanol Production - m3/y	-	67,145	74,605	67,145	134,289	201,434	63,337	190,011
Power Consumption - MWh/y	60,000	97,414	101,571	40,754	81,508	122,262	97,414	122,262
Power Production - MWh/y	212,504	291,866	316,063	362,987	725,974	1,088,060	229,349	898,696
Power Surplus - MWh/y	152,504	194,452	214,492	322,233	644,465	966,698	131,935	776,434
Steam Demand - tonne/h	176.6	230.1*	302.1	51.5	102.9	154.4	226.0*	142.0
Steam Production - tonne/h	283.6	230.1*	443.5	194.4	388.7	583.1	226.0*	489.7
Boilers Capacity - tonne/h	283.6	283.6	443.5	194.4	388.7	583.1	283.6	489.7
Power Capacity - MW	53.4	39.5	79.5	43.2	86.4	129.6	31.7	107.0

*Maximum steam demand and production during the sugarcane season (167 days/y) when both facilities (1G and 2G) are under operation.

CL = co-location; GF = greenfield; BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant; Y = year operation (350 days); S = season operation (167 days)

3.3.4. Capital Investment

In the case of co-location (Scenarios 1, 2 and 6), no capital was considered for infrastructure (Figure 3.5). It was assumed that the existing infrastructure present in the autonomous distillery will be shared to accommodate the 2G ethanol plant. Additionally, the actual boiler capacity in the autonomous distillery (283.6 tonne/h) running 167 days/year (Scenario 0) is enough to accommodate the steam demand for Scenarios 1 and 6 when biomass and solid residues are burned in the CHP area during 350 days of operation. As a result, total capital investment in Scenario 1 (co-location) is 57% of the capital expenditure in Scenario 3 (greenfield) for the same cellulosic ethanol production due to capital savings with infrastructure and boilers. However, surplus power in Scenario 1 represents only 60% of the power surplus in Scenario 3, because in the co-location scenario, the annual process steam demand (1G and 2G plants) is higher than in the greenfield scenario, resulting in less excess steam available to produce power.

When the co-located 2G ethanol plant operates during sugarcane season (Scenario 2), 90% more capital is necessary when compared to Scenario 1 due to additional capital expenses with process equipment (bigger process installed capacity) and boilers. Capital investment in Scenario 7 is 95% of the capital investment in Scenario 5 due to lower production of steam and power resulting in less capital for boilers and turbogenerators.

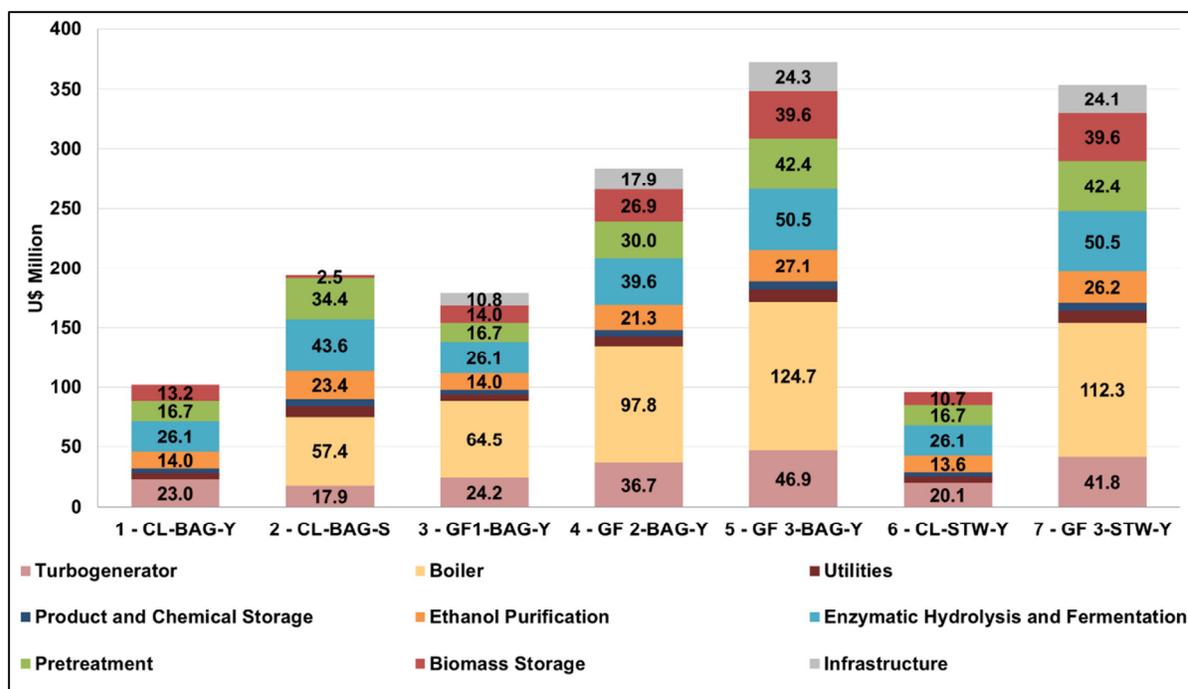


Figure 3.5. Capital Investment for Studied Scenarios (2015 US\$)

3.3.5. Operating Costs and IRR

The operating costs are presented in Table 3.10. For Scenarios 1, 2 and 6 (co-location), the operating costs to purchase bagasse is zero because the biomass is already available at the existing distillery. However, the surplus electricity (accounted as a negative cost or credit) for the co-location scenarios is discounted by the surplus power sold in Scenario 0, which reduces the amount of power sold to the grid.

The operating costs for Scenarios 1 and 6 (co-location) are approximately the same when electricity cost is not accounted. The main difference is the electricity revenue, which is bigger for Scenario 1 (Figure 3.6). As a result, the higher costs in Scenario 6 considerably decreases the IRR when compare to Scenario 1. Same conclusions can be addressed to Scenarios 5 and 7 (greenfield) (Figure 3.7). Figure 3.8 presents the economic impact of plant operating days. Operating costs without expenses with maintenance, operating materials and

depreciation for Scenarios 1 and 2 are basically similar (differences are related to biomass degradation losses). However, higher capital and maintenance expenses when both 1G and 2G facilities operate only during sugarcane season considerably increases overall costs and decreases IRR of Scenario 2.

Table 3.10. Operating Costs for Cellulosic Ethanol Production – US/L

Scenarios	1 CL-BAG-Y	2 CL-BAG-S	3 GF1-BAG-Y	4 GF2-BAG-Y	5 GF3-BAG-Y	6 CL-STW-Y	7 GF3-STW-Y
Sugarcane Bagasse	0.000	0.000	0.182	0.182	0.182	0.000	0.193
Sugarcane Straw	0.113	0.102	0.113	0.113	0.113	0.120	0.120
Enzyme	0.093	0.093	0.093	0.093	0.093	0.091	0.091
Chemicals	0.027	0.027	0.027	0.027	0.027	0.028	0.028
Electricity	-0.038	-0.050	-0.289	-0.289	-0.289	0.020	-0.246
Maintenance, Materials, Others	0.076	0.130	0.134	0.106	0.092	0.076	0.093
Labor	0.016	0.015	0.016	0.008	0.005	0.017	0.006
Depreciation	0.152	0.260	0.267	0.211	0.185	0.151	0.186
Overhead, Administration	0.014	0.014	0.014	0.014	0.014	0.014	0.014
Total - US/L ethanol	0.453	0.590	0.557	0.465	0.423	0.516	0.484
IRR - %	10.5%	2.0%	4.6%	9.7%	12.1%	5.7%	8.4%
Capex - US/L ethanol	1.52	2.60	2.67	2.11	1.85	1.51	1.86
Ethanol - L/OD tonne biomass	296.7	296.7	296.7	296.7	296.7	279.9	279.9

CL = co-location; GF = greenfield; BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant; Y = year operation (350 days); S = season operation (167 days)

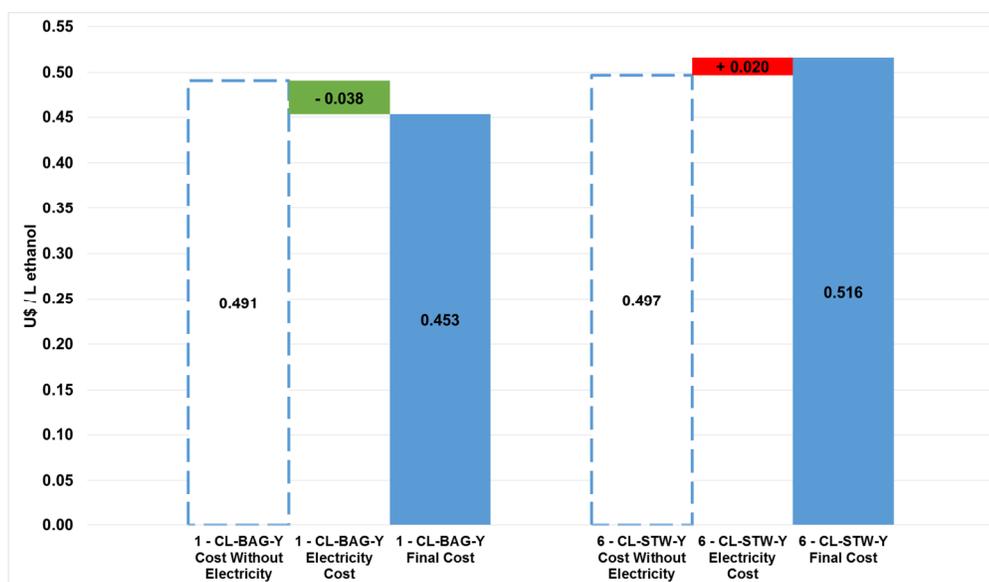


Figure 3.6. Operating Costs for Scenarios 1 and 6 - Impact of Electricity Production

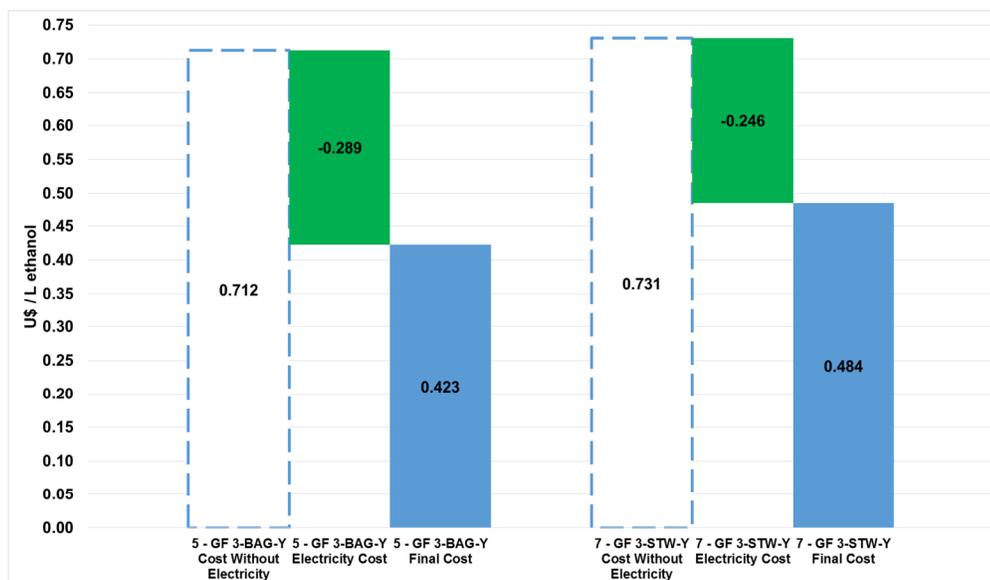


Figure 3.7. Operating Costs for Scenarios 5 and 7 - Impact of Electricity Production

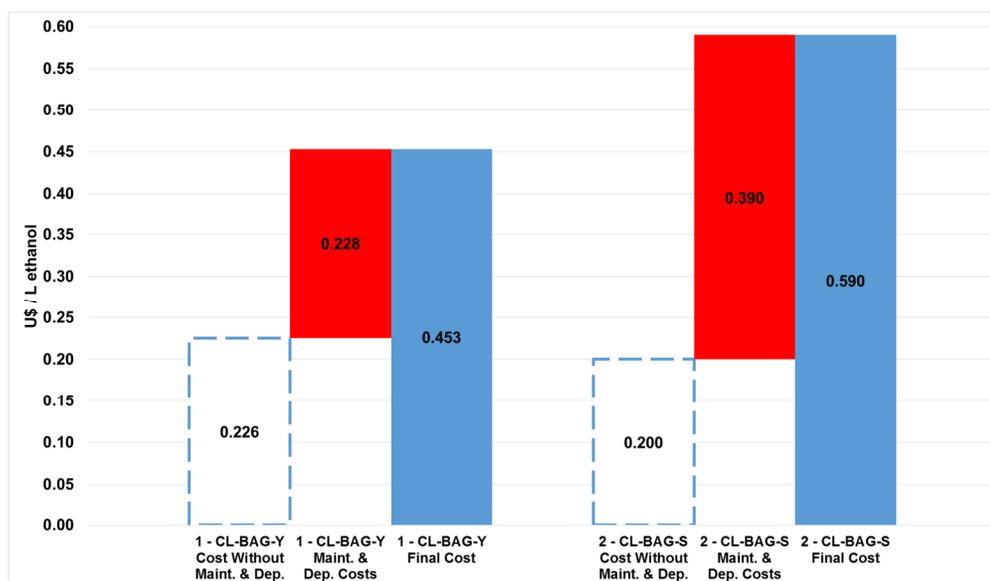


Figure 3.8. Operating Costs for Scenarios 1 and 2 - Impact of Maintenance, Operating Materials and Depreciation

The IRR comparison between Scenario 1 and Scenario 3 clearly shows the benefits of co-location. Greenfield plants presented similar or better IRR than co-location (Scenario 1)

when the production of cellulosic ethanol was double or triple due savings with economy of scale (Scenarios 4 and 5). However, higher capital investment and higher risks associate with large bioethanol facilities (biomass availability, biomass cost, biomass storage, technology reliability) are drawbacks for greenfield plants.

3.4. Conclusions

Among the evaluated scenarios, co-location combined with bagasse conversion to ethanol and straw conversion to steam and power (Scenario 1) has presented as the most promising option based on IRR. Higher sugar yield when bagasse is converted to ethanol, higher power production when straw is burned in biomass boilers and capital savings when cellulosic ethanol plant is co-located inside an existing sugarcane mill have contribute for its better financial performance. Greenfield plants (Scenarios 4 and 5) can achieve similar or better IRR only when the plant capacity is double or triple of the co-location capacity. However, higher risks associate with large cellulosic ethanol plants (high capital investment, high volume of biomass, emerging and non-reliable technology) decrease the attractiveness of greenfield plants. Moreover, the fact that sugarcane mills usually operate only during 6 to 8 months per year creates the opportunity to use available facilities after sugarcane season. In this direction, the correct use of available sugarcane bagasse and straw according to their sugar content, recalcitrance and heating value; co-location of cellulosic ethanol plant (use of existing infrastructure), possible use of 1G process equipment after sugarcane season and possibility of process integration between 1G ethanol mills and 2G ethanol plants, create the opportunity to maximize ethanol and power production and minimize capital expenses and operating costs of cellulosic ethanol facilities.

4. Effect of Mechanical Refining and Delignification on Enzymatic Hydrolysis Yield and Total Sugar Recovery

4.1. Introduction – Mechanical Fibrillation and Delignification

Enzymatic hydrolysis of carbohydrates is one of the bottlenecks for the production of biofuels and biochemicals derived from monomeric sugars when lignocellulosic biomass is used as a feedstock. High capital expenses with reactors due to long reaction times (around 1 - 4 days), high costs of enzymes (US\$5 - US\$6 per kg protein) and low carbohydrate conversion associated with enzymatic hydrolysis have a significant impact in the overall economics of biorefineries. The combination of substrate characteristic and composition, type and conditions of pretreatment, type and dosage of enzymes, and process conditions will affect the enzymatic digestibility of biomass (Alvira et al., 2010).

Many factors influence enzymatic hydrolysis, and they can be related to enzyme, process and substrate related factors. Enzyme factors limiting enzymatic hydrolysis are basically related to the composition of the enzymatic cocktail and enzyme dosage. Enzymatic hydrolysis of cellulose is performed by 3 major groups of cellulases: a) endoglucanase: attacks region of low crystallinity in the cellulose to create free chain-ends. b) exoglucanase: remove cellobiose units from the free chain-ends. c) β -glucosidase: hydrolyses cellobiose to produce glucose. Ancillary enzymes used to hydrolyse hemicellulose can also be applied (glucuronidase, acetyl esterase, xylanase, β -xylosidase, arabinase, galactomannanase, glucomannanase, others). Literature shows that the use of mixture of cellulases produced by different microorganisms, addition of β -glucosidases and addition of hemicellulases is able to increase biomass digestibility significantly (Sun & Cheng, 2002).

Process factors affecting enzymatic hydrolysis are related to process conditions (pH, temperature, consistency, presence of inhibitors) and process configuration (SHF: Separate Enzymatic Hydrolysis and Fermentation; SSF: Simultaneous Saccharification and Fermentation; SSCF: Simultaneous Saccharification and Cofermentation; CBP: Consolidated Bioprocessing). Enzymes are optimally active at a specific pH and temperature (usually around pH 4 to 5 and temperature range of 40°C to 50°C) and different enzymes have different optimum conditions (Van Dyk & Pletschke, 2012). High substrate consistency may cause substrate inhibition, problems with mixing and mass transfer, which impacts negatively enzymatic hydrolysis performance (Taherzadeh & Karimi, 2007). The presence of inhibitory compounds formed during pretreatment (phenolic components, organic acids, furfural, HMF) and during fermentation of sugars may decrease enzymatic hydrolysis yield. Moreover, the activity of enzymes can be affected by their own products (oligosaccharides, disaccharides and monomers) when they reach high concentrations (Van Dyk & Pletschke, 2012). Type of biochemical process may also limit enzymatic hydrolysis. SHF allows the execution of enzymatic hydrolysis at its optimum condition. However, some drawbacks are possible inhibition of cellulase by sugars and risk of contamination due to the long time required for enzymatic hydrolysis. SSF minimizes the inhibition effects caused by cellobiose and glucose because hydrolyzed sugars are immediately consumed by microorganisms. On the other hand, the difference of optimum temperatures between enzymatic hydrolysis (45°C to 50°C) and fermentation (30°C to 37°C for ethanol fermentation) and possible cellulase inhibition by fermentation products come as drawbacks. Hydrolysis is usually the rate-limiting step in SSF. Fermentation products and enzymes are produced in a single reactor by a single culture of microorganisms in the case of CBP (Taherzadeh & Karimi, 2007).

The substrate related factors limiting the enzymatic hydrolysis are: a) cellulose crystallinity: it is considered an important factor but cannot explain biomass recalcitrance by itself. Usually crystallinity can be decreased by reduction of particle size or can be changed by pretreatments; b) cellulose degree of polymerization (DP): it is related to crystallinity. DP can be reduced by xylan removal or enzymatic hydrolysis; c) surface area: it measures the accessibility of substrate to the enzymes. It can be increased by pretreatments; d) hemicellulose content: removal of hemicellulose increases pore size of the substrate, increasing cellulose accessibility and hydrolysis probability; e) particle size: reduction of particle size increases surface area and cellulose accessibility; f) porosity: it is used to understand the relation between pore size and enzyme size. Enzymes can get trapped in the pores if the internal area is much larger than the external area. As a result, an increase in porosity improves enzymatic hydrolysis; g) cell wall thickness: represents a barrier for enzyme and liquid penetration; h) variation of glucan accessibility with conversion. i) lignin barrier (Alvira et al., 2010).

In order to overcome physical, chemical, structural and compositional barriers associated with a substrate, a pretreatment can be used to break down the lignin structure and disrupt the crystalline structure of cellulose, improving enzyme accessibility for a better cellulose digestibility. However, pretreatment is an expensive unit operation and many factors must be evaluated during the selection of a low-cost and effective pretreatment process: highly digestible pretreated material, no significant sugar degradation, minimum production of toxic compounds (carboxylic acids, furans, phenolic), low cost/size reactors, minimum solid waste, low moisture content, high sugar concentration, fermentation compatibility, lignin recovery, low energy consumption (Alvira et al., 2010). Among various options, autohydrolysis is a

cost effective technology, where the absence of external catalysts creates the possibility to reduce capital, operational costs and environmental impacts when compared to acid pretreatments (Carvalho, Duarte, & Girio, 2008).

Moreover, the lignin content of lignocellulosic biomass is significant (up to 25% in hardwoods, 30% in softwoods, 22% in sugarcane bagasse, 18% in corn stover) and several reasons has been addressed to describe why lignin is an important limiting factor for enzymatic hydrolysis (Van Dyk & Pletschke, 2012).

- Lignin acts as a physical barrier limiting the access of enzymes to the substrate;
- Non-productive enzyme adsorption to lignin decreasing the productive hydrolysis of substrate;
- Lignin direct inhibits enzymatic hydrolysis;
- Lignin blocks the progress of cellulase in the cellulose chain;
- Type and distribution of lignin in the substrate also impact enzymatic hydrolysis.

Different strategies can be used to overcome these issues, such as, addition of proteins (BSA) or additives (surfactants) and lignin extraction using enzymatic means (lignin peroxidase, manganese peroxidase and laccase) or chemical means (acid, steam or alkaline treatments) (Alvira et al., 2010).

Alkaline delignification effectively solubilize lignin with minor cellulose and hemicellulose solubilization. Alkaline delignification causes swelling and increases in internal surface area. The removal of lignin decreases DP and crystallinity favoring enzymatic hydrolysis (Carvalho et al., 2008), (Alvira et al., 2010).

Besides lignin content, another important parameter for enzymatic hydrolysis is the accessibility of substrate to enzymes (Barakat, de Vries, & Rouau, 2013). As mentioned

before, the modification of many substrate related factors (removal of hemicellulose and lignin, increase of surface area and pore size, reduction of particle size) directly increases substrate accessibility which may result in improvement of enzymatic digestibility. In this context, mechanical refining represents a feasible and efficient alternative to improve substrate accessibility.

Mechanical refining is a low capital and well established technology used by paper industry to improve the bonding ability of fibers allowing the formation of a strong fiber network. It can also be used to overcome recalcitrance by opening up the biomass structure, which increases surface area resulting in better substrate accessibility and carbohydrate conversion (J. Park et al., 2015).

Mechanical refining acts with 3 mechanisms that changes fiber structure and morphology: cutting (fiber length reduction), shearing (fibrillates surface of fibers), compression (crushes and fibrillates internal fibers). Each one occurs simultaneously but at different levels depending on the refining technology (Chen et al., 2013). As a result, refining improves fiber absorbency and porosity (Chen et al., 2012). Literature reports that mechanical refining can be used to enhance sugar recovery using low enzyme dosage (Chen et al., 2012), (Jones, Venditti, Park, Jameel, & Koo, 2013) and to reduce pretreatment severity and enzyme load reaching same level of sugar recovery (Jones, Venditti, Park, & Jameel, 2014), (Ertas, Han, Jameel, & Chang, 2014).

Therefore, the objective of this work is to evaluate the carbohydrate conversion during enzymatic hydrolysis when PFI refining and oxygen delignification are applied on sugarcane bagasse and sugarcane straw after autohydrolysis pretreatment. The global sugar recovery obtained with different levels of refining and delignification will be determined to compare

both options. Finally, a fundamental study will evaluate the modification of substrate properties (morphology, particle size, swellability, pore area and crystallinity) when mechanical refining is applied to the pretreated biomass.

4.2. Materials and Methods

4.2.1. Biomass

Sugarcane bagasse and straw were kindly provided by CTBE (Brazilian Bioethanol Science and Technology Laboratory). Those feedstocks were air dried, characterized (Table 3.6) using a Laboratory Analytical Procedure published by (National Renewable Energy Laboratory, 2011a) and stored at room temperature.

4.2.2. Pretreatment (Autohydrolysis)

Autohydrolysis pretreatment was executed in a 300 L stainless steel reactor using 15 kg using 1:10 solid:liquid ratio. After pretreatment, the liquid fraction was collected and analyzed to determine the sugars, organic acids and degradation products using HPLC (National Renewable Energy Laboratory, 2006). The solid fraction was washed with sufficient tap water to eliminate any dissolved biomass and until neutral pH was achieved. The washed solid was also characterized.

4.2.3. Delignification (Oxygen Delignification)

Delignification was performed using pretreated and washed substrates stainless steel batch reactors using 100 OD grams of pretreated biomass. The reaction was executed during

1 hour at 100°C and 10% consistency under 100 psig of oxygen pressure. Six different charges of sodium hydroxide were used (0.5; 1.5; 3.0; 5.0 and 10% w/w – mass of NaOH / mass of OD biomass) in order to achieve different levels of delignification. After each batch, samples were washed using sufficient tap water to remove any residue of chemicals and dissolved biomass and until neutral pH was achieved.

4.2.4. Mechanical Refining (PFI Refining)

Mechanical refining was applied on pretreated and washed substrates using a PFI refiner. PFI refiner is a batch equipment where biomass is beaten between a roll with bars and a smooth-walled beater housing, both rotating in the same direction but at different speeds. The refining action is achieved through the differential rotational action and the application of loading between the roll and housing during for a specified number of revolutions. Pretreated and washed biomass was refined using a PFI refiner according to (Technical Association of Paper and Paper Industry, 2000). Each batch of refining was performed with 30 OD grams of biomass at 10% consistency. Four refining intensities (2000, 4000, 6000 and 8000 revolutions) were evaluated. An additional experiment was performed using a pulp disintegrator. Pretreated substrates were disintegrated to evaluate if a better dispersion of fibers improves the enzymatic hydrolysis. Morphology, particle size, swellability, pore volume and crystallinity were evaluated for all refined samples according to the following procedures.

4.2.4.1. Morphology (Light Microscope)

An image analyzer system, consisted of a light microscope (Nikon E200), a 2.0 Megapixel Scientific Camera CCD video camera (Luminera Infinity 2), and an Image-Pro Premier 9.1 software, was used to elucidate the morphology of the pretreated and refined samples, in order to understand the physical modification (fiber bundles separation, fiber cutting, external fibrillation and internal delamination) promoted by mechanical refining. Samples were diluted in deionized water and placed on a glass slide. Images at 100 times magnification (fiber bundles) and 400 times magnification (single fibers) were taken using 1616 x 1216 pixels of resolution.

4.2.4.2. Particle Size (Fiber Quality Analyzer and Bauer-McNett)

The HiRes Fiber Quality Analyzer (FQA) from OpTest Equipment Inc. uses circular polarized light to measure fiber size. This equipment was used to measure length, width and percentage of fines for the samples. Samples were diluted (~ 1 mg/L) and disintegrated before each analysis. Particles with size ranging from 0.03 to 10.0 mm were measured and 10.000 particles were analyzed for each FQA run. Fines were defined as particles with size between 0.03 to 0.2 mm. Fiber width was measured for particles with size bigger than 0.2 mm. Particle length was measured as the true contoured length and reported as the length weighted length $L_w = \frac{\sum n_i L_i^2}{\sum n_i L_i}$ where n is the number of fibers and L is the contour length.

A Bauer McNett classification was used to determine the percentages by weight of the fibers retained in each screen (14, 28, 48, 100, 200 mesh or 1.19, 0.595, 0.297, .149, .074 mm) according to (Technical Association of Paper and Paper Industry, 1995). This instrument comprises five narrow tanks semi cylindrically shaped and full with water, each

tank having a vertical screen. The five tanks are supported in a framework, one below the other, in a cascade arrangement. A vertical and cylindrical agitator with short paddles rotates causing the suspension on each tank to flow across the screen and circulate horizontally around the tank. An overflow weir is provided at the outgoing side of each screen, and a short pipe leads to the next tank with a finer screen, at a slightly lower level, or from the last tank, to waste. A constant headbox above the tanks supplies water at the rate of 11.35 L/min. The consequent motion of the water serves to keep the fibers from settling and to present them repeatedly to the screen through which they will pass if their length is less than twice the screen opening. 10 OD grams of sample were classified during 20 minutes. After classification, sample fractions present in each tank were filtered using pre dried and weighted Whatman pads (cat. # 1004 110). After filtration, the samples were oven dried at 105°C and the weight of each fractions was determined. The cumulative weight distribution was calculated using the weight of biomass passing through each screen.

4.2.4.3.Swellability (Water Retention Value)

Water retention value (WRV) was used to estimate the swelling capacity of fibers by the measurement of the water retained by a wet and swollen sample after centrifugation. With the centrifugation and consequent elimination of excess water, the remained water in the sample can be defined as: a) bond water (BW), including non-freezing bond water (NFBW) and freezing bond water – (FBW) that cannot be remove by centrifugation; b) free water - capillary water (FW) that is held by surface tension inside fibers capillaries and can be partially removed by centrifugation (Lavric, Tomsic, Simoncic, Warmoeskerken, & Jovic, 2012). WRV was executed according to (Technical Association of Paper and Paper Industry,

2011). A pulp suspension was placed into filtering glass tube of medium porosity (22 mm diameter) to yield 1400 g/m^2 (approximately 0.5233 OD grams). The filtering glass tube was centrifuged for 30 min at 0.9 relative centrifugal force to gravity. After centrifugation, sample was oven dried at 105°C . The weights of the wet centrifuged sample (m_{wc}) and the oven-dried sample (m_{od}) were measured to calculate $WRV = (m_{wc} - m_{od}) / m_{od}$.

4.2.4.4. Pore Volume (Thermoporometry - DSC)

Calorimetric Thermoporometry using Differential Scanning Calorimetry (DSC) was performed to assess the pore area. DSC experiments are able to differentiate 3 categories of water absorbed in biomass: non-freezing bound water (NFBW), freezing bound water (FBW), free water (FW) (S. Park, Venditti, Jameel, & Pawlak, 2006). FW is bulk water and it is measured with ice melting at 0°C . FBW is confined inside the biomass in a way that shifts the ice melting temperature and it is measured with ice melting below 0°C (Driemeier, Mendes, & Oliveira, 2012). Water present in the capillaries has a depressed melting temperature due to the lower pressure at a curved interface in cavities. This temperature has a relationship with the pore diameter which allows the evaluation of pore size and pore area. NFBW is represented by the first layers of water adjacent to the biomass surface. As the water movement is restricted by its association with the surface, NFBW does not freeze (S. Park et al., 2006).

Analysis were kindly executed by CTBE (Brazilian Bioethanol Science and Technology Laboratory) using A DSC TA Q200 with an auto sampler and RCS90 cooling unit, according to the procedure presented by (Driemeier et al., 2012) with some corrections introduced by (Driemeier, Oliveira, & Curvelo, 2016).

4.2.4.5. Crystallinity (X-ray Diffraction)

X-ray diffraction was used to estimate the crystallinity index (CI) of samples. The wide angle diffraction data were acquired using a Rigaku SmartLab x-ray diffractometer (CuK α radiation). The diffraction angle of 2θ was measured from 5 to 41° with a step size of 0.05° and 5 seconds of exposure at each step. Measurements were performed at room temperature. Crystallinity index was calculated, based on the peak height method proposed by (Segal, Creely, Martin, & Conrad, 1959), as the ratio between the intensity of the crystalline peak ($I_{002} - I_{am}$) and the total intensity (I_{002}), where I_{002} is the maximum intensity of 002 lattice diffraction (2θ around 22.5°) and I_{am} is the intensity related to the amorphous contribution (2θ around 18.4°).

4.2.4.6. Enzymatic Hydrolysis

Enzymatic hydrolysis was performed in 50 mL tubes using 5 FPU/g (dry basis) of pretreated and washed substrate, 10% consistency, pH 4.8~5.0 and 50°C. Novozymes Cellic CTec 2 supplemented with 1/9 Cellic HTec 2 was used as the enzyme cocktail. Sodium acetate buffer was used for pH control. The incubator (Fine PCR COMBI- D24) was maintained at 50 °C and 15 rpm. During enzymatic, samples were taken at 24, 48, 72 and 96 hours. Each sample was centrifuged for 10 min at 4400 rpm using an Eppendorf Centrifuge 5702. The supernatant was used for sugar determination (National Renewable Energy Laboratory, 2006).

4.3. Results and Discussion

4.3.1. Delignification

Table 4.1, Table 4.2, Figure 4.1 and Figure 4.2 present the composition, mass yield and enzymatic hydrolysis conversion for bagasse and straw after delignification. Delignification effectively removed lignin from biomass. However, delignified samples with total carbohydrate to lignin ratio between 2.3 and 3.7 did not present improved carbohydrate conversion during enzymatic hydrolysis indicating that the lignin removal was not enough to improve the accessibility of substrate to enzymes. Significant improvement in sugar conversion (30% for bagasse and 24% for straw) was observed only when lignin content was reduced to 9.6% (bagasse) and 12.8% (straw) and a high carbohydrate to lignin ratio was achieved. Improvements in enzymatic hydrolysis after pretreatment and delignification of sugarcane biomass was also observed elsewhere (Oliveira et al., 2014), (Silva et al., 2011).

Table 4.1. Bagasse Delignification (Biomass Composition and Mass Yield)

Bagasse Delignification	Glucan %	Pentosan %	Lignin %	Ash %	Total %	Mass Yield %	C/L Ratio*
Undelignified	56.5 ± 0.3	7.4 ± 0.0	28.1 ± 0.4	4.1 ± 0.2	96.1	-	2.3
0.5% w/w NaOH	58.0 ± 0.4	7.1 ± 0.1	26.9 ± 0.1	4.4 ± 0.0	96.4	92.2 ± 3.9	2.4
1.5% w/w NaOH	59.4 ± 0.4	6.7 ± 0.0	25.3 ± 0.7	5.2 ± 0.2	96.6	88.5 ± 4.7	2.6
3.0% w/w NaOH	63.7 ± 2.1	6.4 ± 0.2	22.3 ± 0.0	5.4 ± 0.7	97.8	83.6 ± 3.9	3.1
5.0% w/w NaOH	66.5 ± 0.4	5.9 ± 0.0	19.5 ± 0.2	5.2 ± 0.4	97.1	80.4 ± 1.5	3.7
10.0% w/w NaOH	75.8 ± 0.1	4.6 ± 0.0	9.6 ± 0.4	5.5 ± 0.0	95.5	60.4 ± 2.5	8.4

*C/L Ratio = Carbohydrate to Lignin Ratio

Table 4.2. Straw Delignification (Biomass Composition and Mass Yield)

Straw Delignification	Glucan %	Pentosan %	Lignin %	Ash %	Total %	Mass Yield %	C/L Ratio*
Undelignified	47.6 ± 0.9	9.3 ± 0.1	27.9 ± 0.2	10.2 ± 1.1	95.0	-	2.0
0.5% w/w NaOH	51.1 ± 0.3	9.4 ± 0.8	26.1 ± 0.9	9.3 ± 0.2	95.9	86.0 ± 2.9	2.3
1.5% w/w NaOH	51.8 ± 0.4	9.1 ± 0.2	24.4 ± 0.2	12.2 ± 1.0	97.5	82.9 ± 4.2	2.5
3.0% w/w NaOH	54.8 ± 0.7	8.7 ± 0.5	22.9 ± 0.2	9.3 ± 0.7	95.7	80.7 ± 3.0	2.8
5.0% w/w NaOH	60.7 ± 0.7	8.0 ± 0.5	19.4 ± 0.1	8.4 ± 0.1	96.5	75.1 ± 2.4	3.5
10.0% w/w NaOH	64.9 ± 1.7	5.6 ± 0.0	12.8 ± 1.7	12.8 ± 0.2	96.1	59.5 ± 0.6	5.5

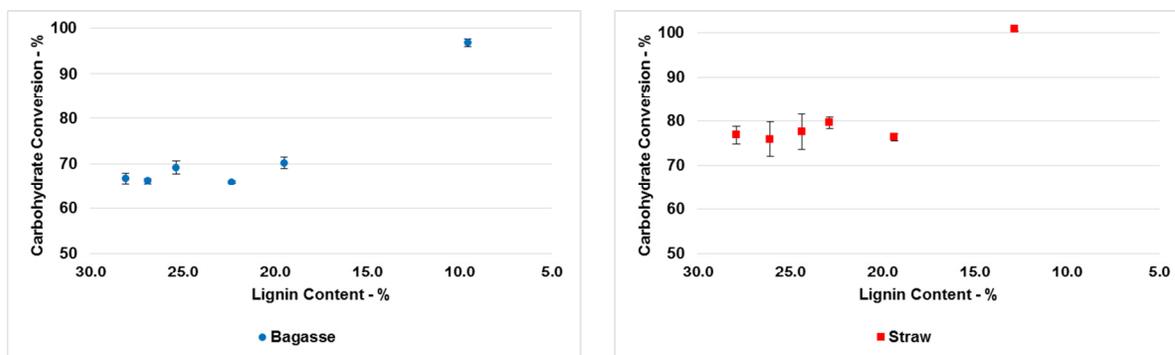


Figure 4.1. Enzymatic Hydrolysis Conversion for Delignified Samples - Carbohydrate Conversion vs Lignin Content (5 FPU/g substrate - 96h)

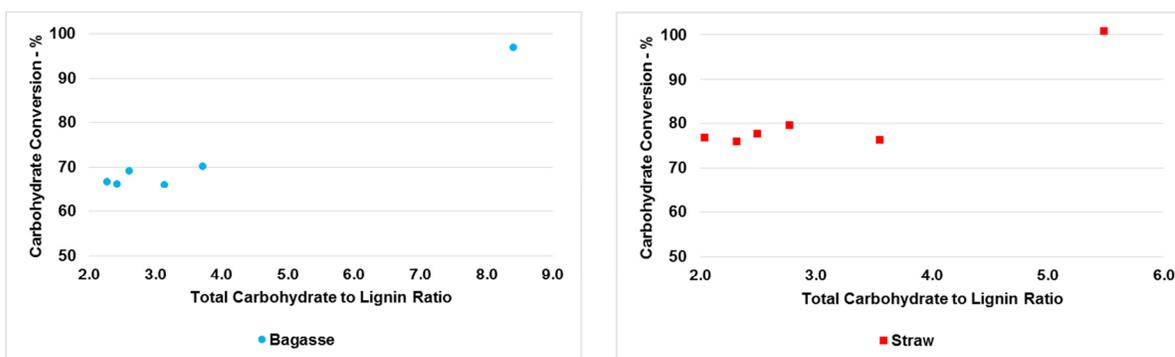


Figure 4.2. Enzymatic Hydrolysis Conversion for Delignified Samples - Carbohydrate Conversion vs Total Carbohydrate to Lignin Ratio (5 FPU/g substrate - 96h)

4.3.2. Mechanical Refining

Figure 4.3 shows the enzymatic hydrolysis conversion after mechanical refining. Mechanical refining improved enzymatic hydrolysis by 10% when bagasse was refined at 8k revolutions. When the same refining intensity was applied to pretreated straw, carbohydrate conversion was improved by 6%. As reported by (Jones et al., 2013), it is expected that unrefined samples with high enzymatic hydrolysis conversion will present lower improvement in sugar conversion after refining. The disintegration of samples in a pulp

blender did not improve enzymatic hydrolysis conversion, elucidating that the simple dispersion of fibers cannot improve the accessibility of enzymes to substrate.

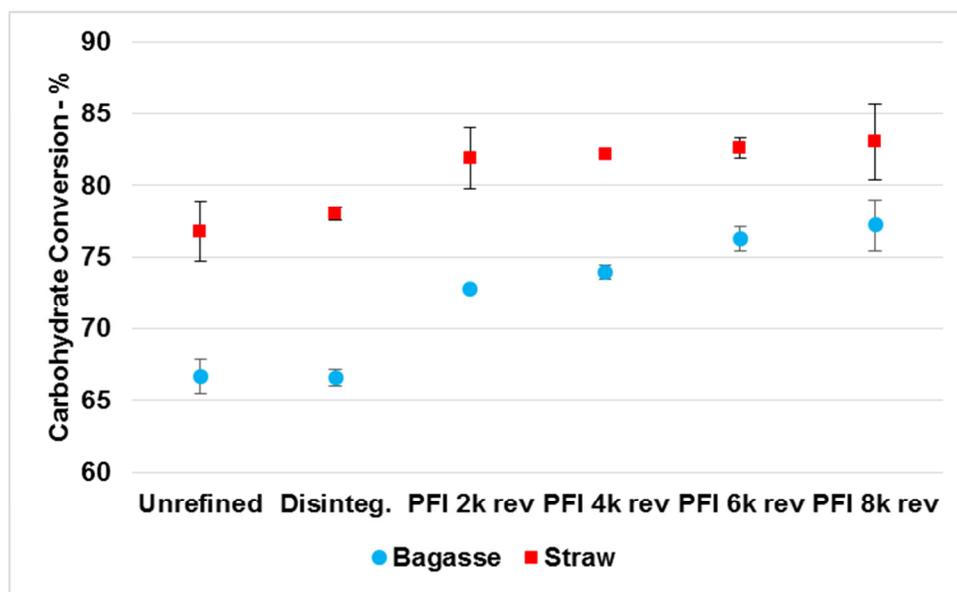


Figure 4.3. Enzymatic Hydrolysis Conversion for Refined Samples (5 FPU/g substrate; 96h)

4.3.3. Sugar Recovery and Sugar Yield

Table 4.3 presents the sugar recovery (Equation 4.1) and sugar yield (Equation 4.2) after pretreatment, delignification/refining and enzymatic hydrolysis. In most of the studied conditions, sugar recovery for straw is same or better when compared to bagasse. However, the higher content of carbohydrates in bagasse results in better sugar yields. Although the delignification can significantly improve carbohydrate conversion in enzymatic hydrolysis when lignin content is reduced to around 10 %, carbohydrate degradation considerably impacts the total carbohydrate recovery. Therefore, the improvement in enzymatic hydrolysis does not compensate the carbohydrate loss (around 25%) during delignification, possibly caused by peeling reaction. It is known that in alkaline medium, peeling reaction

successively removes monomeric units from the end of cellulose chain where a carbonyl group is present. The reaction continues until a competing reaction, known as stopping reaction, creates an end unit without a carbonyl group (McDonough, 1989). Other disadvantages of delignification are the high capital (towers, washers) and high operating costs (chemical, water and effluent treatment). As a result, mechanical refining presents as a simpler, cheaper and more effective technology to improve carbohydrate recovery and produce low cost sugars from cellulosic biomass.

$$\text{sugar recovery (\% w/w)} = \frac{(\text{sugar recovered in pretreatment} + \text{sugar recovered in enzymatic hydrolysis})}{\text{sugar in original biomass}} * 100$$

Equation 4.1

$$\text{sugar yield (\% w/w)} = \frac{(\text{sugar recovered in pretreatment} + \text{sugar recovered in enzymatic hydrolysis})}{\text{original biomass}} * 100$$

Equation 4.2

Table 4.3. Sugar Recovery and Sugar Yield after Pretreatment, Delignification and Refined Samples

Delignification / Refining	Sugar Recovery - %		Sugar Yield - %	
	Bagasse	Straw	Bagasse	Straw
Undelignified/Unrefined	71.5	74.4	48.2	44.6
0.5% w/w NaOH	68.4	69.7	46.1	41.8
1.5% w/w NaOH	69.1	69.3	46.6	41.6
3.0% w/w NaOH	67.2	71.1	45.3	42.6
5.0% w/w NaOH	69.6	69.6	46.9	41.7
10.0% w/w NaOH	76.2	72.8	51.4	43.6
PFI 2k rev	75.7	77.7	51.0	46.6
PFI 4k rev	76.4	77.9	51.6	46.7
PFI 6k rev	78.0	78.1	52.6	46.9
PFI 8k rev	78.7	78.4	53.1	47.1

4.3.4. Morphology

The comparison among unrefined (Figure 4.4, Figure 4.5) and refined samples (Figure 4.7, Figure 4.8) show the structural modifications that mechanical refining has caused to the

pretreated biomass. It is possible to identify a higher level of longitudinal delamination in the case of refined samples. External fibrillation is present in lower extent. The transversal disruption of fibers resulting in fiber cutting can also be identified. Mechanical refining also causes the separation of fiber bundles, considerably present in unrefined samples (Figure 4.6).

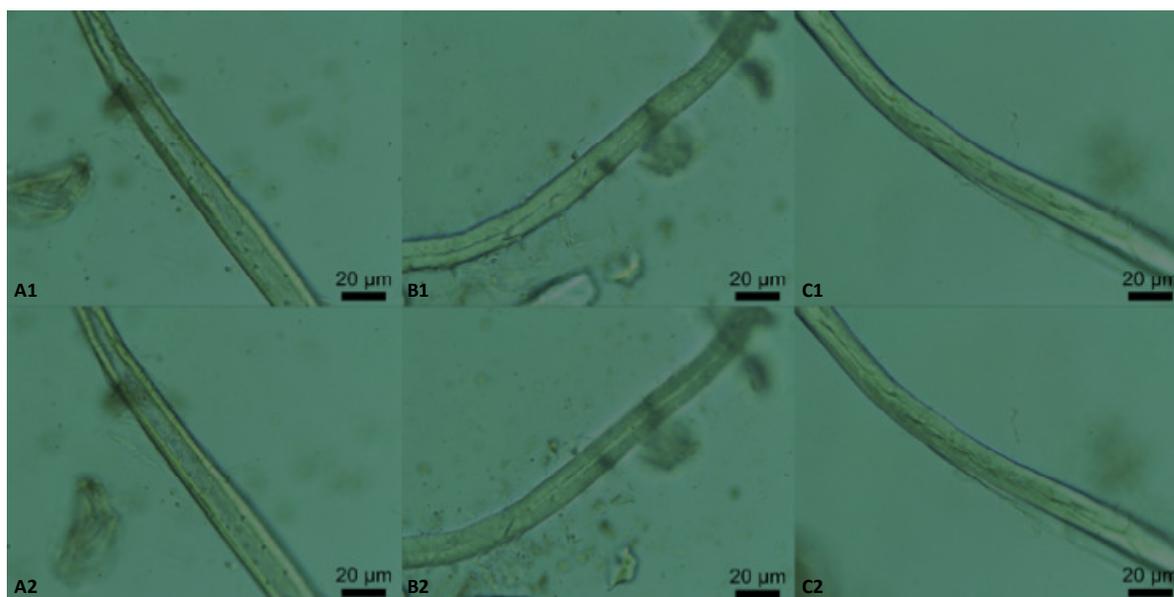


Figure 4.4. Microscope Image - Unrefined Bagasse

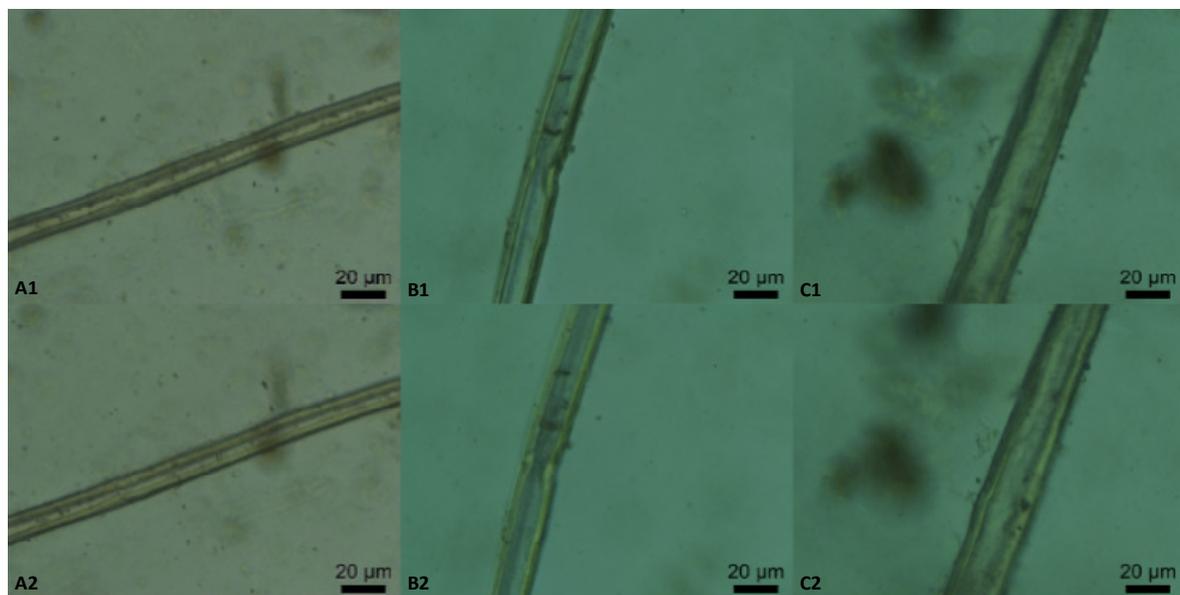


Figure 4.5. Microscope Image - Unrefined Straw

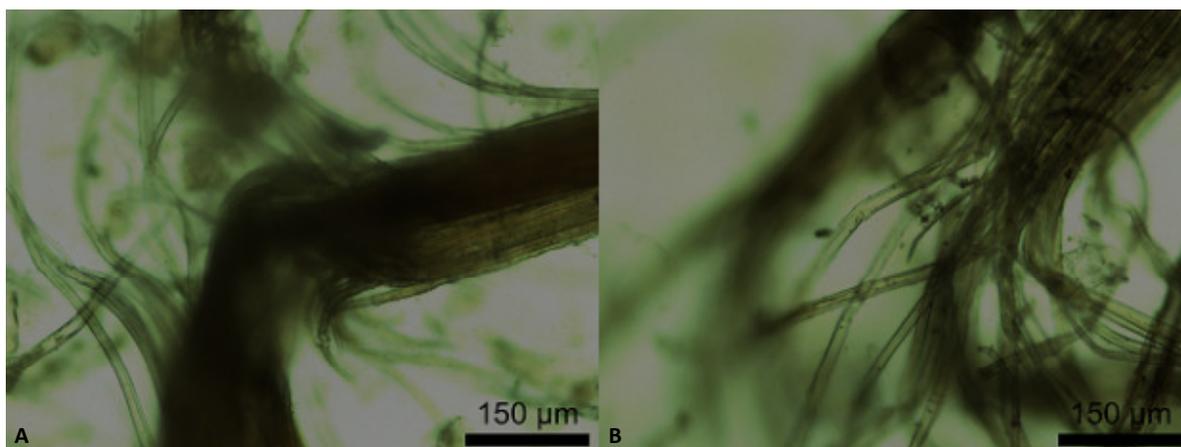


Figure 4.6. Microscope Image - Bundles of Fibers: A) Unrefined Bagasse; B) Unrefined Straw

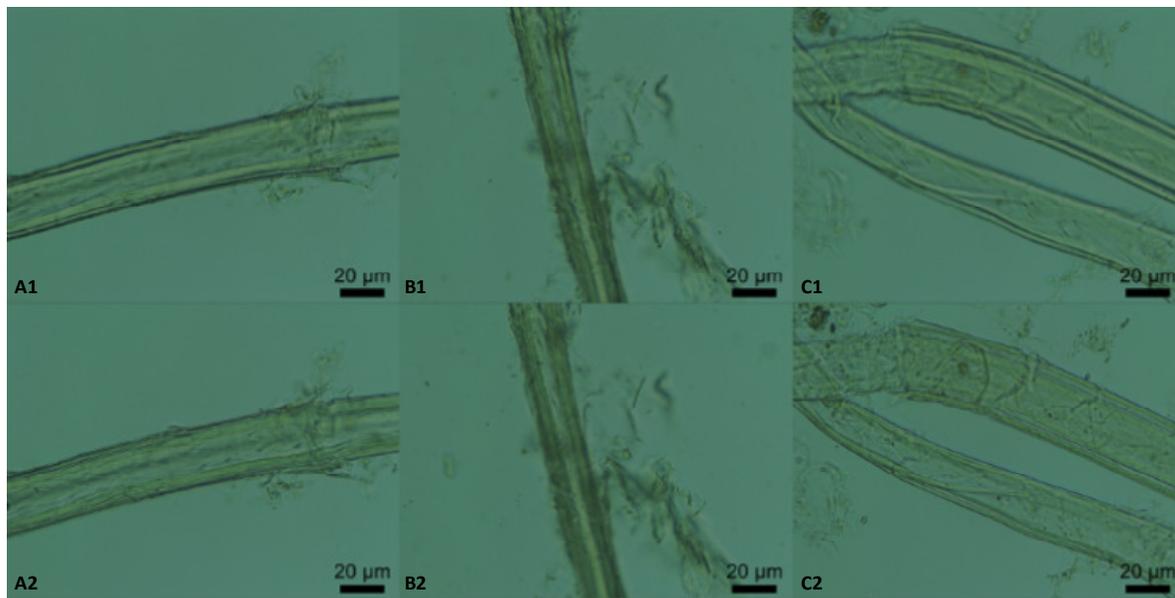


Figure 4.7. Microscope Image - Refined Bagasse - PFI 8k rev

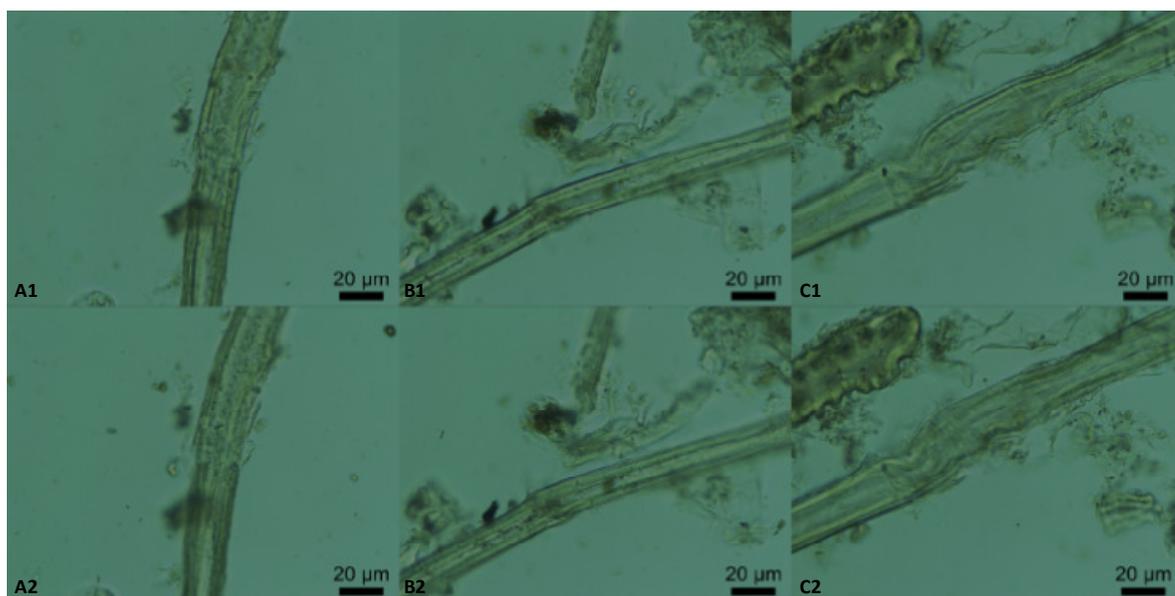


Figure 4.8. Microscope Image - Refined Straw - PFI 8k rev

4.3.5. Particle Size

The length weighted length decreases with the increase of refining intensity, confirming that PFI refining promotes fiber cutting (Figure 4.9, Figure 4.10). It is important to highlight

that some level of fiber cutting may happen during the disintegration of samples before FQA analysis, especially in the case of unrefined samples. A decrease in the measured width, when the unrefined samples are compared to the refined samples, can be observed (Figure 4.9, Figure 4.10). The incomplete separation of fiber bundles during disintegration of the unrefined samples might explain the differences in fiber width when compared to refined samples. Although percentage of fines (< 0.2 mm) based on the number of particles measured with FQA is high for both biomasses (74% for bagasse, 80% for straw), the cumulative mass distribution (Figure 4.11) shows that the mass content of fines (< 0.2 mm), is around 35% for pretreated bagasse and 55% for pretreated straw. Usually, sugarcane bagasse is composed by fibers (55 to 60%) and a remaining fine fraction that is reach in pith or parenchymatous tissue (30 to 35%) (J. A. Lois-Correa, 2012), (Sanjuan, Anzaldo, Vargas, Turrado, & Patt, 2001).

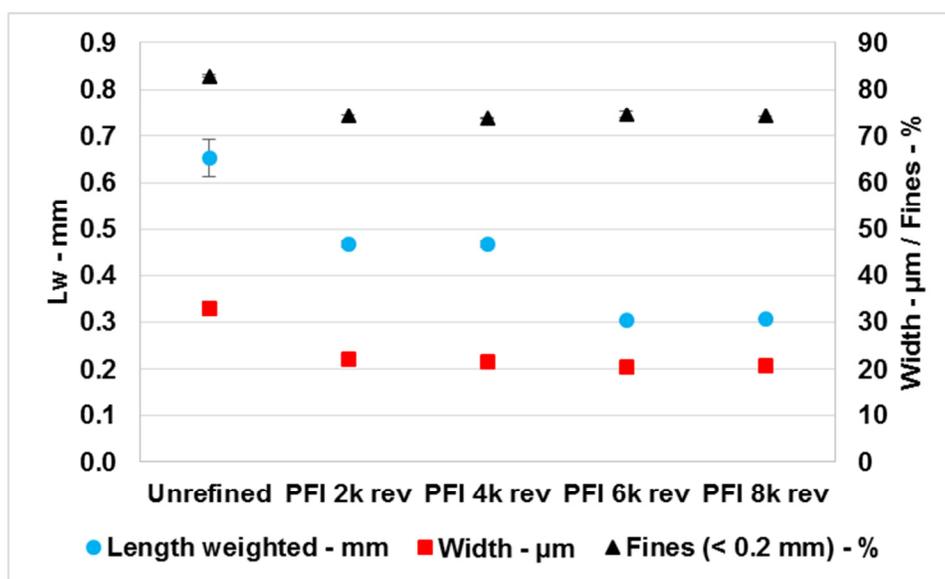


Figure 4.9. Fiber Quality Analysis – Unrefined and Refined Bagasse (L_w = Length Weighted Length)

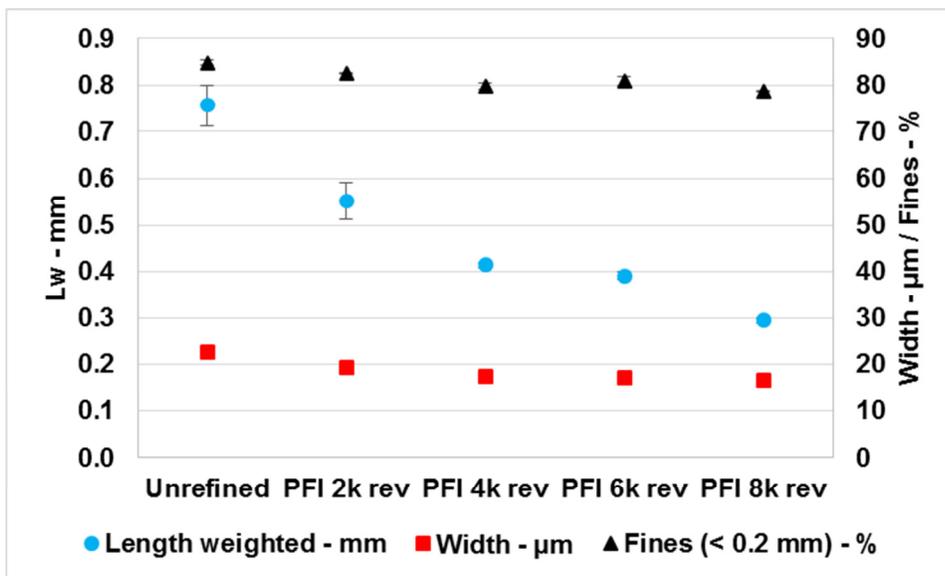


Figure 4.10. Fiber Quality Analysis – Unrefined and Refined Straw (L_w = Length Weighted Length)

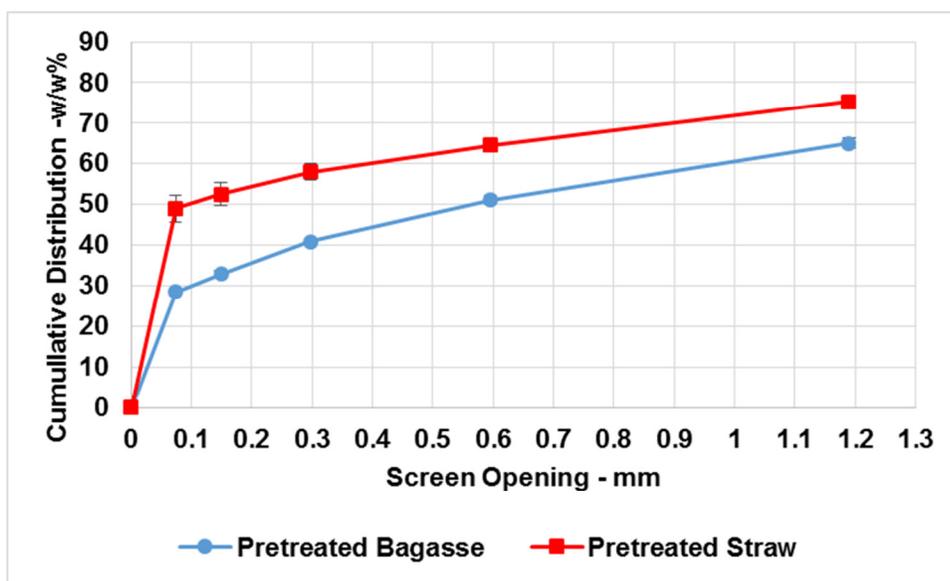


Figure 4.11. Bauer McNett - Cumulative Mass Distribution based on the weight of biomass passing through each screen

4.3.6. Swellability

Figure 4.12 clearly shows the increase of samples swellability with the increase of refining intensity. The enhancement of swellability is more preminent between unrefined

and refined samples. Among refined samples, swellability improves at a lower extent with the increase of refining intensity. Internal delamination, external fibrillation and fiber cutting allows a better accessibility of water within the fibers pores and fiber capillaries.

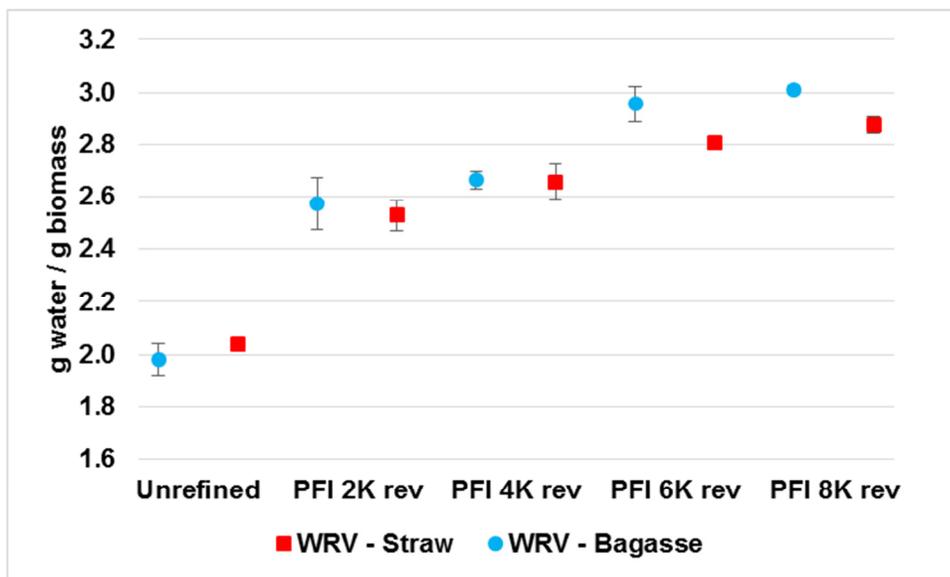


Figure 4.12. Water Retention Value - Unrefined / Refined Bagasse and Straw

4.3.7. Pore Area

Figure 4.13 presents the change in pore area with the increasing in refining intensity calculated from thermoporometry profiles. Signals only originated from pores greater than 10 nm where considered as an indication for potential increase in substrate accessibility to enzymes, which are approximately 5 nm in size (Driemeier et al., 2016). It can be observed gains in pore area with the refining, especially in the case of straw. However, additional gains with the increase of refining intensity are not observed.

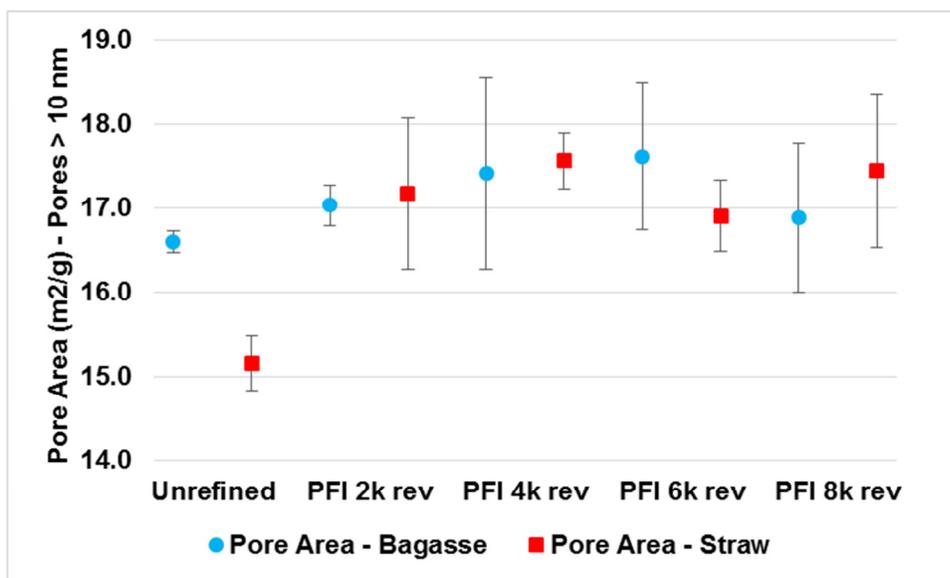


Figure 4.13. Pore Area - Unrefined / Refined Bagasse and Straw

4.3.8. Crystallinity

The X-ray diffraction normalized spectra (Figure 4.14, Figure 4.15) for unrefined and refined samples are similar and it is not possible to affirm that refining has changed the crystallinity of pretreated samples. CI was calculated according to the peak height method proposed by (Segal et al., 1959) to estimate the relative crystallinity among the studied samples (Tabela 4.1). Peaks with high intensity when 2θ is around 21.0 to 21.5 and 26.5 to 27.0 can be explained by the significant content of sand (silicon dioxide - quartz and calcium carbonate - aragonite) in the samples, especially for straw (American Mineralogist, 2016).

Tabela 4.1. Crystallinity Index - Unrefined / Refined Bagasse and Straw

Crystallinity Index - %	Bagasse	Straw
Unrefined	71.2	68.1
PFI 2k rev	69.0	67.9
PFI 4k rev	69.0	68.5
PFI 6k rev	69.1	69.9
PFI 8k rev	69.1	65.8

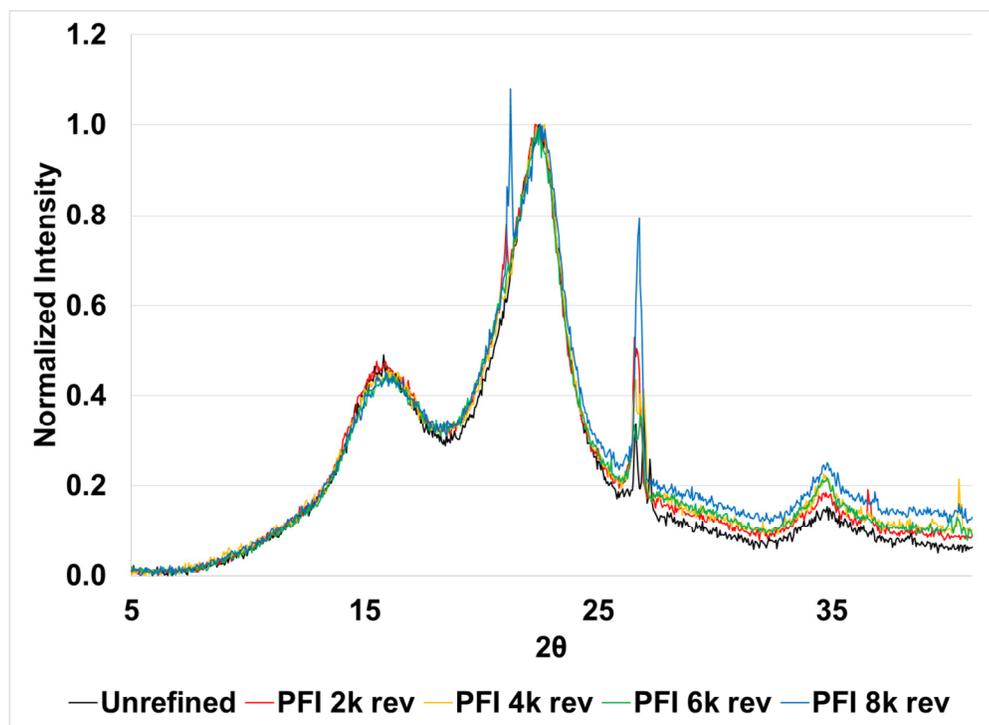


Figure 4.14. Normalized X-Ray Diffraction Spectra - Unrefined and Refined Bagasse

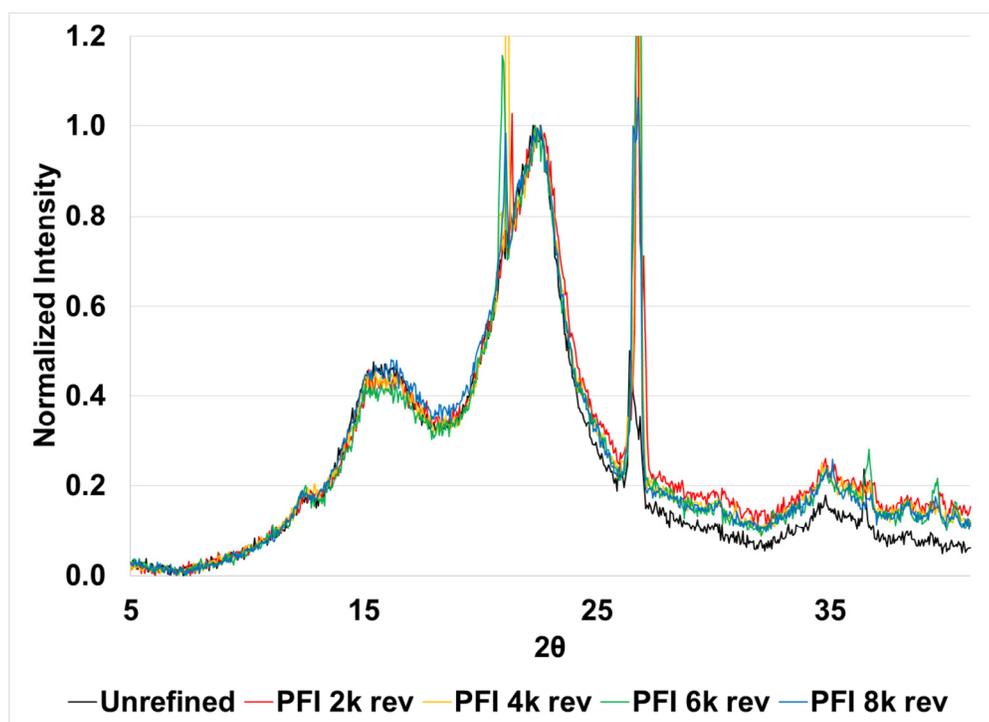


Figure 4.15. Normalized X-Ray Diffraction Spectra – Unrefined and Refined Straw

4.4. Conclusions

Biomass delignification and mechanical refining proved to be effective process alternatives to improve carbohydrate conversion during enzymatic hydrolysis for pretreated sugarcane bagasse and straw by overcoming lignin barrier and improving substrate accessibility to enzyme, respectively. Delignification significantly enhanced enzymatic hydrolysis (25% to 30%) when lignin content was reduced to around 10% and when high carbohydrate to lignin ratio was achieved. However, improvement in enzymatic hydrolysis does not compensate for the carbohydrate loss (25%) due to peeling reaction, compromising the sugar recovery. Additionally, no gain in enzymatic hydrolysis was observed when carbohydrate to lignin ratio was below 3.7. On the other hand, mechanical refining improved sugar recovery (bagasse up to 10%, straw up to 5%), confirming its potential as a promising, simpler and cheaper technology when compared to delignification. The higher content of fibers and lower enzymatic digestibility of unrefined bagasse can possibly explain the better improvement in carbohydrate conversion with mechanical refining when compared to straw. Moreover, the higher content of carbohydrates in bagasse compensates for its lower sugar recovery, resulting in a better sugar yield when compared to straw.

The modification of physical properties when pretreated samples were refined can be used to explain the improvement of enzymatic digestibility. Fiber bundles disintegration, internal delamination and some extent of external fibrillation were elucidated by microscope images. According to FQA results, the increase of refining intensity was followed by a reduction of fiber size. Biomass swellability (WRV) was enhanced with the increase of refining intensity due to better accessibility of water to carbohydrates. Porometry using DSC elucidated the increase of FBW (freezing bound water) and consequent increase of pore area

(pores > 10 nm) for the refined samples. However, additional gains with the increase of refining intensity were not clearly observed. X-ray diffraction did not show any evidence for changes in biomass crystallinity. Therefore, mechanical refining can open fiber structure and decrease particle size, which improve surface area, porosity and carbohydrate accessibility, resulting in better substrate accessibility to enzymes.

5. Techno-Economic Analysis of Sugarcane Cellulosic Ethanol Biorefinery Using Mechanical Refining to Increase Sugar Recovery and Reduce Production Costs

5.1. Introduction

Enzymatic hydrolysis of carbohydrates is one of most expensive process steps for the production monomeric sugars and subsequent fermentation to ethanol when lignocellulosic biomass is used as a feedstock. Enzymatic hydrolysis is usually a low yield process (Sun & Cheng, 2002) that requires several days to be performed using expensive cocktail of enzymes (Taherzadeh & Karimi, 2007), which directly impacts production yield, capital expenses, operating costs and overall profitability. As reported by (National Renewable Energy Laboratory, 2011b), capital investment for enzymatic hydrolysis (84 hours of residence time) is around 10% of the total installed equipment cost for a corn stover cellulosic ethanol plant. The reported costs for enzymes are US\$4.24/kg of protein in the case of on-site enzyme manufacturing and US\$ 6.24/kg of protein when enzymes are purchased from a separate facility. Moreover, the operating cost of enzymes, considering enzyme load of 20 mg of protein per g cellulose to achieve 90% of cellulose conversion, was US\$ 0.09/L of ethanol, representing almost 16% of the overall operating cost.

In the case of sugarcane biorefineries, many authors have highlighted that low enzyme dosages (2 to 5 FPU/g of substrate), high carbohydrate conversion (> 70%, 85%) and short residence time (48h) have to be implemented in order to improve the competitiveness of cellulosic ethanol (Albarelli et al., 2014), (Corrêa do Lago et al., 2012), (Dias et al., 2012), (Wang et al., 2014). Moreover, reported costs of enzymes varies from US\$ 0.10 - 0.30/L of

ethanol for pessimist scenarios to U\$ 0.03 - 0.10/L ethanol when future or optimistic scenarios are evaluated (Dias et al., 2011), (Dias et al., 2012), (Mariano et al., 2013), (Obando, Gualdrón, Reno, & Lora, 2010), (Seabra, Tao, Chum, & Macedo, 2010), (Wang et al., 2014).

In this context, mechanical refining represents a good alternative to improve carbohydrate conversion using low enzyme dosage and at a low capital cost. Refining is a very well established technology used in pulp and paper industry to enhance properties of paper. The main purpose of mechanical refining is to separate fiber bundles and fibrillate cell wall to increase surface area, allowing the easy collapse and bonding of fibers during press and drying, forming a strong fiber network (J. Park et al., 2015).

In biochemical processes, mechanical refining can be applied to overcome biomass recalcitrance. Refining opens up biomass structure, generates microfibrils, shortens long fibers, increases surface area and porosity, resulting in better accessibility of enzymes to substrate and better carbohydrate conversion (Ertas et al., 2014), (J. Park et al., 2015).

Capital investment for mechanical refining is relatively low. (Han, 2014) has reported a capital investment (total installed cost) of U\$ 5.3 million dollars for a cellulosic ethanol plant that processes around 60 OD tonne/h of hardwood. However, the electricity consumption might impact the economic benefit of using mechanical refining. (J. Park et al., 2015) has estimated that an industrial disk refiner, operating at 10% to 15% consistency, would require about 100 kwh/OD tonne of pretreated biomass.

Therefore, the objective of this study is to evaluate the economic impact of using mechanical refining to improve biomass digestibility of pretreated sugarcane bagasse and straw. Different residence times and enzyme dosages will also be evaluate under a techno-

economic perspective. Deterministic and probabilistic techno-economic analysis will be executed to compare the financial performance of each studied scenario, identify the most impacting economic drivers and evaluate the impact of their volatility and variability on internal rate of return, net present value and minimum ethanol selling price.

5.2. Materials and Methods

5.2.1. Biomass

Sugarcane bagasse and straw were kindly provided by CTBE (Brazilian Bioethanol Science and Technology Laboratory). Those feedstocks were air dried, characterized (Table 3.6) using a Laboratory Analytical Procedure published by (National Renewable Energy Laboratory, 2011a) and stored at room temperature.

5.2.2. Pretreatment (Autohydrolysis)

Autohydrolysis pretreatment was executed in a 300 L stainless steel batch reactor using 15 kg using 1:10 solid:liquid ratio. After pretreatment, the liquid fraction was collected and analyzed to determine the sugars, organic acids and degradation products using HPLC (National Renewable Energy Laboratory, 2006). The solid fraction was washed with sufficient tap water to eliminate any dissolved biomass and until neutral pH was achieved. The washed solid was also characterized.

5.2.3. Mechanical Refining (PFI Refining)

PFI refiner is a batch equipment where biomass is beaten between a roll with bars and a smooth-walled beater housing, both rotating in the same direction but at different speeds. The refining action is achieved through the differential rotational action and the application of loading between the roll and housing during a specified number of revolutions. Pretreated and washed biomass was refined using a PFI refiner according to (Technical Association of Paper and Paper Industry, 2000). Each batch of refining was performed with 30 OD grams of biomass at 10% consistency. Four refining intensities (2000, 4000, 6000 and 8000 revolutions) were evaluated.

Samples were also disintegrated using 3000 revolutions in a pulp disintegrator to evaluate if effect of fiber dispersion might improve enzymatic hydrolysis.

5.2.4. Enzymatic Hydrolysis

Enzymatic hydrolysis was performed in 50 mL tubes using 5 FPU/g (dry basis) of pretreated and washed substrate, 10% consistency, pH 4.8~5.0 and 50°C. Novozymes Cellic CTec 2 supplemented with 1/9 Cellic HTec 2 was used as the enzyme cocktail. Sodium acetate buffer was used for pH control. The incubator (Fine PCR COMBI- D24) was maintained at 50 °C and 15 rpm. During enzymatic hydrolysis, samples were taken at 24, 48, 72 and 96 hours. Each sample was centrifuged for 10 min at 4400 rpm using an Eppendorf Centrifuge 5702. The supernatant was used for sugar determination (National Renewable Energy Laboratory, 2006).

5.2.5. Process Simulation - Aspen Plus

A steady state process simulation was built using Aspen Plus 8.2.3. The base case simulation represented by an Autonomous Distillery was kindly provided by CTBE (Brazilian Bioethanol Science and Technology Laboratory). The proposed cases represented by the cellulosic ethanol biorefinery were also built in Aspen according to (National Renewable Energy Laboratory, 2011b). Experimental data for autohydrolysis pretreatment, mechanical refining and enzymatic hydrolysis were used in the process simulation.

5.2.6. Studied Cases: Techno-Economic Analysis to Evaluate the Economic Impact of Mechanical Refining, Enzymatic Hydrolysis Residence Times and Enzyme Dosages for a Co-Located Sugarcane Cellulosic Ethanol Biorefinery

In this work, 12 scenarios will be studied according to Table 5.1. All cases consider a cellulosic ethanol biorefinery will be co-located in an existing autonomous distillery (Figure 3.1 - base case) that crushes 2,000,000 TC/y and burns all bagasse to produce steam and power.

Scenario 1 to 6 (see Figure 5.1) considers that all bagasse will be converted in the cellulosic ethanol plant. Solid residues and straw will be burned in the CHP area to produce steam to meet the demand of both facilities (1G and 2G plants). Excess steam is converted to power. Excess power is sold to the grid. Differences among Scenarios 1 to 6 is the inclusion of mechanical refining, residence time for enzymatic hydrolysis and enzyme dosage. Scenarios 7 to 12 are similar to Scenarios 1 to 6, however, Scenarios 7 to 12 use straw as

feedstock for ethanol production. It was assumed an additional electricity consumption of 100 kWh/OD tonne of pretreated biomass for mechanical refining.

Table 5.1. Studied Scenarios - Economic Evaluation of Mechanical Refining, Residence Time and Enzyme Dosage

Scenario	Plant Allocation	Biomass to 2G Ethanol	Mechanical Refining	Enzymatic Hydrolysis
1 BAG-5FPU-96h	Co-location 2 MTC/y	Bagasse	NO	5 FPU/g; 96 h
2 BAG-MR-5 FPU-96h	Co-location 2 MTC/y	Bagasse	Yes	5 FPU/g; 96 h
3 BAG-MR-5 FPU-72h	Co-location 2 MTC/y	Bagasse	Yes	5 FPU/g; 72 h
4 BAG-MR-5 FPU-48h	Co-location 2 MTC/y	Bagasse	Yes	5 FPU/g; 48 h
5 BAG-MR-5 FPU-24h	Co-location 2 MTC/y	Bagasse	Yes	5 FPU/g; 24 h
6 BAG-MR-3 FPU-96h	Co-location 2 MTC/y	Bagasse	Yes	3 FPU/g; 96 h
7 STW-5 FPU-96h	Co-location 2 MTC/y	Straw	NO	5 FPU/g; 96 h
8 STW-MR-5 FPU-96h	Co-location 2 MTC/y	Straw	Yes	5 FPU/g; 96 h
9 STW-MR-5 FPU-72h	Co-location 2 MTC/y	Straw	Yes	5 FPU/g; 72 h
10 STW-MR-5 FPU-48h	Co-location 2 MTC/y	Straw	Yes	5 FPU/g; 48 h
11 STW-MR-5 FPU-24h	Co-location 2 MTC/y	Straw	Yes	5 FPU/g; 24 h
12 STW-MR-3 FPU-96h	Co-location 2 MTC/y	Straw	Yes	3 FPU/g; 96 h

BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant;
MR = mechanical refining; 5FPU - 3 FPU = enzyme dosage FPU/g substrate (dry basis);
24h - 48h - 72h - 96h = enzymatic hydrolysis residence time in hours.

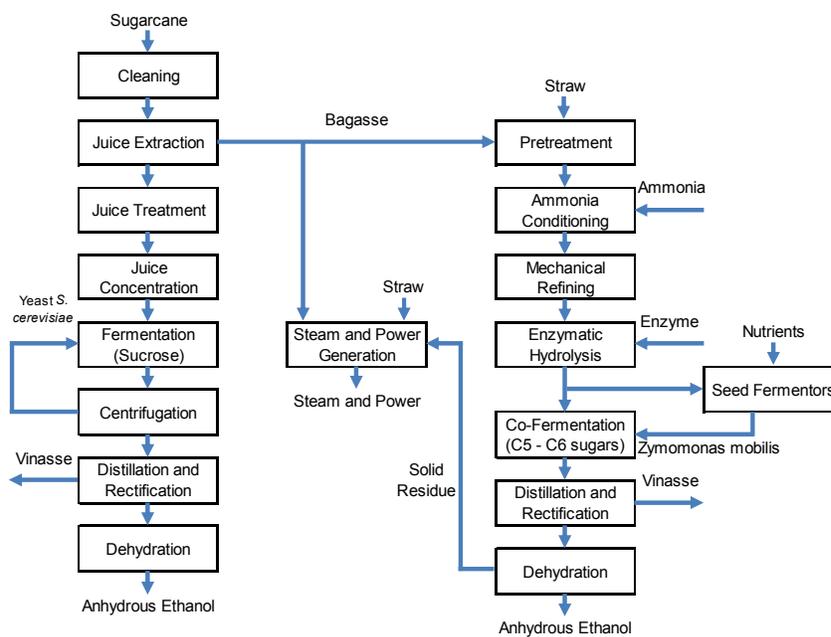


Figure 5.1. Scenario 1 to 6 - Bagasse to Cellulosic Ethanol; Straw to CHP

5.2.7. Economic Analysis and Financial Assumptions

The mass and energy balances obtained from simulations were used as input data for the economic analysis. A traditional cash flow was built using a spreadsheet. Internal rate of return (IRR) and net present value (NPV) were used as financial metrics to evaluate the return on investment. IRR is defined as the discount rate that gives a zero NPV. The NPV is defined as the sum of discounted free cash flow at target rate of return at each project year.

5.2.7.1. Capital Investment

Capital investment was estimated using data reported by (National Renewable Energy Laboratory, 2011b). Capital investment for mechanical refining was assumed to be US\$ 1,000,000 to refine 36 OD tonne/h of pretreated biomass (Han, 2014). Equipment cost were scaled using 0.60 as scaling exponent for all areas, except for storage (1.0) and pretreatment (0.85). “Producer Price Index Industry Data” for “Other Heavy Machinery Rental and

Leasing” was used to escalate equipment costs to 2015. Scaled and escalated equipment cost were multiplied by 2.6 to calculate the total installed costs for each process step. Biomass Storage includes a concrete floor and trucks to store and transport biomass to CHP area or cellulosic ethanol plant. Bagasse is stored in compacted and covered piles to minimize degradation. Infrastructure (Land, Roads, Offices, Water Plant) is 15% of the installed cost for storage, pretreatment, enzymatic hydrolysis, fermentation and ethanol purification.

5.2.7.2. Financial Assumptions

Table 5.2 brings the financial assumptions adopted in this chapter.

Table 5.2. Financial Assumptions

Start up year	2015
Terminal Year	2030
1G ethanol Plant Operating Hours Per Year	3976
2G ethanol Plant Operating Hours Per Year	8400
Capital Spending in year -3 - %	5
Capital Spending in year -2 - %	15
Capital Spending in year -1 - %	30
Capital Spending in year 0 - %	50
Capital Investment in Maintenance and Improvement - % RAV*	2
Depreciation years	10
Nominal Capacity in 2015 - %	60
Nominal Capacity in 2016 - %	90
Working Capital - % Revenue + Direct Costs**	10
Overhead and Administration Costs- % Revenue	3
Maintenance, Operating Materials, Other Costs - % RAV	5
Taxes - %	34
Project Terminal Value - Multiple of EBITDA*** in year 15	5
Internal Rate of Return - %	12
Ethanol Price - US\$/L	0.46
Electricity Price - US\$/MWh	60.32
Bagasse Opportunity Delivered Cost - US\$/OD tonne	48.70
Bagasse Moisture - %	50
Straw Delivered Cost - US\$/OD tonne	31.84
Straw Moisture - %	15
Enzyme Delivered Cost - US\$/kg protein	5.00

*Capital investment for continuous improvement and reduction of costs as a percentage of RAV (Replacement Asset Value)

**Direct Costs = Biomass, Chemical, Enzyme and Electricity

***EBITDA = Earns Before Interests, Taxes and Depreciation

All costs and prices are in 2015 Dollars - US\$1.00 = R\$3.33

5.2.7.3. Deterministic Analysis, Probabilistic Analysis and MESP

Techno-economic analysis of capital projects is usually performed by combining a process model (conversion process of raw materials into desired products) with a financial model (cash flow). Input parameters related to raw materials, products and process (e.g. raw material cost, product price, reaction yield) are fed to the techno-economic model and output parameters, which usually are financial metrics (e.g. NPV, IRR), are used to evaluate and compare projects during the decision making process. Traditionally, techno-economic analysis is based on deterministic analysis (Figure 5.2), where a single value for the inputs (average value, actual value, common value) generates a single value for the outputs.

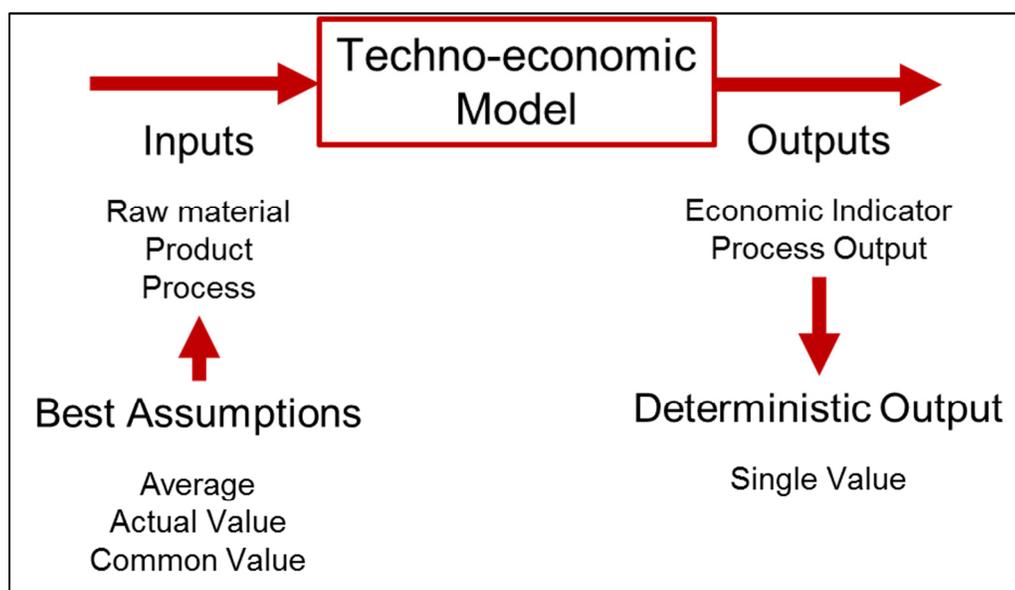


Figure 5.2. Deterministic Techno-economic Analysis

However, variability and volatility of input parameters (e.g. variability of biomass composition, volatility of ethanol price) can significantly impact the outcome. Additionally, during the decision making process, companies have to choose the most robust projects among several options and a better understanding about the uncertainties associated with each project could help investors to choose projects with higher chances of success.

In this context, probabilistic analysis can be performed using a specialized software (@Risk) to give a better understanding about the financial risks associate with the uncertainties of input parameters. In this analysis, the uncertainties of each input parameter is represented by a probability density function created using historical market data, data from literature or applying best judgment. @ Risk continuously samples the probability functions and continuously records the outputs. As a result, this approach allows the observation of the impact of input variability on a financial metric and provides the probability for an output parameter to reach a target value (Treasure et al., 2014) (Figure 5.3).

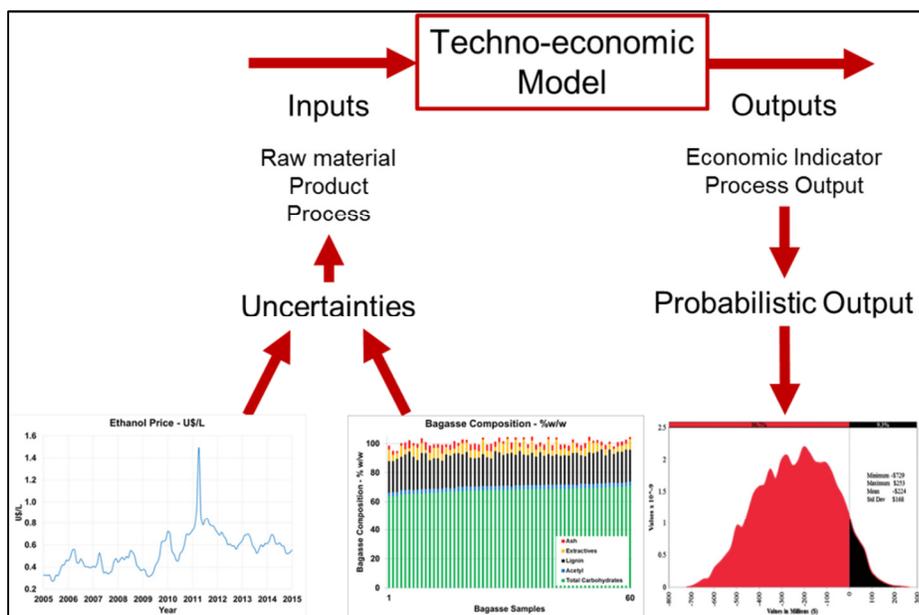


Figure 5.3. Probabilistic Techno-Economic Analysis

Therefore, deterministic techno-economic analysis was performed to compare all 12 scenarios using IRR as a financial metric. Sensitivity analysis was also executed for Scenarios 1 and 2 to monitor IRR while revenue and cost drivers were perturbed under a defined pattern ($\pm 25\%$). Ethanol price, electricity price, straw cost, enzyme cost, chemical cost and capital investment were the revenue and costs drivers evaluated with sensitivity analysis. The most economic impacting parameters defined by sensitivity analysis were used as input for probabilistic techno-economic analysis for Scenarios 1 and 2. Finally, MESP (Minimum Ethanol Selling Price) was calculated using deterministic and probabilistic analysis for Scenario 2.

5.3.Results and Discussion

5.3.1. Enzymatic Hydrolysis Conversion

Mechanical refining improved enzymatic hydrolysis conversion by 6 to 10% in the case of pretreated bagasse (Figure 5.4) and by 5 to 7% in the case of pretreated straw (Figure 5.5). No significant differences in digestibility were observed between unrefined and disintegrated samples, showing that the simple dispersion of fibers is not able to improve enzymatic hydrolysis.

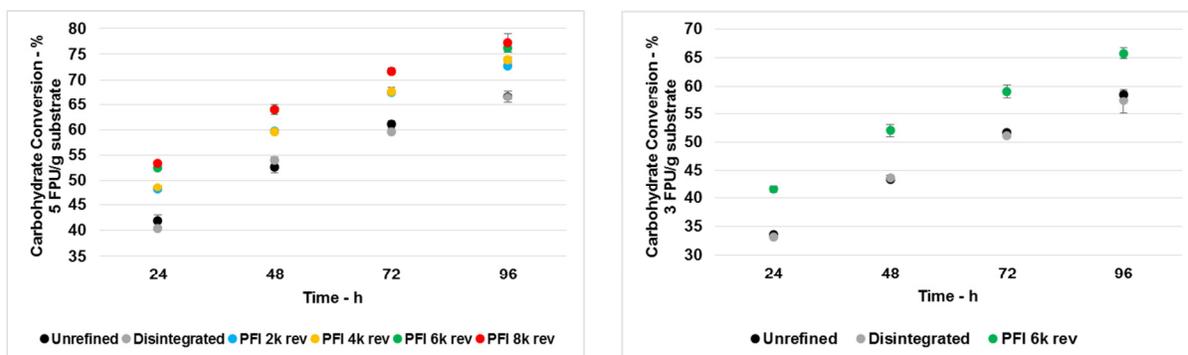


Figure 5.4. Enzymatic Hydrolysis Conversion - Unrefined, Disintegrated and Refined Bagasse - 24 to 96 h; 3 and 5 FPU/g

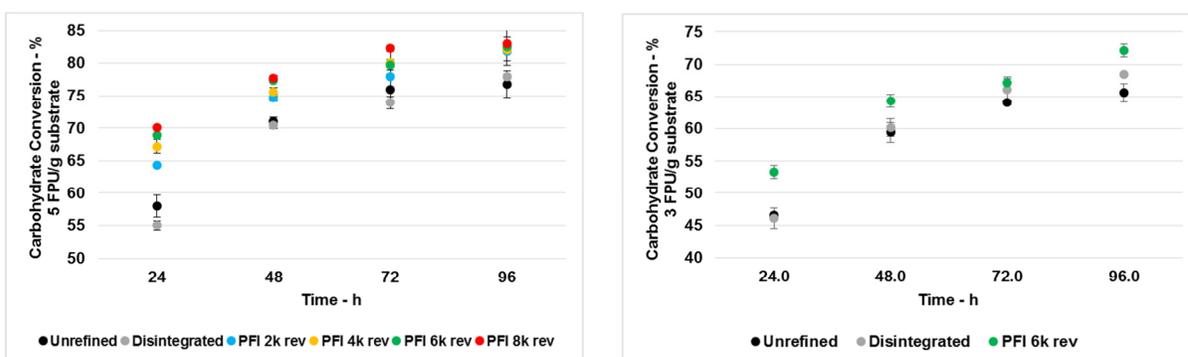


Figure 5.5. Enzymatic Hydrolysis Conversion - Unrefined, Disintegrated and Refined Straw - 24 to 96 h; 3 to 5 FPU/g

5.3.2. Simulation Results

The comparison between Scenarios 0 and 1 shows that 2G ethanol production was increased by 9.7% when pretreated sugarcane bagasse was refined. However mechanical refining has increased the electricity consumption by 16.3% (Table 5.3). The better digestibility of biomass after mechanical refining reduces the amount of solid residue to be burned in the CHP and increases the steam consumption with ethanol distillation, which decreases the power production by 4.3%.

Table 5.3. Simulation Results - Scenarios 1 to 6

Scenario	0	1 BAG-5FPU- 96h	2 BAG-MR- 5FPU-96h	3 BAG-MR- 5FPU-72h	4 BAG-MR- 5FPU-48h	5 BAG-MR- 5FPU-24h	6 BAG-MR- 3FPU-96h
Sugarcane to 1G - tonne/y	2,000,000	2,000,000	2,000,000	2,000,000	2,000,000	2,000,000	2,000,000
Bagasse to 2G - OD tonne/y	-	226,323	226,323	226,323	226,323	226,323	226,323
Bagasse Storage Loss - %	-	10	10	10	10	10	10
Straw to CHP - OD tonne/y	-	226,323	226,323	226,323	226,323	226,323	226,323
Straw Storage Loss - %	-	5	5	5	5	5	5
1G Ethanol Production - m3/y	166,024	166,024	166,024	166,024	166,024	166,024	166,024
2G Ethanol Production - m3/y	-	67,145	73,720	70,593	65,347	57,529	66,429
Refining Power consumption - MWh/y	-	-	15,888	15,888	15,888	15,888	15,888
Total Power Consumption - MWh/y	60,000	97,414	113,301	111,965	110,629	109,293	113,301
Power Production - MWh/y	212,504	291,866	279,199	285,232	294,970	309,958	292,593
Power Surplus - MWh/y	152,504	194,452	165,898	173,267	184,342	200,666	179,292
Steam Demand - tonne/h	176.6	230.1*	231.4*	230.8*	229.7*	228.2*	229.9*
Steam Production - tonne/h	283.6	230.1*	231.4*	230.8*	229.7*	228.2*	229.9*
Boilers Capacity - tonne/h	283.6	283.6	283.6	283.6	283.6	283.6	283.6
Power Capacity – MW	53.4	39.5	37.8	39.2	41.7	45.6	41.3

*Maximum steam demand and production during the sugarcane season (167 days/y) when both facilities (1G and 2G) are under operation.

BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant;
MR = mechanical refining; 5FPU - 3 FPU = enzyme dosage FPU/g substrate (dry basis);
24h - 48h - 72h - 96h = enzymatic hydrolysis residence time in hours.

Table 5.4. Simulation Results - Scenarios 7 to 12

Scenario	0	7 STW-5FPU- 96h	8 STW-MR- 5FPU-96h	9 STW-MR- 5FPU-72h	10 STW-MR- 5FPU-48h	11 STW-MR- 5FPU-24h	12 STW-MR- 3FPU-96h
Sugarcane to 1G - tonne/y	2,000,000	2,000,000	2,000,000	2,000,000	2,000,000	2,000,000	2,000,000
Bagasse to CHP - OD tonne/y	-	226,323	226,323	226,323	226,323	226,323	226,323
Bagasse Storage Loss - %	-	10	10	10	10	10	10
Straw to 2G - OD tonne/y	-	226,323	226,323	226,323	226,323	226,323	226,323
Straw Storage Loss - %	-	5	5	5	5	5	5
1G Ethanol Production - m3/y	166,024	166,024	166,024	166,024	166,024	166,024	166,024
2G Ethanol Production - m3/y	-	63,337	66,563	64,948	63,572	58,726	60,477
Refining Power consumption - MWh/y	-	-	14,525	14,525	14,525	14,525	14,525
Total Power Consumption - MWh/y	60,000	97,414	111,939	110,603	109,266	107,930	111,939
Power Production - MWh/y	212,504	229,349	223,096	226,201	228,975	238,234	234,394
Power Surplus - MWh/y	152,504	131,935	111,158	115,598	119,709	130,304	122,455
Steam Demand - tonne/h	176.6	226.0*	226.6*	226.3*	226.0*	225.1*	225.4*
Steam Production - tonne/h	283.6	226.0*	226.6*	226.3*	226.0*	225.1*	225.4*
Boilers Capacity - tonne/h	283.6	283.6	283.6	283.6	283.6	283.6	283.6
Power Capacity – MW	53.4	24.7	24.6	25.3	25.9	28.2	27.6

*Maximum steam demand and production during the sugarcane season (167 days/y) when both facilities (1G and 2G) are under operation.

BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant;
 MR = mechanical refining; 5FPU - 3 FPU = enzyme dosage FPU/g substrate (dry basis);
 24h - 48h - 72h - 96h = enzymatic hydrolysis residence time in hours.

When pretreated sugarcane straw was refined (Scenarios 7 and 8), 2G ethanol production was increased by 5.1%, power production was decreased by 2.7% and electricity consumption was increased by 14.9% (

Table 5.4).

When enzymatic hydrolysis residence time is reduced (Scenarios 3, 4 and 5; Scenarios 9, 10 and 11), less ethanol is produced and more electricity is generated due to the increase in the amount of solid residue burned in the CHP area. Reduction of enzyme dosage (Scenarios 2 and 6; Scenarios 8 and 12) reduces ethanol production and increases power production due to lower conversion of biomass in enzymatic hydrolysis.

5.3.3. Capital Investment

Capital expenditure with mechanical refining is considerably low (around 2% of the total capital - Figure 5.6 and Figure 5.7). Reduction in enzymatic hydrolysis residence time decreases capital investment with enzymatic hydrolysis and fermentation up to 38% (Scenarios 3, 4 and 5; Scenarios 9, 10 and 11). Lower ethanol production reduces capital expenses with ethanol purification and product storage. Lower digestibility of biomass due to reduction in residence time or enzyme dosage, increases the production of excess steam and investment with turbogenerators.

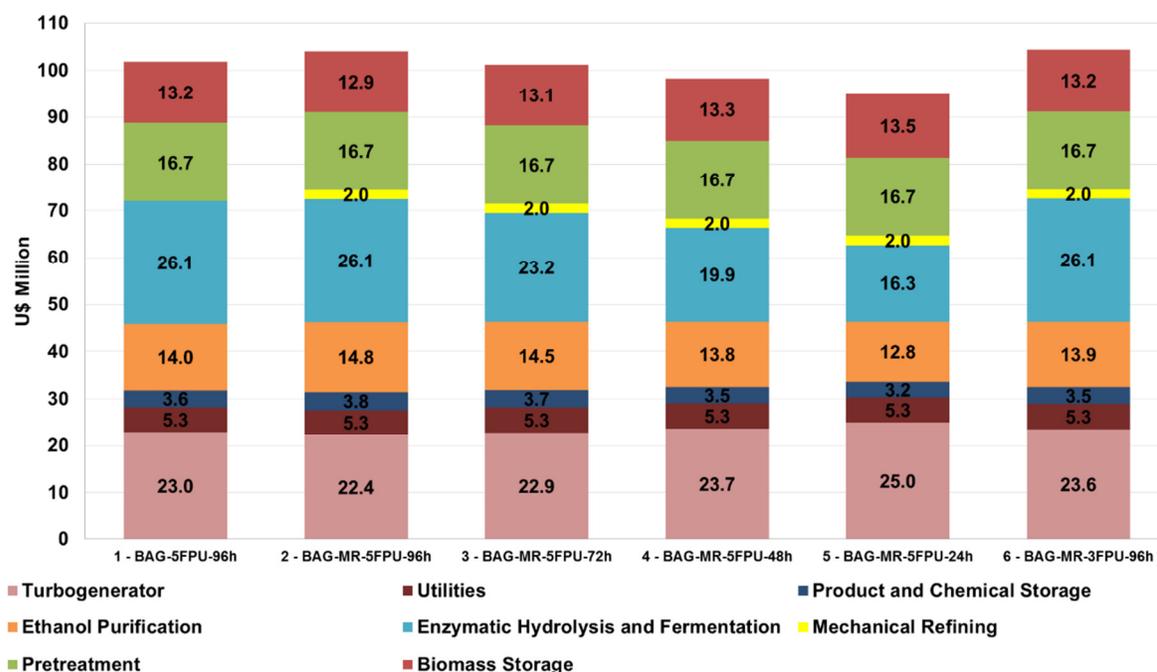


Figure 5.6. Capital Investment - Scenarios 1 to 6.

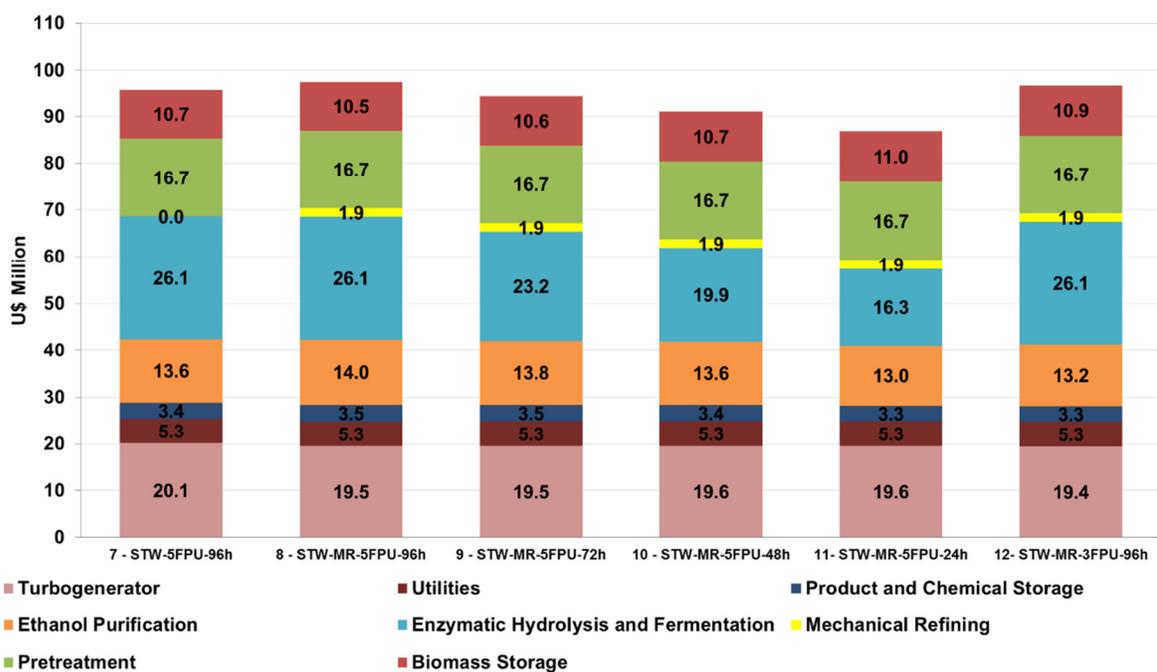


Figure 5.7. Capital Investment - Scenarios 7 to 12

5.3.4. Operating Costs and IRR

The additional ethanol production (~ 9%) and consequent additional revenue obtained when mechanical refining was introduced in the process, has contribute to reduce the operating costs between Scenarios 1 and 2 (bagasse conversion to ethanol) (Table 5.5). On the other hand, additional power consumption and lower power production has caused a reduction in the electricity revenue (reported as a negative cost). Therefore, mechanical refining was able to improve the IRR by 0.8%, showing that the additional ethanol revenue has overcome the additional capital investment and lower surplus electricity (Scenarios 1 and 2). However, when straw was converted to ethanol (Scenarios 7 and 8), the additional ethanol revenue (~ 5%) obtained with mechanical refining was not enough to overcome additional capital and lower power production, resulting in no improvement in IRR (Table 5.6).

Reduction of enzymatic hydrolysis residence time (96h to 24h) does not improve IRR (Scenarios 3, 4 and 5; Scenarios 8, 10 and 11). The benefit of reducing capital investment and the additional surplus electricity does not overcome the reduction in ethanol revenue. Reduction on enzyme dosage (5 FPU/g to 3 FPU/g) does not improve the financial performance significantly (Scenarios 2 and 6; Scenarios 8 and 12).

Table 5.5. Operating Costs and IRR - Scenarios 1 to 6

Scenarios	1 BAG- 5FPU-96h	2 BAG-MR- 5FPU-96h	3 BAG-MR- 5FPU-72h	4 BAG-MR- 5FPU-48h	5 BAG-MR- 5FPU-24h	6 BAG-MR- 3FPU-96h
Sugarcane Bagasse	0.000	0.000	0.000	0.000	0.000	0.000
Sugarcane Straw	0.113	0.103	0.107	0.116	0.132	0.114
Enzyme	0.093	0.085	0.089	0.096	0.109	0.057
Chemicals	0.027	0.024	0.025	0.027	0.031	0.027
Electricity	-0.038	-0.011	-0.018	-0.029	-0.050	-0.024
Maintenance, Materials, Others	0.076	0.071	0.072	0.075	0.082	0.079
Labor	0.016	0.015	0.016	0.017	0.019	0.016
Depreciation	0.152	0.141	0.143	0.150	0.165	0.157
Overhead, Administration	0.014	0.014	0.014	0.014	0.014	0.014
Total - US\$/L ethanol	0.453	0.442	0.449	0.466	0.502	0.440
IRR - %	10.5%	11.3%	10.8%	9.5%	6.8%	11.3%
Capex - US\$/L ethanol	1.52	1.41	1.43	1.50	1.65	1.57
Ethanol - L/OD tonne biomass	296.7	325.7	311.9	288.7	254.2	293.5

BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant;
MR = mechanical refining; 5FPU - 3 FPU = enzyme dosage FPU/g substrate (dry basis);
24h - 48h - 72h - 96h = enzymatic hydrolysis residence time in hours.

Table 5.6. Operating Costs and IRR - Scenarios 7 to 12

Scenarios	7 STW- 5FPU-96h	8 STW-MR- 5FPU-96h	9 STW-MR- 5FPU-72h	10 STW-MR- 5FPU-48h	11 STW-MR- 5FPU-24h	12 STW-MR- 3FPU-96h
Sugarcane Bagasse	0.000	0.000	0.000	0.000	0.000	0.000
Sugarcane Straw	0.120	0.114	0.117	0.119	0.129	0.125
Enzyme	0.091	0.086	0.089	0.091	0.098	0.057
Chemicals	0.028	0.026	0.027	0.028	0.030	0.029
Electricity	0.020	0.037	0.034	0.031	0.023	0.030
Maintenance, Materials, Others	0.076	0.073	0.073	0.072	0.074	0.080
Labor	0.017	0.016	0.017	0.017	0.019	0.018
Depreciation	0.151	0.146	0.145	0.143	0.148	0.160
Overhead, Administration	0.014	0.014	0.014	0.014	0.014	0.014
Total - US\$/L ethanol	0.516	0.514	0.515	0.515	0.534	0.514
IRR - %	5.7%	5.7%	5.5%	5.5%	3.4%	6.2%
Capex - US\$/L ethanol	1.51	1.46	1.45	1.43	1.48	1.60
Ethanol - L/OD tonne biomass	279.9	294.1	287.0	280.9	259.5	267.2

BAG = bagasse to 2G ethanol plant; STW = straw to 2G ethanol plant;
MR = mechanical refining; 5FPU - 3 FPU = enzyme dosage FPU/g substrate (dry basis);
24h - 48h - 72h - 96h = enzymatic hydrolysis residence time in hours.

5.3.5. Sensitivity Analysis

Figure 5.8 shows the sensitivity analysis performed for Scenarios 1 and 2. IRR is very sensitive to Ethanol price and CAPEX. Straw cost and enzyme cost have also high impact on IRR but at a lesser extent. Electricity price and chemical costs are less important. As a result,

any further investigation trying to improve the financial performance should include a better investigation on the parameters with high economic impact. However, traditional sensitivity analysis has some limitations. It evaluates cost and revenue drivers individually and does not account for their volatility or variability. Usually, during the decision making process, companies and investors look for the most attractive options among several projects alternatives. In this process, companies are interested on projects with high return on investment and with high chances of reaching a target financial metric (e.g. IRR, NPV) even when volatilities and variabilities of economic drivers are considered. In order to address those issues, probabilistic analysis was performed using software @Risk.

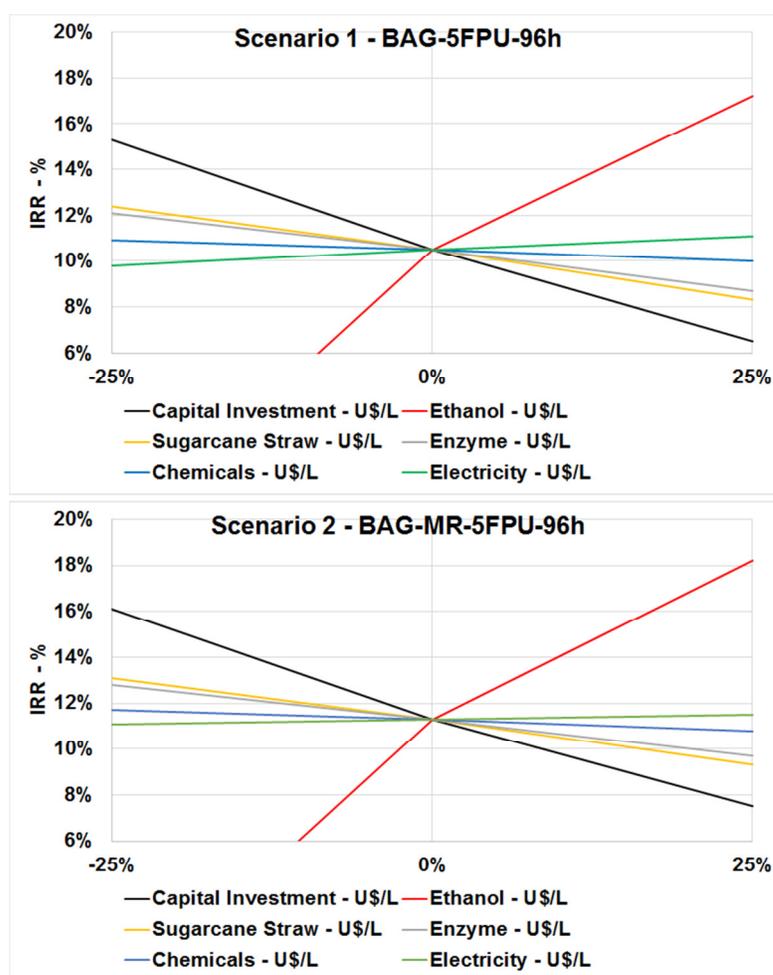


Figure 5.8. Sensitivity Analysis - Scenarios 1 and 2

5.3.6. Probabilistic Analysis

The parameters with high impact on IRR identified during sensitivity analysis were selected for probabilistic analysis (ethanol price, capital investment, straw cost, enzyme cost, electricity cost). Additionally, it is known that biomass chemical composition may vary according to crop variety, weather conditions, soil composition and agricultural practices. Variations on biomass composition will impact the amount of ethanol production and biomass heating value. In this context, variability of bagasse composition was also considered for probabilistic analysis.

5.3.6.1. Probability Density Functions (PDF)

Figure 5.9, Figure 5.10, Figure 5.11, Figure 5.12, Figure 5.13, Figure 5.15, Figure 5.16 and Figure 5.17 present the probability density functions. Historical data collected from 2005 to 20015 was used to build the probability distributions for ethanol and electricity prices. Values were corrected to 2015 using Brazilian inflation index (IPCA - Índice Nacional de Precos ao Consumidor Amplo). Uniform distribution were assumed for capital investment ($\pm 30\%$) and enzyme cost ($\pm 25\%$).

Straw cost is based on (T.F. Cardoso et al., 2015) and it is a function of percentage of collection, transportation distance and sugarcane productivity. Percentage of collection was fixed to 50%. A discrete distribution was selected for transportation distance based on (Companhia Nacional de Abastecimento, 2013). Historical data from 2005 to 2015 was used for sugarcane productivity (Companhia Nacional de Abastecimento, 2016). As a result, the average price of straw is U\$31.77/OD tonne, with a maximum of U\$37.04/OD tonne when lowest productivity and highest transportation distance were considered; and a minimum of

US\$28.36/OD tonne when straw is collected from fields with high productivity and located close to the mill.

Figure 5.14 brings the variability of bagasse composition reported by (Rocha et al., 2015). The average content of carbohydrates is 67.98%, with a maximum of 71.15% and minimum of 63.78%. Lignin content also varies from 18.9% to 26.12%, with an average of 21.56%. There is a significant variation on the biomass composition which will impact the financial performance.

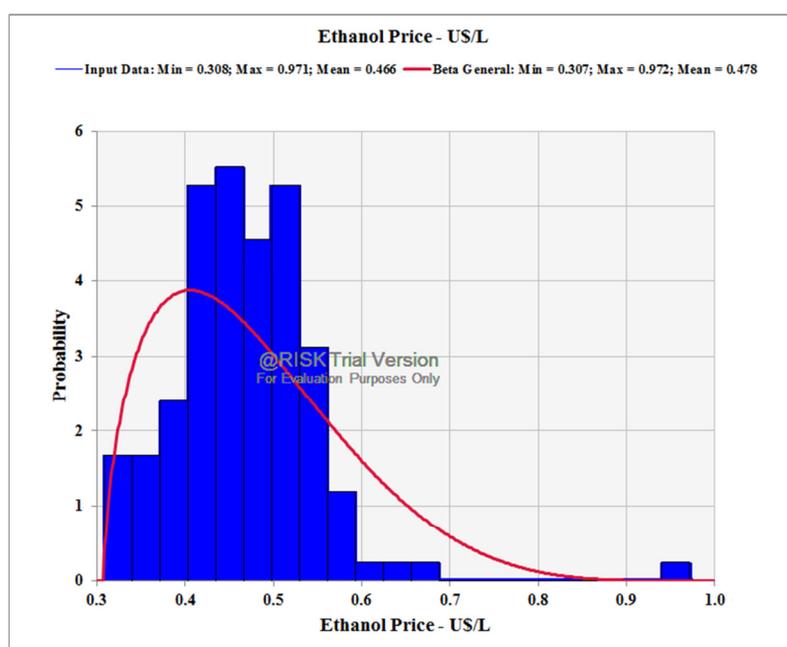


Figure 5.9. Ethanol Price PDF - Historical data from 2005 to 2015 - Values corrected with IPCA

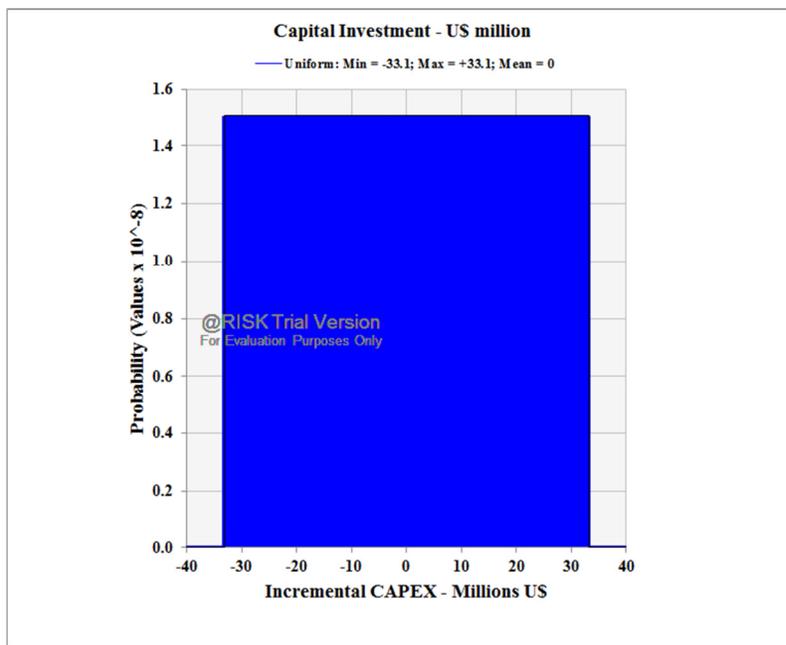


Figure 5.10. Incremental Capex PDF - Uniform distribution +/- 30%

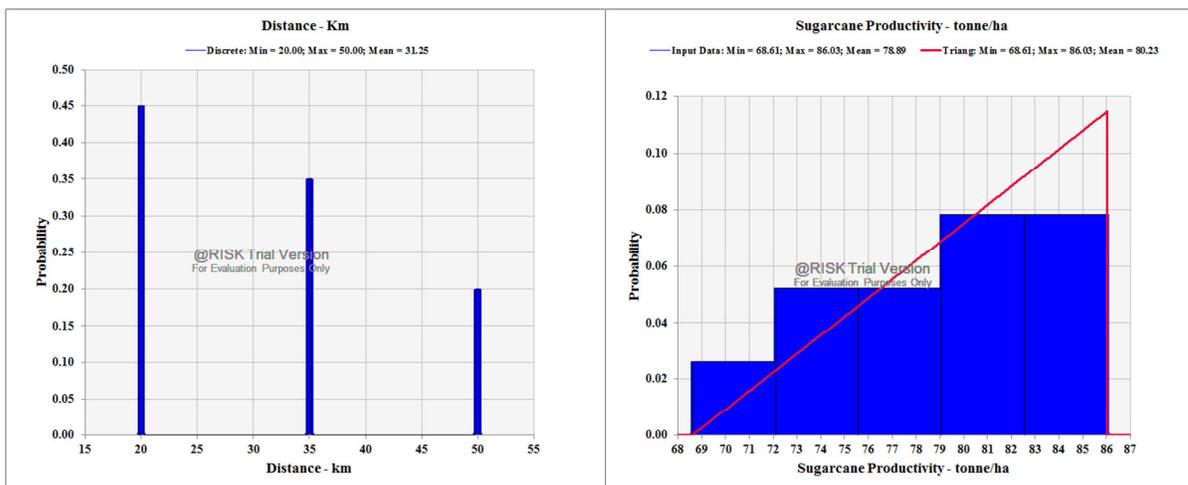


Figure 5.11. Transport Distance PDF - Discrete distribution; Sugarcane Productivity PDF -Triangular distribution

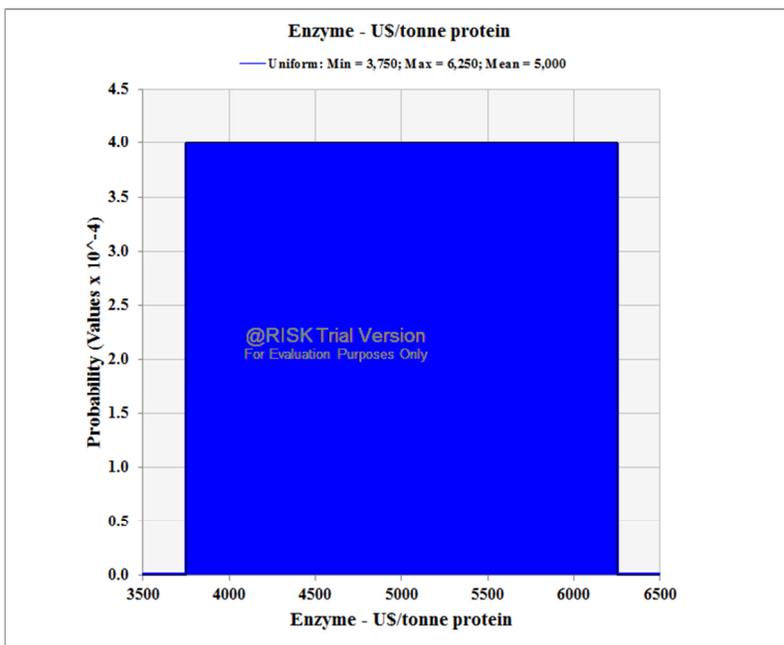


Figure 5.12. Enzyme Cost PDF - Uniform Distribution +/- 25%

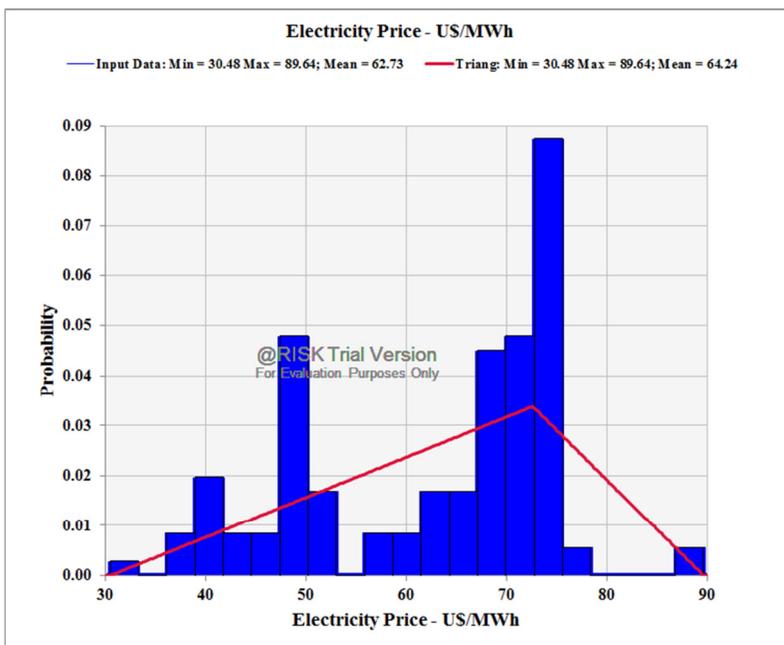


Figure 5.13. Electricity Price PDF - Triangular Distribution – Historical data from 2005 to 2015 auctions - Values corrected with IPCA

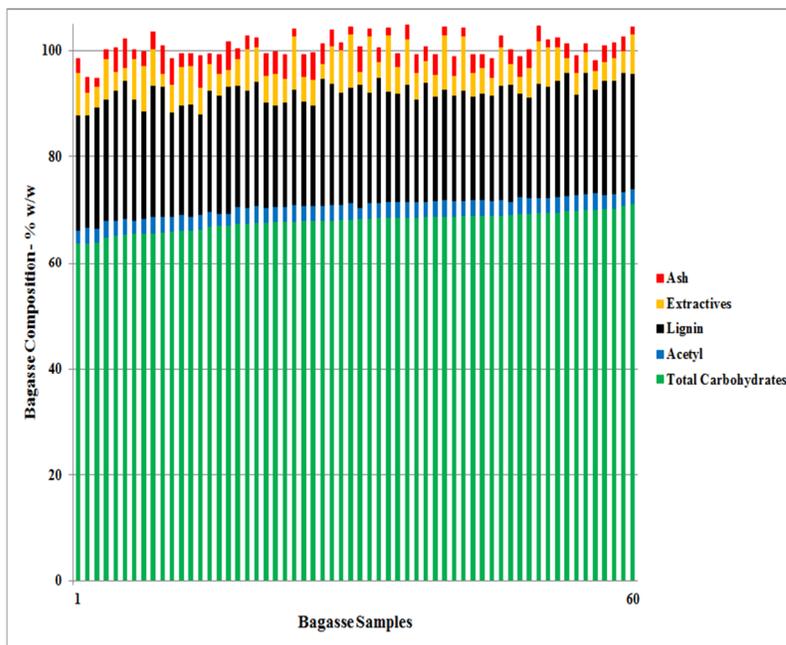


Figure 5.14. Bagasse Composition Variability (Rocha et al., 2015)

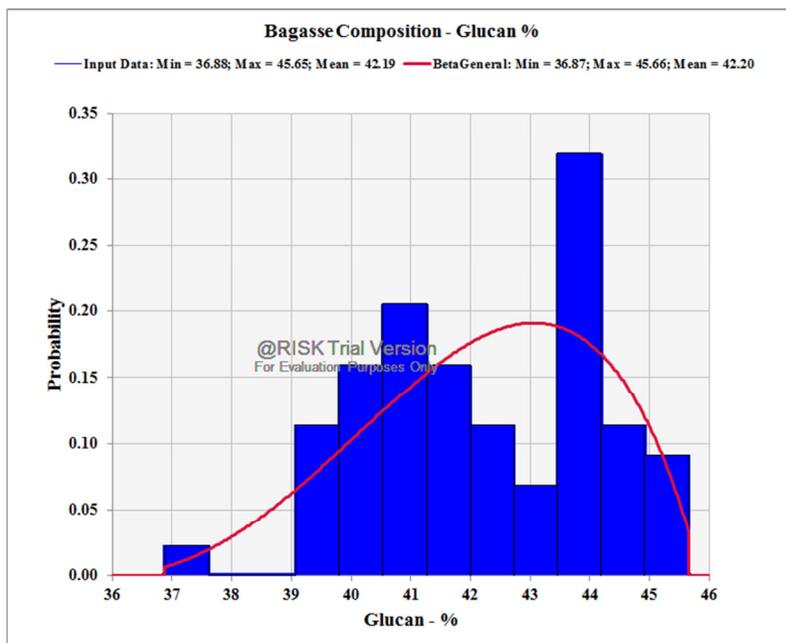


Figure 5.15. Bagasse Composition - Glucan % PDF

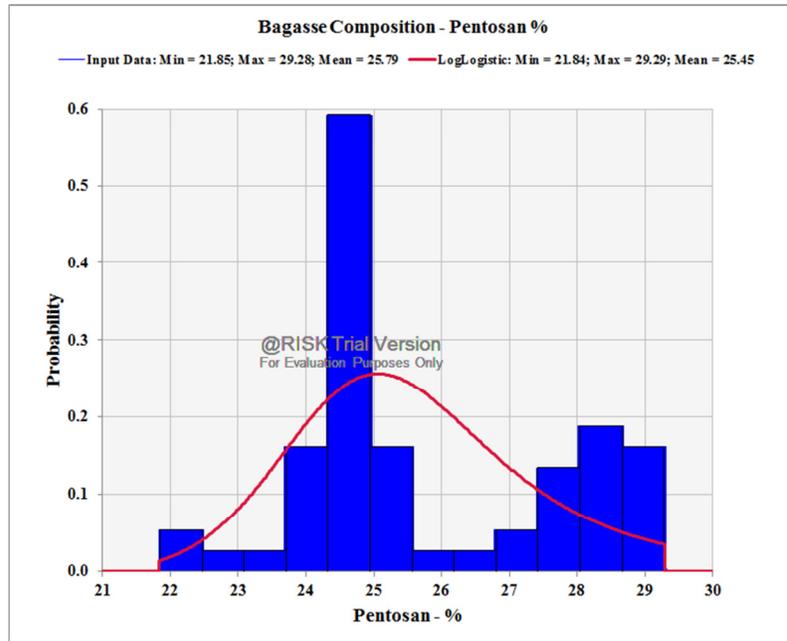


Figure 5.16. Bagasse Composition - Pentosan % PDF

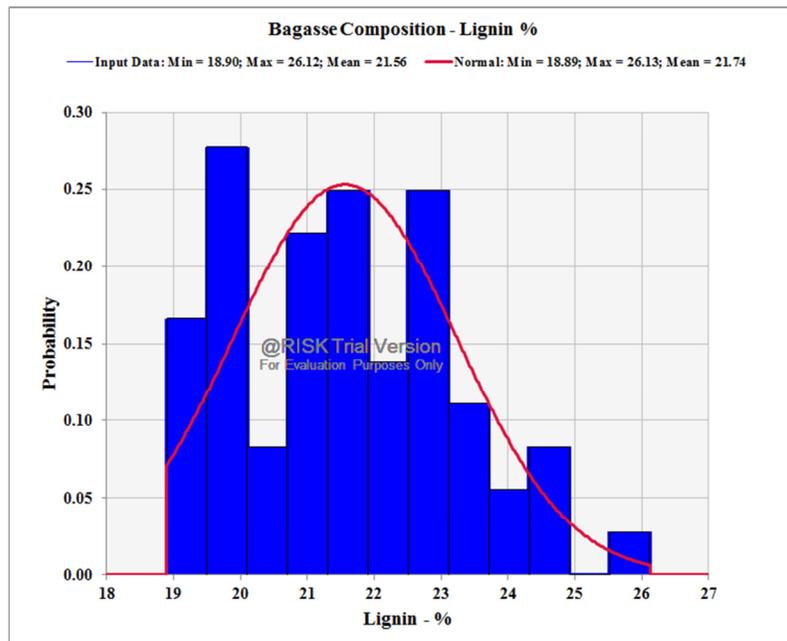


Figure 5.17. Bagasse Composition - Lignin % PDF

5.3.6.2. Probability Distribution of Net Present Value

The probability distributions of NPV with a discount rate of 12% for Scenarios 1 and 2 are presented in Figure 5.18 and Figure 5.19. The volatility and variability of most significant cost and revenue drivers considerably influences the probability distribution of NPV. Scenario 2 has 50.4% chances of having a positive NPV at 12% discount rate, while Scenario 1 has 47.8% chances.

Sensitivity analysis was also performed during probabilistic analysis. Figure 5.20 and Figure 5.21 show the regression coefficients (slope of the sensitivity line obtained between changes in each economic driver and net present value) calculated during probabilistic analysis. The information obtained with sensitivity regression coefficients is very similar to the traditional sensitivity analysis. Besides ethanol price, capital investment and enzyme cost, carbohydrate content is also an important economic driver because it directly impact ethanol production per tonne of biomass. However, lignin content is not so significant.

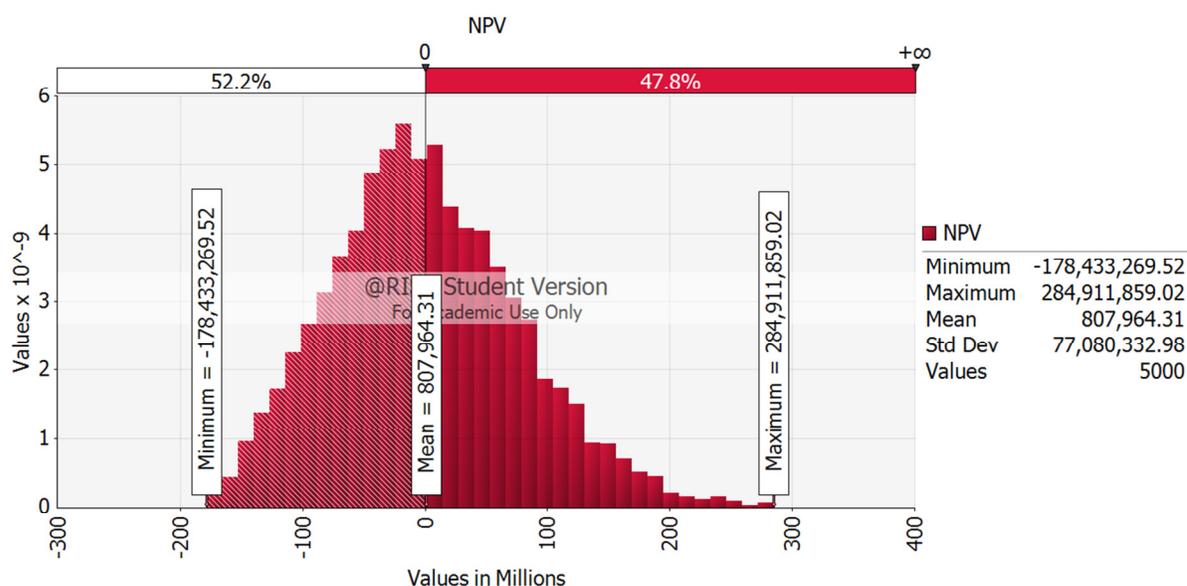


Figure 5.18. Probability Distribution of NPV with a discount rate of 12% for Scenario 1

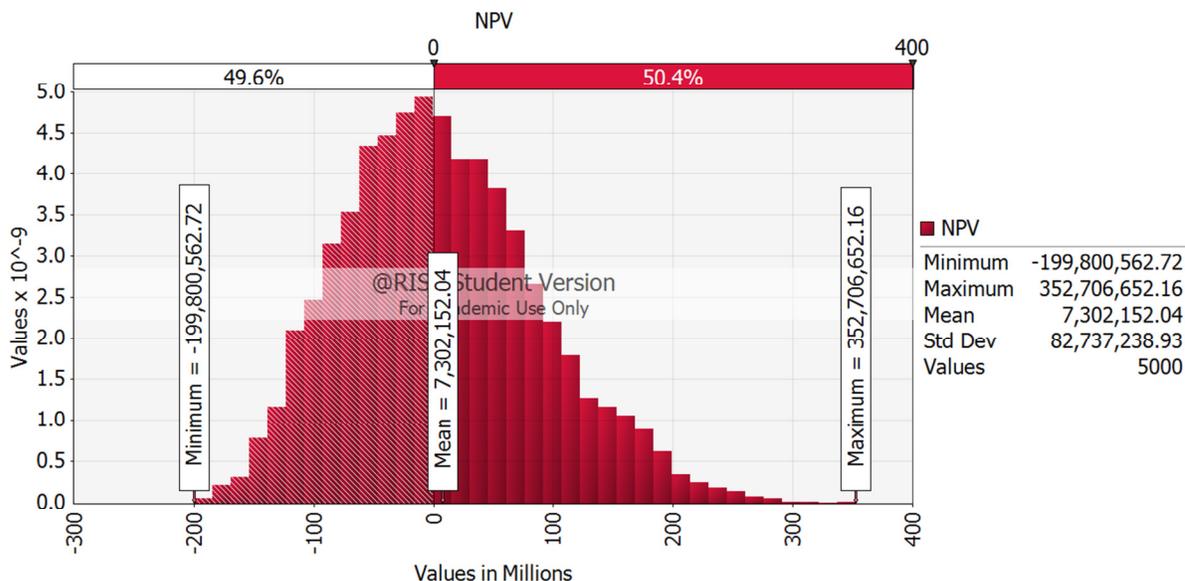


Figure 5.19. Probability Distribution of NPV with a discount rate of 12% for Scenario 2

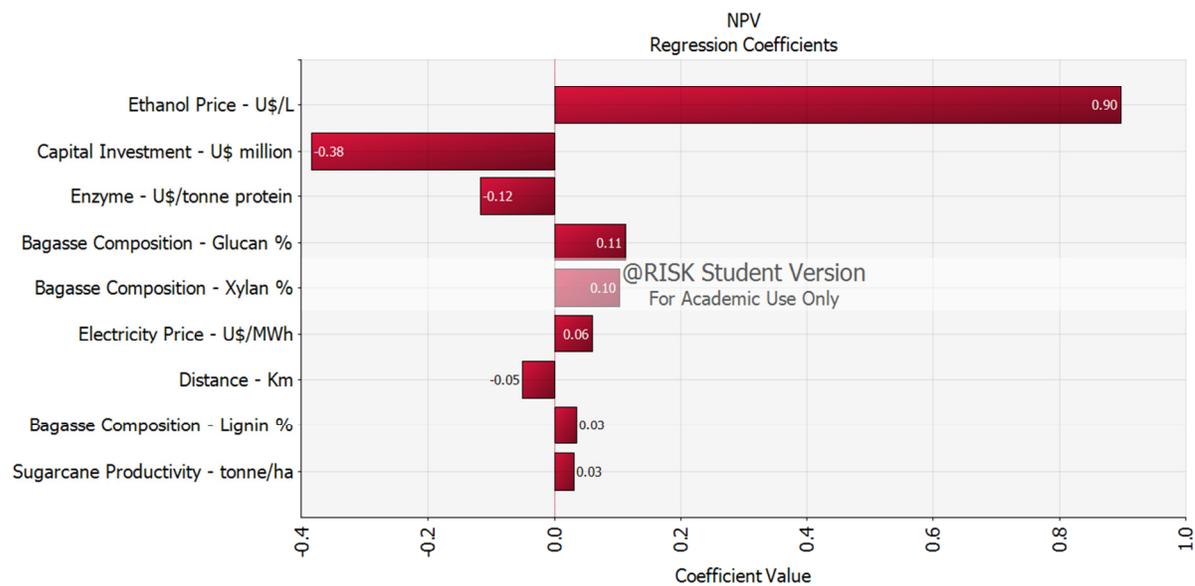


Figure 5.20. Tornado Plot - Sensitivity Regression Coefficients for Scenario 1 NPV 12%

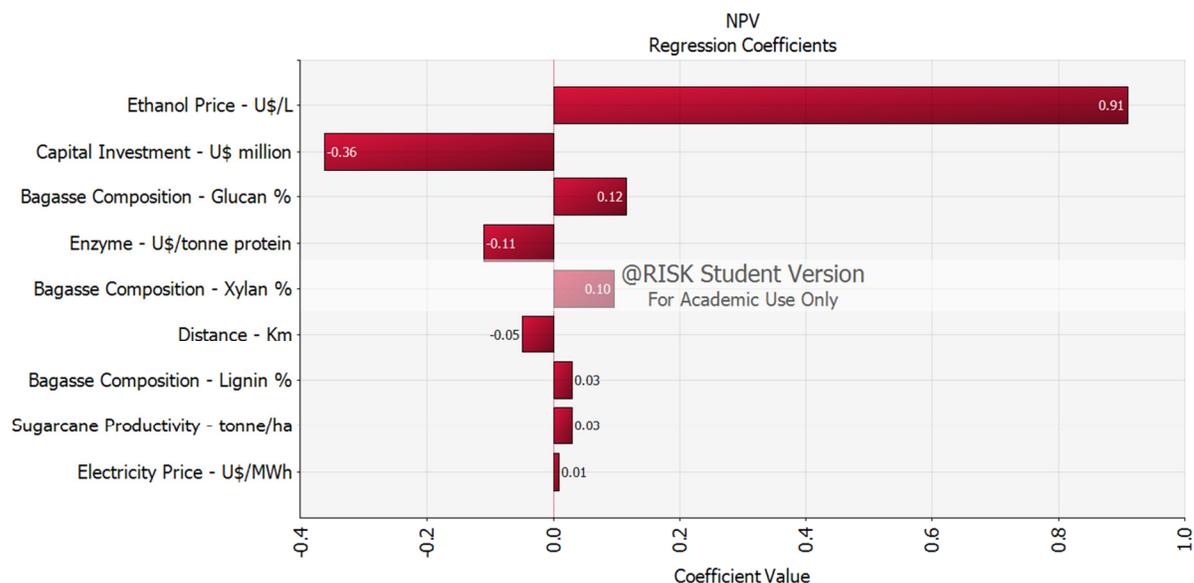


Figure 5.21. Tornado Plot - Sensitivity Regression Coefficients for Scenario 2 NPV 12%

5.3.6.3. Minimum Ethanol Selling Price

Scenario 2 presented a negative value (-MU\$ 6.9) for NPV with a discount rate of 12% when ethanol price is U\$ 0.458/L. Using deterministic analysis to have a value of zero for NPV at 12% discount rate, the calculated MESP is U\$ 0.469/L. However, as discussed before, deterministic analysis is based on average values and does not include the variability and volatility of economic drivers. Therefore, probabilistic analysis can be used to calculate the MESP for 99.9% of chances of having a positive NPV when variability is included in the analysis (Figure 5.22). In this direction, the original probability density function for ethanol price was modified according to Figure 5.23, and a MESP of U\$ 0.565/L was calculated.

Therefore, one cent of dollar per liter of ethanol would be enough to reach the proposed target value for NPV when deterministic analysis was used. However, additional ten cents of dollar per liter of ethanol is necessary to have positive NPV when the uncertainties of input parameters were included in the analysis.

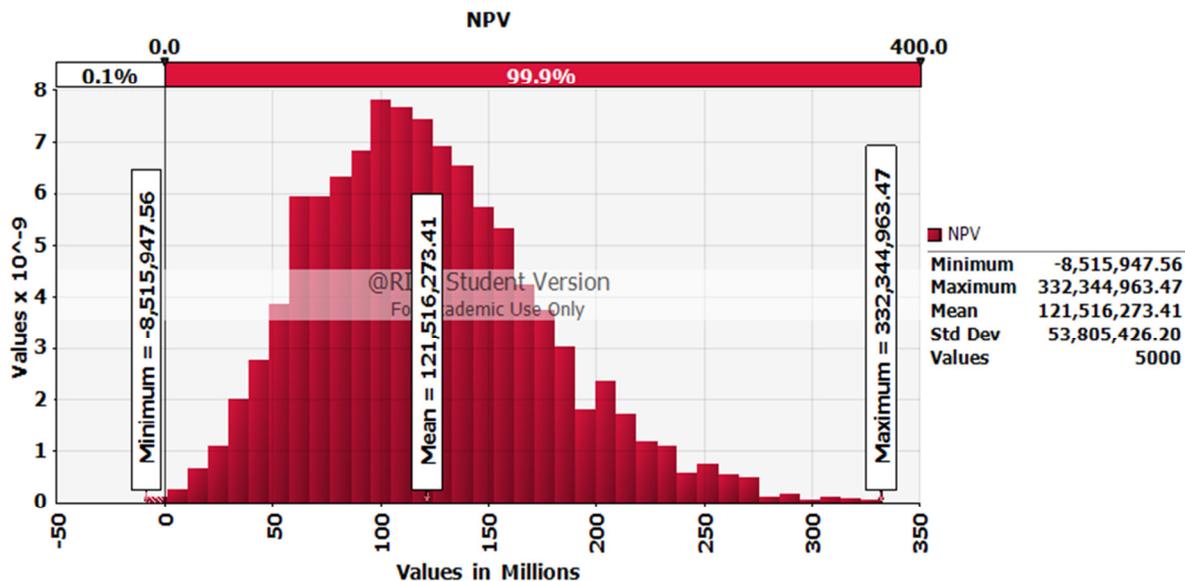


Figure 5.22. Probability Distribution for 99.9% of chances of having a positive NPV with a discount rate of 12% for Scenario 2

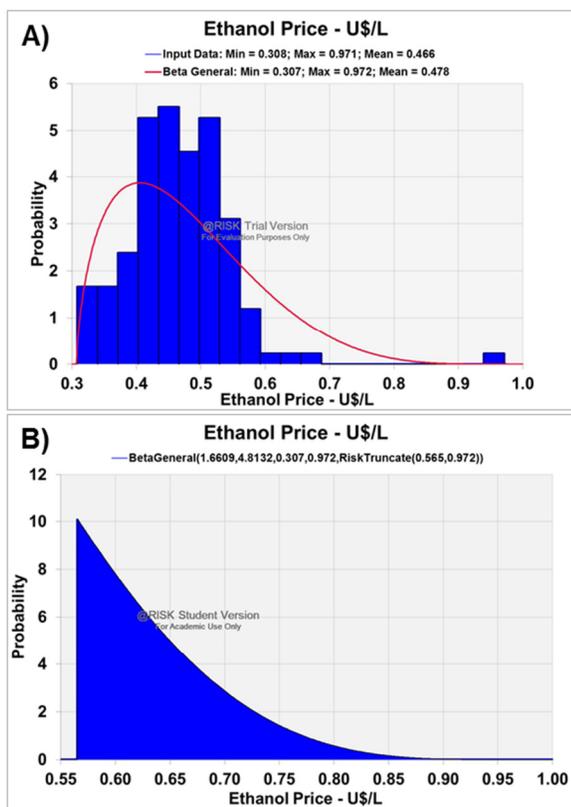


Figure 5.23. Probability Density Function for Ethanol Price. A) Original Distribution; B) Modified Distribution

5.4. Conclusions

In this work, the impact of mechanical refining, enzyme dosage and enzymatic hydrolysis residence time on the economics of a cellulosic ethanol plant to be co-located in existing sugarcane autonomous distillery was evaluated. Reduction of enzymatic hydrolysis residence time from 96h to 24h did not improve the financial performance. Saving with capital investment for enzymatic hydrolysis reactors and the additional surplus electricity obtained by burning a higher amount of enzymatic solid residue did not overcome the reduction in ethanol revenue. Reduction on enzyme dosage (5 FPU/g to 3 FPU/g) did not improve the financial performance significantly.

Mechanical refining proved to be an effective and cheap technology to overcome biomass recalcitrance in order to improve biomass digestibility and ethanol production. However, the additional revenue obtained with a higher production of ethanol, has to overcome the reduction in surplus power due to considerable electricity consumption with mechanical refining. In this context, refining has improved IRR by 0.8% when bagasse was converted to ethanol. However, the low improvement in biomass digestibility when straw was refined did not improve the financial performance.

Deterministic and probabilistic techno-economic analysis can be used in combination to identify the most important costs and revenue drivers and evaluate the impact of their volatility and variability on techno-economic analysis. According to sensitivity analysis, ethanol price, CAPEX, enzyme costs, carbohydrate content and straw cost are most significant economic drivers. Scenario 2 has presented as the most promising option (IRR 11.3%). However, probabilistic analysis showed that Scenario 2 has 50% of chances of having NPV greater than zero at a discount rate of 12% due to the volatility and variability of

cost and revenue drivers. MESP (NPV = 0 for 12% discount rate) calculated for Scenario 2 using deterministic analysis was US\$ 0.469/L, which is one cent of dollar per liter of ethanol more expensive than the market price assumed in this work. However, probabilistic analysis showed that additional ten cent of dollar per liter of ethanol is necessary for 99.9% of changes of having a positive NPV at 12 % discount rate. Therefore, deterministic and probabilistic analysis can be used to identify bottlenecks, potential areas for research and mitigation strategies to improve the economic performance of sugarcane cellulosic ethanol plants.

6. Final Conclusions

This work has covered techno-economic aspects for the production of sugarcane cellulosic ethanol with focus on alternatives to reduce capital investment and operating costs, maximize ethanol and power revenues, and increase sugar recovery during enzymatic hydrolysis.

The first study has compared the financial performance between greenfield and co-located 2G ethanol facilities when sugarcane bagasse and straw are consumed as a feedstock for cellulosic ethanol production or as a fuel for steam and power generation. Higher sugar yield when bagasse is converted to ethanol, higher power production when straw is burned in biomass boilers and capital savings when a co-located cellulosic ethanol plant operates during the 350 days have presented as the most promising alternatives among the evaluated scenarios. Higher risks associate with large cellulosic ethanol plants (high capital investment, high volume of biomass, emerging and non-reliable technology) decrease the attractiveness of greenfield plants.

The second work has evaluated the improvement of enzymatic carbohydrate conversion and global sugar recovery when mechanical refining and delignification were applied on pretreated sugarcane bagasse and straw to increase substrate accessibility to enzymes and reduce lignin content, respectively. A significant enzymatic hydrolysis improvement (25% to 30%) was achieved when the lignin removal resulted in a high carbohydrate to lignin ratio. However, the carbohydrate loss (up to 25%) due to peeling reaction and high costs compromise sugar recovery and economics for delignification. On the other hand, mechanical refining improved sugar recovery by 5% to 10%, confirming its potential as a

simpler and cheaper technology when compared to delignification. Therefore, a fundamental study was addressed to evaluate how and what physical properties (morphology, particle size, swellability, pore area and crystallinity) can explain the improvements in enzymatic hydrolysis when mechanical refining is applied after pretreatment. A light microscope has elucidated fiber bundles disintegration, internal delamination and some extent of external fibrillation. The increase of refining intensity was followed by a reduction of fiber length (FQA) and enhancement of swellability (WRV). Porometry using DSC has elucidated the increase FBW (freezing bound water) and consequent increase of pore area (pores > 10 nm) for the refined samples. However, additional pore area gains with the increase of refining intensity were not observed. X-ray diffraction did not show any evidence for changes in biomass crystallinity. Therefore, mechanical refining can open fiber structure and decrease particle size, which improve surface area, porosity and carbohydrate accessibility, resulting in better substrate accessibility to enzymes.

In the third and last work, a techno-economic study was executed to evaluate the economic impact of mechanical refining, enzymatic hydrolysis residence time and enzyme dosage on sugarcane cellulosic ethanol economics. Deterministic and probabilistic analysis (using specialized software - @Risk) were also performed to identify the most important cost and revenue drivers and evaluate the impact of their volatility and variability on internal rate of return and net present value. Reduction of enzymatic hydrolysis residence time from 96h to 24h did not improve the financial performance. Reduction on enzyme dosage (5 FPU/g to 3 FPU/g) did not improve the financial performance significantly. The additional ethanol production (~ 9%) when mechanical refining was applied to pretreated bagasse compensates for the reduction in the electricity revenue and additional capital investment, improving the

IRR by 0.8% (Scenario 2). However, when straw was converted to ethanol, the additional ethanol revenue (~ 5%) obtained with mechanical refining was not enough to overcome the costs drivers, resulting in no improvement in IRR. According to sensitivity analysis, ethanol price, CAPEX, enzyme costs, carbohydrate content and straw cost are most significant economic drivers. Although Scenario 2 has presented as the most promising alternative with a IRR of 11.3%, probabilistic analysis has shown that Scenario 2 has 50% of chances of having NPV greater than zero at a discount rate of 12% due to the volatility and variability of the main cost and revenue drivers. MESP calculated for Scenario 2 using deterministic analysis was US\$ 0.469/L. However, probabilistic analysis showed US\$ 0.565/L is necessary for 99.9% of changes of having a positive NPV at 12 % discount rate.

Therefore, the combination of experimental work and techno-economic analysis can be used to identify bottlenecks and potential areas for research, propose mitigation strategies and improve process performance in order to increase the profitability of cellulosic ethanol plants. Some of the most important areas to be addressed are: a) development of cheaper and more efficient process technologies and enzymes are necessary to reduce capital investment and operating costs; b) identification and production of sugarcane varieties to improve carbohydrate content and productivity will result in higher ethanol production and reduction of biomass cost; c) Scale up of existing technologies to validate industrial equipment and ethanol yield.

7. Future Work

The co-location of a cellulosic ethanol plant inside an existing sugarcane autonomous distillery using sugarcane biomass as feedstock has presented improved financial performance when compared to greenfield plants. Capital savings with infrastructure and boilers, use of available bagasse and straw, and the possibility of using existing facilities after sugarcane season are the main drivers for the better economic performance of co-located plants. Therefore, a higher level of integration between the existing sugarcane mill and the cellulosic ethanol facility involving ethanol fermentation and purification could be evaluated in future works as a tentative to improve the overall economics. In this context, the possibility of using the existing ethanol distillation system (distillation columns and molecular sieves) for purification of cellulosic ethanol could be evaluate using process simulation and thermodynamic models to address how the chemical composition of cellulosic wine will affect the traditional distillation system. Additionally, the techno-economic evaluation of genetically modified yeasts, capable of fermenting not only the traditional sucrose juice but also cellulosic sugars, could result in additional capital savings with fermentation. In this context, the existing fermentation and ethanol purification areas could be used to produce cellulosic ethanol after sugarcane season.

Mechanical refining has proved to be a relatively cheap technology that can be used to improve enzymatic hydrolysis yield. It is known that the improvement in enzymatic hydrolysis is dependent on type of biomass, pretreatment severity and enzymatic hydrolysis conditions (J. Park et al., 2015). This way, different pretreatment conditions (temperature and time) combined with mechanical refining could be evaluated according to the recalcitrance of

bagasse and straw to maximize sugar yield. Additionally, a techno-economic analysis could be performed to understand how different pretreatment conditions will change capital expenses, operating costs, sugar yield and overall economics.

It is also known that different refining technologies works with different refining mechanisms (fiber cutting, internal delamination and external fibrillation), which will affect the improvement in enzymatic hydrolysis yield. This way, different refining technologies (e.g. disk refiner, extruder, valley beater) combined with refining conditions (e.g. consistency, intensity, plate design, gap) and characterization technics (e.g. particle size, swellability, surface area, morphology) could be evaluated to understand: a) what refining mechanisms are more relevant to enhance enzymatic hydrolysis; b) what physical properties can explain the improvement in enzymatic hydrolysis; c) what refining features or refining technologies can be used in order to maximize the refining effects and enzymatic hydrolysis.

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