UNIVERSIDAD DE LA REPÚBLICA URUGUAY

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SUSTAINABLE PRODUCTION OF FUEL BIOETHANOL FROM SWITCHGRASS IN URUGUAY

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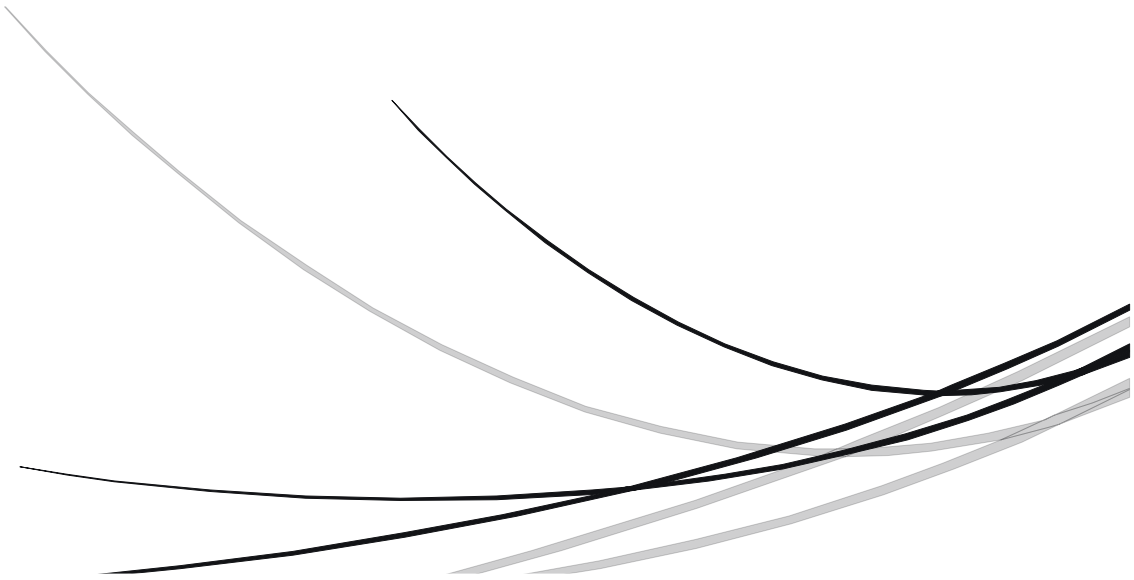


Abstract

Sustainability concerns due to long-term depletion of fossil fuels and climate change are responsible for a renewed interest on biofuels and biorefineries. Fuel bioethanol produced from lignocellulosic materials using modern technology could lead to high greenhouse gases (GHG) emissions savings. Biorefineries integrate the production of materials, chemicals, fuels, and energy. This could maximize the value obtained from biomass and minimize environmental impacts. Switchgrass (*Panicum virgatum L.)* is considered a good source of biomass because of its high productivity, longevity, high efficiency in water and nutrient use, and low production cost. Although several works have studied bioethanol production from switchgrass, a complete analysis of techno-economic and environmental sustainability for the current technology and conditions in Uruguay is necessary to promote the sustainable national production of bioethanol.

In this work, switchgrass was evaluated as a feedstock for the production of bioethanol in a biorefinery located in Uruguay using a liquid hot water (LHW) pretreatment. Material and energy use was determined for different scenarios and process configurations through process modeling. Material and energy results were used in a techno-economic model to analyze the effect of different parameters and configurations on the economics of the process. The minimum ethanol selling price (MESP) obtained for ethanol in a facility producing only ethanol and electricity was within the expected price range for advanced alcohol fuels and could compete with oil prices above 100 $/ barrel. Working on a biorefinery scenario producing furfural, acetic acid, and formic acid as high-value co-products, decreased the MESP. The MESP was sensitive to plant size and to switchgrass composition. Enzyme dosage, solids content, and hydrolysis and fermentation efficiencies are the operating parameters with higher impact on MESP, experimental information on how they are related (e.g. efficiency vs solids content) is necessary for more reliable assessments.

Experimental assays were performed to evaluate the cellulose enzymatic hydrolysis of LHW pretreated switchgrass at high solids content. LHW pretreatment (200ºC, 5 min) proved to be a suitable alternative for a biorefinery approach. It was found that the washing of solids and initial pH had a significant effect on hydrolysis efficiency. The effect of solids content, enzyme dosage, and partial cellulase substitution by xylanase, were studied experimentally. Glucose concentration and hydrolysis efficiency were significantly affected by solids content and enzyme dosage. Very high glucose concentrations (189 g/L) were achieved. High hydrolysis efficiencies were found even for high solids content (>90% for 25% solids content) but only for high enzyme dosage (40-70 $mg_{protein}/g_{glucan}$).

Experimental results were combined with the process and techno-economic models. Maximizing glucose concentration or hydrolysis efficiency did not directly correlate to minimizing the MESP. Enzyme dosage and solids content had a significant effect on MESP and it was found that an enzyme dosage of 37 $mg_{protein}/g_{glucan}$ and a solids content of 21 %, minimized MESP.

A life cycle assessment (LCA) was performed to evaluate GHG emissions and non-renewable fossil energy consumption associated with the production of fuel bioethanol in Uruguay using results from material and energy balances previously obtained. GHG emissions for bioethanol produced in all the scenarios analyzed were lower than the reference emissions for fossil fuel. The biorefinery scenario was better than the ethanol and electricity facility in terms of the environmental impacts and the biofuel produced there could meet GHG reduction requirements. All the factors analyzed (switchgrass composition, enzyme dosage, fermentation and hydrolysis efficiency and solids content) had a significant effect on the environmental performance of fuel bioethanol, enzyme use being the most significant factor. When compared with other works for Uruguay, the ethanol from the scenario with only electricity as co-product had a worst environmental performance than ethanol from sugarcane and sorghum grain. However, the ethanol from the biorefinery scenario performed better. Other scenarios analyzed (e.g. low enzyme dosage) also had a good environmental performance.

Optimal conditions for both economics and GHG emissions were found from models based on experimental data. These conditions (21 % solids w/w, 37 $mg_{protein}/g_{glucan)}$ had a good environmental performance (well to tank: -68 $\pm$ 5 $gCO_{2eq}/MJ_{ethanol,}$ GHG emissions) and good process economics (MESP of 0.84 $/L). Therefore, environmentally sustainable production of ethanol from switchgrass on a biorefinery located in Uruguay (in terms of GHG emissions and fossil energy use) could be possible with the technology and yields currently available. Economic sustainability for current technology and yields depends on oil prices and/or policies (carbon taxes). Scale-up of the experimental results obtained and appropriated industrial equipment are critical aspects of the technical feasibility.

Keywords: Bioethanol, sustainability, switchgrass, life cycle assessment, techno-economic analysis

Resumen

Existe un interés renovado en biocombustibles y biorefinerías debido a problemas de sustentabilidad asociados al agotamiento a largo plazo de combustibles fósiles y al cambio climático. El bioetanol combustible producido a partir de materiales lignocelulósicos con tecnologías modernas podría reducir significativamente las emisiones de gases de efecto invernadero (GEI). Las biorrefinerías integran la producción de materiales, químicos, combustibles y energía. Esta integración podría maximizar el valor obtenido de la biomasa y minimizar los impactos ambientales. El switchgrass (*Panicum virgatum L.)* es considerado una buena fuente de biomasa debido a su alta productividad, longevidad, alta eficiencia en el uso de agua y nutrientes y bajos costos de producción. Aunque existen varios trabajos sobre la producción de bioetanol a partir de switchgrass, es necesario un análisis completo de la sustentabilidad técnico-económica y ambiental para la tecnología disponible y las condiciones de Uruguay para promover la producción nacional de bioetanol combustible.

En este trabajo, se evaluó el uso de switchgrass como materia prima para la producción de bioetanol combustible en una biorrefinería localizada en Uruguay. Se estudió el uso de materiales y energía para diferentes escenarios y configuraciones mediante el modelado del proceso. Los resultados de los balances de materia y energía se utilizaron en un modelo técnico-económico, con el objetivo de analizar el efecto de diferentes parámetros y configuraciones en la economía del proceso. El precio mínimo de venta de etanol en una planta que produce solamente etanol y electricidad estuvo en el rango esperado para alcoholes combustibles de avanzada y podría competir con precios de petróleo superiores a 100 US$/ barril. Producir furfural, ácido acético y ácido fórmico como co-productos de alto valor agregado en un concepto de biorrefinería, redujo el precio mínimo de venta del etanol. El precio mínimo de venta fue sensible a la escala de producción y a la composición del switchgrass. Las eficiencias de hidrolisis y fermentación, el contenido de sólidos y la dosis de enzima son los parámetros operativos con más impacto en el minimo precio de venta. Información experimental sobre cómo se relacionan (ej. eficiencia vs contenido de sólidos) es importante para obtener resultados más confiables.

Se realizaron ensayos experimentales para evaluar la hidrolisis enzimática de la celulosa a altos contenidos de sólidos obtenidos luego de un tratamiento de auto-hidrólisis. El pretratamiento de auto-hidrólisis (200ºC, 5min) fue una alternativa adecuada para el enfoque de biorefinerías. El lavado de los sólidos pretratados y el pH inicial tuvieron un efecto significativo en la eficiencia de hidrólisis. Se estudió experimentalmente el efecto del contenido de sólidos, dosis de enzima y sustitución parcial de celulasas por xilanasas. Tanto la concentración final de glucosa como la eficiencia de hidrólisis se vieron afectadas por contenido

de sólidos, y por la dosis de enzima. Se obtuvieron concentraciones altas de glucosa (189 g/L). Se encontraron eficiencias de hidrólisis elevadas incluso para contenidos de sólido altos (>90% para 25% solidos) pero sólo para concentraciones altas de enzima (40-70 $mg_{proteina}/g_{glucano}$). Los resultados experimentales se utilizaron en el modelo del proceso y en el modelo técnico-económico. Se encontró que maximizar la concentración de glucosa o la eficiencia de hidrólisis no se correlaciona directamente con una reducción del precio mínimo de venta. La dosis de enzima y el contenido de sólidos tuvieron un efecto significativo sobre el precio de venta, una dosis de 37 $mg_{protein}/g_{glucan}$ y un contenido de sólidos de 21 % minimizaron el precio de venta.

Se realizó un análisis de ciclo de vida para evaluar las emisiones de gases de efecto invernadero y el consumo de energía fósil no renovable asociados a la producción de bioetanol combustible en Uruguay, utilizando los resultados de los balances de materia y energía obtenidos previamente. En todos los casos estudiados el bioetanol combustible presentó emisiones de GEI menores que las de referencia para combustibles fósiles. La biorrefinería presentó un mejor desempeño ambiental que la planta que produce etanol y electricidad, y el etanol producido allí podría cumplir con los requerimientos de reducciones de GEI. Todos los parámetros estudiados (composición del switchgrass, dosis de enzima, eficiencias de hidrolisis y fermentación, y contenido de sólidos) tuvieron un efecto significativo en el desempeño ambiental del bioetanol combustible, siendo el uso de enzima el más significativo tanto en las emisiones de GEI como en el uso de energía fósil. Comparado con otros estudios realizados para Uruguay el etanol de la planta que sólo produce etanol y electricidad tuvo un peor desempeño que el etanol de caña de azúcar y sorgo grano. Sin embargo, el etanol producido en la biorrefinería tuvo un mejor desempeño ambiental. Otros escenarios analizados también presentaron un buen desempeño ambiental.

Se encontraron las condiciones que optimizan simultáneamente las emisiones GEI y la economía del proceso a partir de los modelos basados en datos experimentales. Estas condiciones (21 % solidos m/m, 37 $mg_{proteina}/g_{glucano}$) tuvieron en buen desempeño ambiental ("cuna a tanque": -68 $\pm$ 5 $gCO_{2eq}/MJ_{etanol,}$ emisiones GEI) y económico (precio de venta 0.84 US$/L). La producción ambientalmente sustentable de etanol a partir de switchgrass en una biorrefinería ubicada en Uruguay, podría realizarse con la tecnología y rendimientos actuales. La sustentabilidad económica en estas condiciones depende del precio del petróleo y de las políticas (ej. bonos de carbono). El escalado y el diseño de equipos industriales es un aspecto crítico de la viabilidad técnica del proceso.

Palabras clave: Bioetanol, sustentabilidad, switchgrass, análisis de ciclo de vida, análisis técnico económico

Contents

ACKNOWLEDGMENTS **2**

ABSTRACT **3**

RESUMEN **5**

CONTENTS **7**

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS **9**

1. CHAPTER 1: STATE OF THE ART **11**

1.1. BIOFUELS 12
1.2. BIOETHANOL PRODUCTION 14
1.3. SWITCHGRASS AS FEEDSTOCK FOR BIOETHANOL 22
1.4. SUSTAINABILITY AND BIOREFINERIES 24

2. CHAPTER 2: AIMS AND OBJECTIVES **27**

2.1. AIMS 28
2.2. OBJECTIVES 28

3. CHAPTER 3: MODELING, SIMULATION AND TECHNO-ECONOMIC ANALYSIS OF THE INDUSTRIAL PROCESS **29**

3.1. INTRODUCTION 30
3.1.1. Process simulation *30*
3.1.2. Techno-economic analysis *30*
3.2. DESCRIPTION OF THE DEVELOPED MODELS AND THE SIMULATED SCENARIOS 33
3.2.1. Ethanol and electricity production process *33*
3.2.2. Biorefinery process *45*
3.2.3. Conditions and basis for the other scenarios studied *48*
3.2.4. Economic parameters and considerations *54*
3.3. RESULTS AND DISCUSSION 57
3.3.1. Economics of the ethanol and electricity production process *57*
3.3.2. Effect of the use of hemicellulose *59*
3.3.3. Effect of feedstock and enzyme costs *61*
3.3.4. Effect of feedstock composition *62*
3.3.5. Effect of hydrolysis parameters *64*
3.3.6. Effect of fermentation parameters *67*
3.3.7. Effect of solids content *69*
3.3.8. Process variables affecting carbon dioxide emissions and energy balance on the industrial stage *71*
3.4. CHAPTER CONCLUSIONS 73

4. CHAPTER 4: EXPERIMENTAL STUDY OF THE CELLULOSE ENZYMATIC HYDROLYSIS AT HIGH SOLIDS CONTENT **74**

4.1. INTRODUCTION 75

4.2. MATERIALS AND METHODS 77
4.2.1. Feedstock 77
4.2.2. Pretreatment 77
4.2.3. Enzymatic hydrolysis 78
4.2.4. Analytical methods 82
4.2.5. Calculations 83
4.2.6. Statistical analyses 84
4.2.7. Function optimization 84
4.3. RESULTS AND DISCUSSION 85
4.3.1. Switchgrass: particle size and composition 85
4.3.2. Liquid hot water pretreatment 86
4.3.3. Study of solids washing effect 87
4.3.4. Study of the effect of initial pH for different solids content 88
4.3.5. Study of the effect of solids content enzyme dosage and substitution of cellulase by xylanase 90
4.4. CHAPTER CONCLUSIONS 95
5. CHAPTER 5: ENVIRONMENTAL ASSESSMENT 96
5.1. INTRODUCTION 97
5.1.1. Life cycle assessment (LCA): definition and procedure 97
5.1.2. Regulatory framework for the environmental assessment of biofuels 104
5.2. MATERIALS AND METHODS 105
5.2.1. Bases for the life cycle assessment 105
5.2.2. Switchgrass production inventory 109
5.2.3. Ethanol production system 113
5.2.4. Sensitivity analyses 114
5.2.5. Monte Carlo Analysis 115
5.2.6. Statistical analyses and optimization 117
5.3. RESULTS AND DISCUSSION 118
5.3.1. Inventory analysis 118
5.3.2. Impact evaluation and life cycle interpretation 121
5.4. CHAPTER CONCLUSIONS 139
6. CHAPTER 6: CONCLUSIONS 141
7. CHAPTER 7: REFERENCES 145

List of abbreviations, acronyms and symbols

AFEX	Ammonium fiber expansión
ALUR	Alcoholes del Uruguay
ANCAP	Administración nacional de combustibles, alcohol y portland
ANOVA	Analysis of variance
BP	British petroleum
BCA	Bicinchoninic acid assay
C4	Four carbon (refers to the initial molecule formed during photosynthesis)
C5	Five carbon molecules
C6	Six carbon molecules
CAFI	Consortium for applied fundamentals and innovation
CBP	Consolidated bioprocessing
CED	Cumulative energy demand
CIP	Cleaning in place
COD	Chemical oxygen demand
CSL	Corn steep liquor
DAP	Diammonium phosphate
DB	Declaining balance
E	Enzyme dosage
EROI	Energy return on investment
EU	European Union
EEMAC	Estación Experimental “Dr. Mario A. Cassinoni”
F	Fermentation efficiency
FCI	Fixed capital investment
FPU	Filter paper units
G	Glucose concentration
GHG	Greenhouse gases
GREET	Greenhouse gases, regulated emissions, and energy use in transportation
GWP	Global warming potential
H	Cellulose hydrolysis efficiency
HMF	Hydroxymethyl furfural
HPLC	High performance liquid chromatography
HSD	Honest significant difference
IEA	International energy agency
ILUC	Indirect land use change
INIA	Instituto nacional de investigación agropecuaria
IPCC	International panel on climate change
IRENA	International renewable energy agency
ISO	International organization for standarization
LCA	Life cycle assessment
LCI	Life cycle inventory
LHV	Lower heating value
LHW	Liquid hot water
LSD	Least significant difference
LUC	Land use change
MESP	Minimum ethanol selling price
NREL	National renewable energy laboratory
PEG	Polyethylene glycol
RI	Refractive index
RO	Reverse osmosis
S	Solids content for the enzymatic hydrolysis stage
SSF	Simultaneous saccharification and fermentation
TCI	Total capital investment
TDC	Total direct cost
TEA	Techno-economic analysis

THF	Tetrahydrofuran
TRL	Technology readiness level
US	United States
UTE	Administración nacional de usinas y transmisiones eléctricas
UV	Ultraviolet
WTT	Well to tank
X	Cellulase substitution by xylanase
x1	Normalized solids content
x2	Normalized enzyme dosage
x3	Normalized cellulase substitution by xylanase

Chapter 1: State of the art

"If I have seen further than others it is by standing upon the shoulders of giants"

Sir Isaac Newton

1.1. Biofuels

The perspective of fossil fuel depletion, added to global warming concerns and the possibility of more security in energy supply have generated worldwide interest on research towards renewable energy alternatives (Warner & Jones, 2017).

Energy used by the transportation sector accounts for twenty percent of the world's energy consumption, half the oil consumption, and causes one fifth of its greenhouse gases emission (IRENA, 2016). Its demand is expected to grow 25 % in the next 25 years (BP, 2018). To achieve sustainability in this scenario, it is necessary to research and promote renewable energy alternatives for the transportation sector such as biofuels and electric vehicles (with a majorly renewable power mix).

Biofuels are the only one of this options that currently applies to aviation, ships and heavy machinery. Aviation alone is responsible for almost 3% of carbon emissions worldwide (IRENA, 2016).

Conventional biofuels (obtained from crops that could be used in the production of food and/or feed) have raised concerns about their impact on food prices, and on the use of land for agricultural and forest products. These issues could be mitigated using advanced biofuels, derived from agricultural and forestry residues, non-food/feed energy crops or organic waste. The concept of advanced biofuels also involves the achievement of large greenhouse gases emissions savings and the use of modern technology.

Current facilities for advanced biofuels at demonstration and commercial levels have a production capacity of 1 billion liters per year, planned facilities would add 2 billion liters per year, which would represent only 0.12% of the current demand for liquid fuel in the transportation sector (IRENA, 2016). Therefore, the development of more and better advanced biofuels is of foremost importance.

Other advantages associated with biofuels production involve: local market opportunities, additional revenue streams for the agricultural and forestry sectors, diversification of feedstock sources, more efficient use of resources, industrial and agricultural technology innovation, and economic growth (Canadian Renewable Fuel Association, 2014).

Summarizing, the replacement of liquid fossil fuels with advanced biofuels could help achieve energy independence and improve the security of price and supply. It could contribute to rural development, generating work in depressed rural areas and diversifying agricultural production. It could mitigate climate change, by reducing greenhouse gas emissions and promote sustainable practices through the energy production system.

Uruguay depends heavily on imported fossil fuels. The transportation sector represents twenty-seven percent of the total energy consumption in the country, which relies almost exclusively on petroleum-derived fuels (Ministerio de Industria, Energía y Minería, 2016). In 2007 the "*Ley de Agrocombustibles* (N° 18195)" law was approved with the goal of promoting and regulating the production, commercialization and use of biofuels, setting horizons for the substitution of fossil fuels by national fuels, considering the greenhouse gas emission reductions set by the Kyoto protocol at the United Nations Framework Convention on Climate Change, and contributing to the sustainable development of the country. It established that from 2014 a minimum of 5% ethanol should be included in gasoline and 2% of biodiesel on the regular diesel (http://www.alur.com.uy/empresa/ley-agrocombustible.php access 04/2018). In this context four biorefineries are operating in the country, all belonging to ALUR S.A., they produce biodiesel, bioethanol, animal feed, energy, and sugar. Two of these facilities produce bioethanol. One is installed in *Bella Unión* which uses mainly sugarcane and small amounts of sweet sorghum as feedstocks. In 2015, 23000 metric tonnes (t) of sugar, 15000 MWh of electricity, and 32000 m^3 of bioethanol, equivalent to a 5-8% substitution on gasoline, were produced. The second facility started operating in 2015, and has the capacity to produce 70000 m^3 of ethanol per year and 7000 t of animal feed, from grain sorghum (http://www.alur.com.uy/agroindustrias/ access 04/2018). In 2016, the minimum values for biofuel substitution were surpassed and a 10% ethanol on gasoline, and 7% biodiesel in gasoil were achieved, reducing greenhouse gases emissions in 270000 t (http://www.alur.com.uy/noticias/sostenibilidad-combustible-gala-2017/Presentacion%20GEI.pdf access 05/2018), but due to economic problems ANCAP decided to maintain the substitution percentages at the minimum required by the law to reduce costs (https://www.carasycaretas.com.uy/biocombustible-una-decision-politica/ access 05/2018). Therefore, it is fundamental to study the sustainability of new alternatives for biofuel production in Uruguay that could help achieve the social, environmental and economic goals.

1.2. Bioethanol production

Ethanol or ethyl alcohol is a simple alcohol with the chemical formula CH_3CH_2OH. It can be produced via petrochemical processes or via fermentation of sugars contained in biomass. When it is produced from biomass it is called bioethanol.

Bioethanol can be obtained from biomass, which contains carbohydrates in the form of: soluble fermentable sugars (sugarcane, sugar beet and sweet sorghum); polysaccharides like starch (corn, maize, grain sorghum, rice, potato, sweet potato, cassava) or hemicellulose and cellulose (wood, perennial grasses, agricultural residues), after converting the more complex compounds to fermentable sugars (Baeyens et al., 2015; Balat, 2011; Mussatto et al., 2010). The necessary stages to convert these different types of biomass are shown in Figure 1.1.

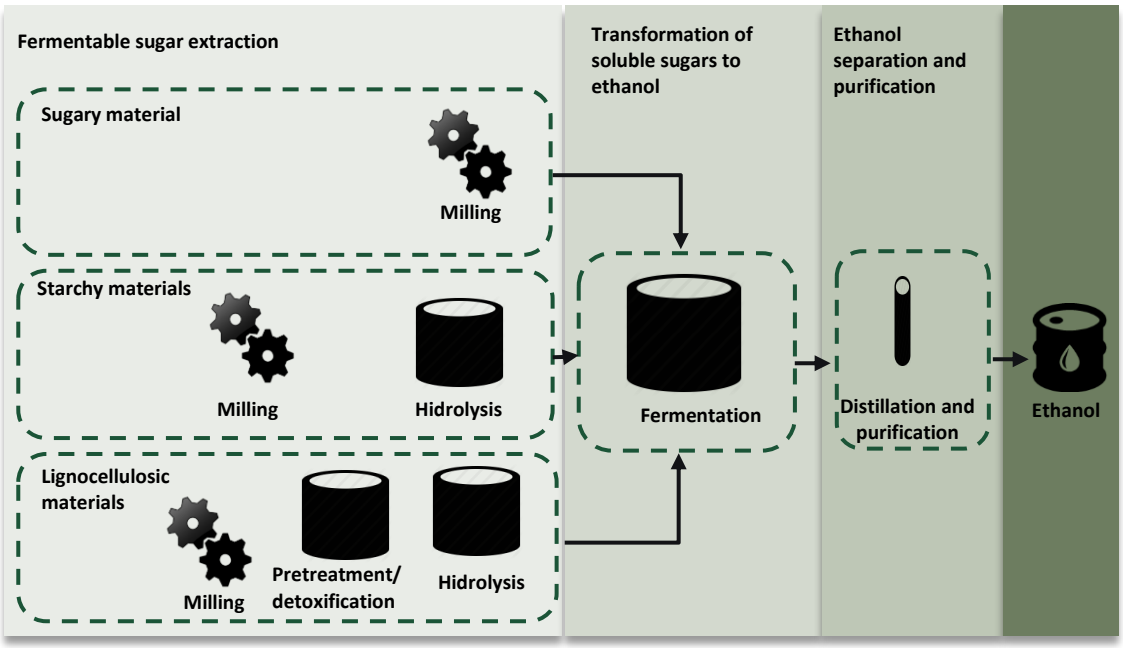


Figure 1.1. Stages for the convertion of different types of biomass to ethanol.

The first stage, fermentable sugar extraction highly depends on the complexity of biomass. It can comprise just one process (like juice extraction in sugar cane) or several processes (like milling, pretreatment, detoxification, and hydrolysis for lignocellulosic biomass). After fermentable sugars have been obtained, they are transformed to ethanol in a fermentation process. This ethanol is later separated and purified to comply with quality requirements for its use as biofuel. Current production of bioethanol is mostly based on sugar cane (in Brazil) and corn (in the United States).

For bioethanol to be considered an advanced biofuel, it should be produced from either agricultural and forestry residues, non-food or feed energy crops, or organic waste using modern

technology in a way that reduces greenhouse gases emissions relative to regular fuels. These materials are mostly comprised of lignocellulosic biomass.

Lignocellulosic biomass presents advantages over other feedstocks like low cost, great availability and not competing with food production and animal feed (Hamelinck et al., 2005; Limayem & Ricke, 2012; Mussatto et al., 2010; Sun & Cheng, 2002). The main components of lignocellulosic biomass are cellulose, hemicellulose, and lignin. Cellulose constitutes 35-50 % of the biomass. It is a polysaccharide consisting of linear chains of D-glucose linked through β-(1,4)-glycosidic bonds. These chains associate to others creating cellulose fibrils (Mood et al., 2013). The intermolecular and intramolecular hydrogen bonds that link cellulose fibers are responsible for cellulose being insoluble in water and most organic solvents (Swatloski et al., 2002). Hemicelluloses (20-35 % of the biomass) are branched polymers containing pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids). As a consequence of their amorphous branched structure and lower molecular weight, they are easier to hydrolyze (Mood et al., 2013) than cellulose. Xylan is the most abundant hemicellulose component. The content and chemical structure of xylans vary depending on the type of biomass. Hemicelluloses are located in secondary cell walls covering cellulose fibrils. Therefore, hemicelluloses must be removed to increase the digestibility of cellulose, making them more available for enzymatic hydrolysis (Agbor et al., 2011). Hemicelluloses are quite sensitive to operating conditions like temperature and retention time. These parameters must be controlled in order to avoid the formation of undesired products such as hydroxymethyl furfurals (HMF) and furfurals, known inhibitors of the fermentation process (Palmqvist & Hahn-Hägerdal, 2000). Lignin usually accounts for 15 to 25% of the biomass and is an aromatic polymer synthesized from phenylpropanoid precursors consisting mostly of syringyl, guaiacyl and p-hydroxy phenol, linked together in a complex matrix (Mood et al., 2013). A schematic representation of this structure is shown in Figure 1.2.

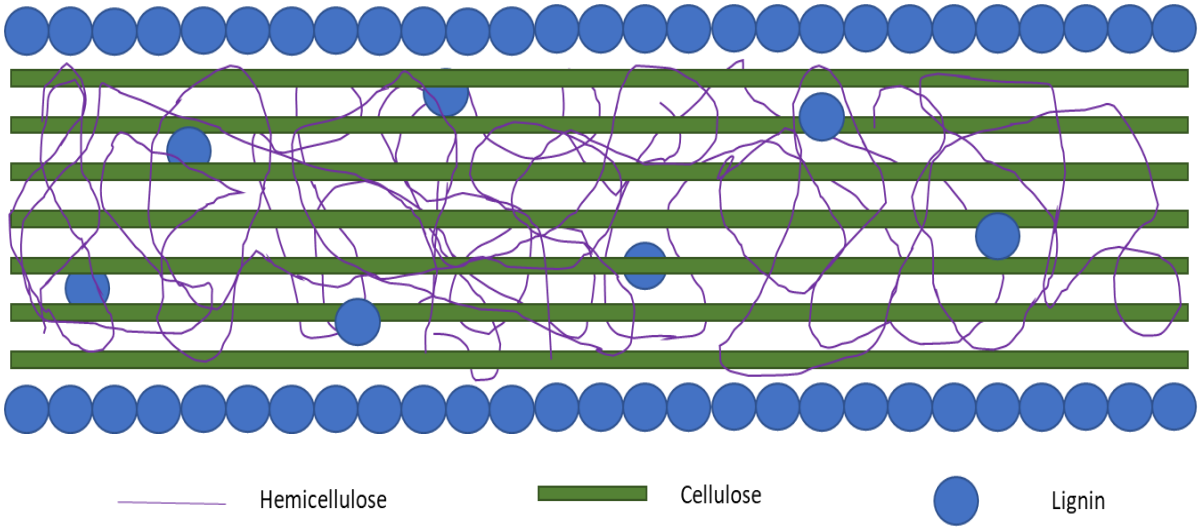


Figure 1.2. Schematic structure of lignocellulosic materials. Adapted from Mood et al. (2013).

Due to the lignocellulosic structure, obtaining fermentable sugars from these materials is a critical and difficult part of the production process. It involves particle size reduction (milling), pretreatment (to make cellulose and hemicellulose accessible by liberating them from their matrix with lignin), detoxification (necessary only if inhibitors are generated on the previous stage), and hydrolysis (depolymerization of carbohydrates to obtain fermentable sugars from cellulose and/or hemicellulose). Pretreatment (with or without detoxification) and hydrolysis are the most expensive and technically challenging process steps (Martín Pérez et al., 2017). The main technical barriers in pretreatment relate to: insufficient separation of cellulose and lignin (reducing cellulose accessibility, affecting the subsequent hydrolysis and overall conversion efficiency), ability to handle large size biomass, formation of undesired inhibitors of the fermentation and sugar losses, high use of chemicals and / or energy, and high capital and operational costs (Chiaramonti et al., 2012; Van Dyk & Pletschke, 2012). Several technologies for pretreatment are in operation and under development in an attempt to tackle these barriers, which are summarized in Table 1.1. TRL refers to Technology Readiness Levels an index of the maturity level of a technology ranging from 1 (basic principles observed and reported) to 9 (system proved through successful operations).

Table 1.1. Summary of different pretreatment technologies. Adapted from E4tech et al. (2015).

Technology	TRL	Opportunities	Barriers	Mitigations	Notes
Steam explosion	6 - 8	• Cost-effective. • High glucose yields. • Lignin and hemicelluloses removal. • Low environmental impact.	• Catalyst needed to optimize pre-treatment. • Formation of inhibitors and toxic compounds.	• Development of new catalysts and microorganisms more tolerant to inhibitors.	• Suitable for variety of herbaceous and woody feedstocks. • At 1st commercial plant scale.
Dilute acid pretreatment	5 - 7	• Good hemicellulose removal.	• Degradation by-products and inhibitors. • Corrosion.	• Develop microorganisms more tolerant to inhibitors and new enzymes. • Reduce pretreatment severity.	• Particularly suited for low lignin feedstocks.
Concentrated acid hydrolysis	4 - 5	• No enzymes needed Good hemicellulose removal.	• High chemical use and capital cost. • Corrosion and toxic hazard. • Degradation by-products and inhibitors.	• Recovery and reuse of chemicals. • Developing new catalysts and more tolerant microorganisms.	• Suitable for variety of feedstocks.
Auto-catalysis/ hydrothermal	4 - 6	• No chemical use or residues. • High glucose yields.	• Higher operating temperature. • Inhibitor formation.	• Develop methods to add value to lignin.	• Scale up to pilot scale. • Suitable for low % lignin.
Organosolv treatment	4 - 6	• Lignin and hemicellulose hydrolysis.	• High capital and operating costs. • Solvent may inhibit cell growth.	• Develop methods to add value to lignin. • Recovery and reuse of chemicals.	• High quality lignin co-product.
Alkaline pretreatment	5 - 7	• Low capital costs. • Low inhibitor formation. • High glucose yields.	• Residue formation. • Need to recycle chemicals. • Enzyme adjustment needed.	• New enzyme development. • Recovery and reuse of chemicals.	• Suitable for smaller scale facilities.
Ammonia Fibre Explosion (AFEX)	3 - 5	• No need for small particles. • Low inhibitor formation. • High accessible surface area.	• High cost due to solvent.	• Recovery and reuse of chemicals.	• Suitable for smaller decentralized plants. • Not effective for high % lignin.
Supercritical (CO_2) pretreatment	2 - 4	• Increases accessible surface area. • Low inhibitors or residues.	• Does not affect lignin and hemicelluloses. • High pressure, high capex	• Develop methods to add value to lignin. • Improve process technology.	• Continuous technology. • Suitable for small scale plants.
Ionic liquids	2 - 3	• Effective dissolution of all components. • Low degradation products.	• Expensive technology and recovery required.	• Develop methods to add value to lignin. • Recovery and reuse of chemicals. • Develop process technology.	
Microbial/fungi	3 - 4	• Low energy requirement. • No corrosion. • Lignin and hemicelluloses removal.	• Time consuming. • Some saccharide losses.	• Development of robust microorganisms.	
Mechanical milling	5 - 6	• Reduces cellulose crystallinity. • No inhibitors or residues.	• High energy consumption. • Poor sugar yields.	• Process integration, combine with mild chemical treatments.	

Liquid hot water (LHW) is one of the most frequently used thermal pretreatments in which biomass is treated with water at high temperatures and pressure for a short period of time. This treatment achieves hemicellulose and extractives solubilization, with partial solubilization of lignin and does not require the addition of chemicals (Hendriks & Zeeman, 2008; Laser et al., 2002;Zhuang et al., 2015). The use of chemicals is associated with added costs (purchase of chemicals, recovery or treatment) and an increase in the environmental impact (chemicals production and transportation). This pretreatment also leads to a lower reactor cost as it does not need to be designed for corrosive conditions. LHW could be applied to wet or fresh lignocellulosic material (Ruiz et al., 2013; Sun et al., 2015). It has been reported that with this pretreatment, hemicellulose is solubilized to oligomers with a minimum monomer formation. This constitutes an advantage, as oligomers can be used for the production of chemical with a higher added value than bioethanol, and the degradation of monomers to aldehydes that could inhibit the fermentation is avoided (Alvira et al., 2010; Mosier et al., 2005; Sun et al., 2015; Zhuang et al., 2015). Mild conditions are required to limit degradation of the cellulose and hemicelluloses obtained by removing the extractives and the lignin (holocellulose) and the formation of inhibitors.

Enzymatic hydrolysis should be tailored to the specific biomass characteristics, due to the fact that pH, enzyme dosage, and enzyme mix necessary to achieve high yields depend on the composition of the pretreated material. This restricts the flexibility of the process in terms of feedstock variation (Van Dyk & Pletschke, 2012). The most challenging aspects of the hydrolysis process are: limited solids loading (that lead to low product concentration) and enzyme performance (Humbird et al., 2010; Weiss et al., 2013). Hydrolysis at high solids content will be discussed further on Chapter 4. Many research projects are directed to improve the commercial enzyme cocktails and to develop new enzymes, both by companies and academia. Some of the current bioethanol facilities produce their own enzymes on site (U.S. Energy Information Administration, 2016).

Fermentation involves the transformation of the soluble hexose and/or pentose sugars to ethanol by yeasts or bacteria. The fermentation of hexoses is a well-established process on an industrial scale. Research in this area focuses on finding new or genetically modified microorganisms that: can ferment both hexoses and pentoses, have more tolerance to ethanol, have good performance at high temperatures, have higher ethanol yields or are able to hydrolyze and ferment biomass on the same step (Harun et al., 2011; Kurylenko et al., 2016; Qiu & Jiang, 2017; Thammasittirong et al., 2013).

The last steps, ethanol separation and purification, are usually done using a couple of distillation columns that recover ethanol with a composition that approaches the azeotrope with water, and membranes or molecular sieves for further purification. This step can be energy intensive if the ethanol concentration after fermentation is low. There is some research focusing on advanced, less energy intensive separation processes, but they are still at a laboratory scale (U.S. Energy Information Administration, 2016).

The different steps described (pre-treatment, hydrolysis, and fermentation) can be integrated in an effort to: make energy savings, lower enzyme usage, achieve better conversions and/or decrease capital cost. Processes where enzymatic hydrolysis and fermentation are integrated (as simultaneous saccharification and fermentation [SSF]) are well developed. The use of microorganisms capable of both hydrolysis and fermentation of cellulose polymers is called consolidated bioprocessing (CBP). It could lead to the advantages mentioned before, but it is currently in the research and development phase (Raftery & Karim, 2017; Yee et al., 2012).

Regarding the industrial production of lignocellulosic ethanol, there are several facilities around the world working at a demonstration level (TRL 6-7) or at an early commercial phase (TRL 8) using agricultural residues and energy crops (see Table 1.2). Lignocellulosic ethanol via fermentation is the most technologically developed and cheapest of the advanced biofuels (IRENA, 2016). It is expected that the demonstration and early commercial facilities give some guarantees in technology performance, facilitating investment in commercial facilities.

Table 1.2. Operational facilities at TRL>6 that produce lignocellulosic ethanol via sugar fermentation.

(http://demoplants.bioenergy2020.eu/ access on 03/2018).

Facility (Location)	Feedstock	Technology brief	Ethanol production (t/y)	TRL
Thomaston GP3+ Biorefinery (US)	Any woody or non-woody biomass.	Hydrothermal fractionation producing cellulosic sugars.	180	6-7
Anhui BBCA Biochemical (China)	Corn cob/corn stover		5,000	6-7
AS BALI Demo Biorefinery (Norway)	Sugarcane bagasse, straw, wood, energy crops.	Chemical pretreatment, saccharification fermentation of hexoses, fermentation or chemical conversion of pentoses.	110	6-7
AS ChemCell Ethanol (Norway)	Sulfite spent liquor from spruce wood pulping.	After concentration of the SSL, the sugars are fermented.	15,800	8
Cane Technology Center (Brazil)	Bagasse		2,400	8
Chempolis Biorefining Plant (Finland)	Straw, empty fruit bunch, bagasse, and wood residues.	"Formicobio" technology for the production of cellulosic ethanol and biochemicals. Fractionation with a biosolvent.	5,000	6-7
Sunliquid (Germany)	Wheat straw	Technology for pretreatment, enzyme production, fermentation of C5 and C6 sugar ethanol purification through adsorption-desorption.	1,000	6-7
COFCO Zhaodong Co. (China)	Corn stover		500	6-7
DuPont Cellulosic Ethanol Demonstration plant (US)	Corn stover, cobs and fibre; switchgrass.	NH3 and steam pretreatment, enzymatic hydrolysis.	750	6-7
Gevo (US)	Corn		54,000	8
GranBio Bioflexl(Brazil)	Sugarcane bagasse and straw.	Beta Renewables' PROESA process and Chemtex services.	65,000	8
Henan1 (China)	Wheat/corn stover		10,000	8
Henan 2 (China)	Lignocellulosic crops.		30,000	8
Iogen Corporation (Canada)	Agricultural residues like wheat straw; corn stover, sugar cane bagasse.	Pretreatment: modified steam explosion. Cellulase enzyme tailored to the feedstock, fermentation converts C6 and C5 sugars.	1,600	6-7
Jilin Fuel Alcohol (China)	Straw		3,000	6-7
Ethanolix GOT (Sweden)	Waste		4,000	6-7
Advanced Biofuels Project Liberty (US)	Agricultural residues	Integrated technology package that converts corn crop residue to cellulosic bio-ethanol.	75,000	8
2G Futurol Project (France)	Woody and agricultural by-products, residues, energy crops.		2,700	
Quad Country Biorefionery (US)	Corn kernel fiber		6,000	8
Raizen Energia (Brazil)	Bagasse		31,600	8
Renmatix (US)	Wood chips, switchgrass.	Microorganisms engineered to produce cellulose enzymes and ferment sugars to ethanol.	500	6-7
Shandong Zesheng Biotech Co. (China)	Straw		3,000	6-7
SP/EPAP (Sweden)	Wood chips; sugarcane bagasse, wheat, corn stover, energy grass, recycled waste.	Two step diluted acid + enzyme hydrolysis.	160	6-7
Woodland Biofuels Demo (Canada)	Wood waste		601	6-7
ZeaChem (US)	Poplar trees, wheat straw	Non-GMO bacteria ferment cellulosic sugars. Combines biological and thermochemical processes.	750	6-7

Overall, the main obstacles for further scale-up of advanced bioethanol are high capital costs and high costs for enzymes. In addition, many pretreatment pathways are developed for one feedstock, and pretreatment conditions (as well as enzymes) need to be modified when other lignocellulosic feedstocks are used, limiting process flexibility. Process integration, plant optimization, improved control systems and a reduction in losses are expected to increase overall plant efficiency from 37% to 42% by 2030 (IRENA, 2016). The major cost factors for bioethanol production are feedstock, enzymes and energy demand. Therefore, it is necessary to use feedstocks with high carbohydrate content, efficient transformation processes energetically optimized and with a cheap accessible energy source (Kwiatkowski et al., 2006).

1.3. Switchgrass as feedstock for bioethanol

As previously mentioned advanced biofuels based on lignocellulosic feedstocks could greatly expand the number of sources available for fuels in the transport sector while mitigating sustainability risks associated with land use change and competition with food production. Feedstock cost, availability (through a stable supply chain), and a sustainable production are important factors to be considered when selecting a feedstock for advanced fuels (Simmons et al., 2008).

Lignocellulosic feedstocks can be classified into three categories:

Agricultural residues: a potentially very large feedstock source, with generally low costs associated with collection and transport, although costs might rise when competing uses for the feedstock exist. It should be noted that a fraction of the agricultural residues needs to remain in the field in order to maintain good soil characteristics.

Forest residues: have a lower potential than agricultural residues but it could be substantial in areas with commercial forestry activities. Costs are relatively low, but they are currently used for heat and electricity generation. The supply of this resource depends on the demand for forest products and is limited by economic and sustainable extraction rate. Just as with agricultural residues, a fraction of the residues needs to remain in the forest to maintain soil quality.

Non-food energy crops: quick growth crops, cultivated with the purpose of energy generation. They have a wide range of potential due to the differences in crop production methods, land use, and environmental constraints. Feedstock costs in this category can be quite high, but they vary due to differences in crop yields between crops and regions. Supply chains for energy crops need to be developed. They are currently used for heat and/or power generation, and they may compete for land with other crops.

Perennial grasses belong to the non-food energy crop category. They can grow on marginal soils, which are not apt for food production and currently not used, decreasing their cost and environmental impact. They have advantages over annual crops, like low establishing cost, decreasing soil erosion potential, increasing water quality and improving wildlife (Keshwani & Cheng, 2009; McLaughlin et al., 2002; Siri-Prieto, 2012).

Switchgrass (*Panicum virgatum L.)* is a C4 summer perennial grass from North America. It is considered a good source of biomass because of its high productivity, longevity, high efficiency in water and nutrient use, and low production cost amongst other characteristics. Since 1985 switchgrass is being studied as an energy crop for ethanol production and electricity generation. The crop can reach a height of 2.5 m with leaves of 30 to 90 cm length. It has high yields on marginal soils and has a low incidence of diseases and plagues. Switchgrass has been

compared to other lignocellulosic crops like *Miscanthus sp*., showing advantages like its easy propagation and lower implementation cost. Its high glucose content translates to a high potential for ethanol production and its low ash content is good for generating energy by burning in a combustor (Keshwani & Cheng, 2009).

In Uruguay, experimental switchgrass crop (Alamo variety) showed an average productivity of 15.5 t/ha for single harvest (Siri Prieto et al., 2017), comparable to the 15 t/ha reported in the literature review (Keshwani & Cheng, 2009). This translates to a theoretical ethanol potential of 3800 L/ha based on approximate cellulose content.

Bioethanol production from switchgrass was studied in several works that focused on different aspects of the production process (Bai et al., 2010; Garlock et al., 2011; Haque & Epplin, 2012; Ioelovich & Morag, 2012; Laser & College, 2009b; Paap et al., 2013; Papa et al., 2015; Pimentel & Patzek, 2005; Tao et al., 2011; Wyman et al., 1992). Findings of these and other works will be discussed in the following chapters.

1.4. Sustainability and biorefineries

Sustainable development is defined as development that "meets the needs of the present without compromising the ability of future generations to meet their own needs", and it should consider environmental, social and economic aspects (Brundtland, 1987). In 2015, the United Nations manifested their commitment to sustainable development, defining 17 goals to achieve by 2030. Goal 7 is to ensure access to affordable, reliable, sustainable and modern energy, and its targets include increasing the share of renewable energy. Goal 13 is to take urgent action to combat climate change and its impacts (https://www.un.org/sustainabledevelopment/ access 02/2018).

Regarding the economic aspect, the economic viability of the biorefinery can be selected as an indicator of sustainability. Therefore techno-economic analysis of the whole biorefinery is a tool to assess its economic sustainability (Gnansounou & Pandey, 2017).

Regarding social sustainability, existing criteria are mainly qualitative and difficult to define. Social acceptability, social well-being, energy security and external trade, resource conservation, rural development, and rural workforce training are some of the indicators that can be chosen to assess the social impact of biorefineries. Social acceptability depends on the social risk related to changes in land ownership and rights of the working class, social accountability and information sharing with all the actors involved, and stakeholders' participation in the decision-making process. Social well-being includes social prosperity (expected increase in revenues and its social distribution), safety, and health and food security. Energy security is comprised of energy dependency, energy affordability, and net energy balance. Resource conservation includes the rational use of fossil fuels, water, and protection of the landscape and cultural heritage (Gnansounou & Pandey, 2017). Social concerns about advanced biofuels are usually the same as for conventional biofuels because that distinction is not clear for all the social actors involved. The usual concerns revolve around human rights (involuntary resettlement, land grabbing and reduced access to resources), food insecurity (especially true in developing countries) and cost to the consumer. Communication strategies to inform the difference between advanced and first generation biofuels, as well as specific decisions to choose production practices that avoid these problems, could eliminate said concerns. The policy has an important role to play in providing a framework with guarantees on the social sustainability of biofuels (IRENA, 2016).

Environmental sustainability for a given product/service or process considers the issues of: land use (land planning, agricultural practices and impact on land fertility and productivity),

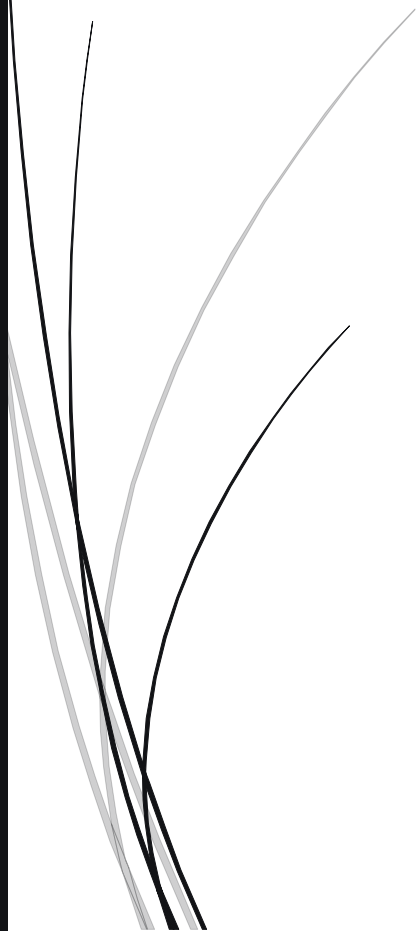
disturbances of local environment (resource depletion, pollution), biodiversity (conserving diversity and protecting ecological systems and habitats) and disturbances on global environment (greenhouse gas emissions, ozone depletion)(Gnansounou & Pandey, 2017). Compliance to sustainability criteria is easier for wastes and residues other than agricultural, fisheries and forestry residues. The effect of indirect land use change and the carbon debt that may result from forest biomass use is still under debate (Plevin et al., 2010). Nevertheless, advanced biofuels could achieve the greenhouse gas (GHG) emissions reduction target of 60% in comparison to the fossil fuel reference value set by the European Commission's 2009 Renewable Energy Directive (European Parliament, 2009; Rathore et al., 2013). The use of carbon tax in fuel markets could promote the emergence of an advanced biofuels industry (Timilsina et al., 2011). In order for biofuels to meet the sustainability criteria worldwide, robust methodologies to assess these impacts need to be created and regulated through appropriate policy and sustainability standards (World Energy Council, 2010). In the European Union (EU), advanced biofuels must comply with sustainability criteria, demonstrated through voluntary third-party certification, by the "Roundtable on Sustainable Biomaterials", "International Sustainability and Carbon Certification System" and other programs.

Sustainability concerns due to long-term depletion of fossil fuels, threatened climate change and their irreversible damages are responsible for a renewed interest on the biorefinery concept (Gnansounou & Pandey, 2017).

The definition of biorefinery originates from an analogy to the petroleum refinery, where many products are produced from crude oil. A biorefinery can be broadly defined as a facility that produces a range of products from biomass (FitzPatrick et al., 2010). The United States (US) National Renewable Energy Laboratory (NREL) defined it as a facility that integrates biomass conversion processes and equipment for the production of fuels, power, and value-added chemicals from biomass (Gnansounou & Pandey, 2017). Task Group 42 of the International Energy Agency (IEA) included sustainability in the concept, defining it as "the sustainable processing of biomass into a spectrum of marketable products and energy" (IEA, 2009). By integrating the production of materials, chemicals, fuels, and energy, biorefineries could help to maximize the value obtained from the biomass. Biorefineries can be classified into energy-driven, or product-driven. Energy-driven biorefineries focus in the production of biofuels/energy with the biorefinery aspect adding value to co-products, while product-driven biorefineries target the production of food, feed, chemicals, and/or materials (IEA, 2009). Product diversification can reduce market-based risks and improve process economics through combining fuel production with specialty chemicals production (fuels can achieve greater economies of scale than specialty chemicals, and low volume high-value chemicals increase the

revenues). Lignocellulosic feedstock biorefineries are promising due to the potential flexibility to a wide range of low-cost feedstocks. Policies and business models are important factors to assure the continuous development and cost decline of biorefineries (FitzPatrick et al., 2010; IRENA, 2016). Biorefineries should aim to minimize burdens on the environment, as sustainability is one of their fundamental drivers, this can be achieved through technology development associated with new high-value products, more efficient transformation processes and the appropriate agricultural practices. These characteristics make biorefineries a promising production model to achieve economic and environmental sustainability while valorizing the biomass (Gnansounou & Pandey, 2017).

Chapter 2: Aims and objectives



"A light here required a shadow there."

Virginia Woolf

2.1. Aims

The general objective is to contribute to promote the sustainable national production of bioethanol fuel through efficient transformation processes, the flexible use of diverse agricultural raw materials and the generation of other products with high added value (biorefinery concept). This work focuses specifically on the use of switchgrass, an energy crop developed for the production of bioenergy which is experimentally cultivated in Uruguay with promising results. To fulfill this objective, experimental, techno-economic and environmental studies were performed.

2.2. Objectives

- Determination of the chemical composition of an experimental switchgrass (Alamo variety) developed as an energy crop and produced in Uruguayan territory. Evaluation of its potential as raw material for the production of bioethanol based on the carbohydrate content.
- Development of a process model using a simulation software (Aspen Plus®), experimental and literature data; that describes the material and energy use in different scenarios and process configurations in an ethanol production plant from switchgrass as a raw material and in a biorefinery that produces ethanol as a main product and other co-products with higher added value.
- Techno-economic model and analysis of ethanol production from switchgrass in Uruguay. This analysis aims to identify aspects with significant influence over the economy of the process.
- Experimental evaluation of enzymatic hydrolysis for the conditions found to have a great impact on process economics and/or energy consumption. This evaluation has the purpose of finding the most promising operational conditions with current technology for ethanol production, from an economic and environmental point of view.
- Environmental evaluation of the production process, through a life cycle assessment model that calculates greenhouse gases emissions and non-renewable energy consumption for a production facility in Uruguay.

Chapter 3: Modeling, simulation and techno-economic analysis of the industrial process

"All models are wrong, but some are useful".

George Box

3.1. Introduction

3.1.1. Process simulation

Process simulation has been successfully used to model and predict energy and material balances for several industrial processes. Amongst other applications process simulation can be used to estimate the effect of variations on feedstock, operating conditions, process configuration, and innovative technologies. Several authors have reported models that simulate the production of bioethanol from different feedstocks, developed using software such as: SuperPro Designer®, Intelligen Inc., Scotch Plains, NJ (Ferrari et al., 2013; Nghiem et al., 2011) and Aspen Plus®, Aspen Technologies Inc., Cambridge, MA (Dias et al., 2012; Dutta et al., 2009; Humbird et al., 2011; Kazi et al., 2010; Larnaudie et al., 2016; McLaughlin et al., 2002; Quintero et al., 2013; Sánchez & Cardona, 2012; Tasić & Veljković, 2011; Wang et al., 2013). These simulations provide material and energy balances that have been used as the basis for techno-economic and environmental analysis of different biofuel production processes including ethanol (Humbird et al., 2011; Laser & College, 2009b; Quintero et al., 2013).

3.1.2. Techno-economic analysis

Techno-economic analysis (TEA) is a fundamental tool that provides guidance for research and development of new technology, integrating process modeling and engineering design with economic analysis. It can be used to identify bottlenecks in a process and critical aspects for the improvement of process economy or the technical feasibility of the process. When the critical aspects are identified, it is possible to perform experimental studies to find the best operating conditions of the process.

There are detailed techno-economic models that depict the proper process behavior, results, and economics for bioethanol production, that can be used to demonstrate the economic impact of production parameters (Tao & Aden, 2009). This was demonstrated by comparing techno-economic analysis derived from these models with published market values for ethanol from different feedstocks like corn (0.40 $/L market, 0.41 $/L theoretical) and sugarcane (0.30 $/L market, 0.34 $/L theoretical) (Humbird et al., 2011). Despite the good prediction of market value, the best use of this analysis is to compare the cost of production from technological modifications and process improvements, rather than using the absolute value as a basis for decision-making. Most of the techno-economic models follow the conceptual design basis developed by the National Renewable Energy Laboratory (NREL). In the specific case

of lignocellulosic ethanol most studies base their models and compare their results with the techno-economic model developed by NREL in 2002 (Aden et al., 2002), which was later updated in 2011 (Humbird et al., 2011; Laser & College, 2009b; Vaskan et al., 2018).

Table 3.1 shows the results of techno-economic studies of bioethanol production from lignocellulosic materials. Production costs were not normalized to a consistent cost-year, but cost-year varies between 2007 and 2010. Therefore, these variations should not affect the analysis.

Table 3.1. Parameters and results of different techno economic analysis of bioethanol production from lignocellulosic materials. Adapted from Humbird et al. (2011).

Feedstock	Pretreatment	Plant size (dry $t_{feedstock}$/day)	Feedstock price ($/dry t)	Ethanol yield (L/dry t)	Cost year	Minimum selling price ($/L)	Source
Corn stover	Dilute acid	2000	51	341	2007	0.4	Aden et al. (2002)
Corn stover	Dilute acid	2000	59	299	2007	0.57	Humbird et al. (2011)
Corn stover	Dilute acid, LHW, AFEX	2000	75	159-273	2007	0.90-1.17	Kazi et al. (2010)
Corn stover	Dilute acid	2000	60	197-280	2009	0.93-1.21	Klein-marcuschamer et al. (2010)
Corn stover	AFEX	2000	40	300-325	2008	0.21-0.25	Sendich et al. (2008)
Corn stover	AFEX, several conditions	770	45	295	2008	0.49-0.58	Bals et al. (2011)
Switchgrass	AFEX	4535	44	367-397	2006	0.17-0.22	Laser & College (2009a)
Straw, eucaliptus, poplar, switchgrass	Dilute acid.	1450-1800	57-127	265-318	2007	0.56-0.77	Gnansounou & Dauriat (2010) (Review)
Hardwood	Dilute acid	2000	65	284	2007	0.91-1.1	Piccolo & Bezzo (2009)
Poplar and “high glucan”	Dilute acid	907-1451	50-88	254-401	2007	0.32-0.71	National Academy of sciences (2009)
Sugarcane bagasse	Dilute acid with steam explosion	743	40	305	2007	0.52	Gubicza et al. (2016)
Miscanthus x giganteus	Dilute acid	2000	80-100	330	2007	0.65-0.71	Boakye-boaten et al. (2017)
Palm empty fruit bunches	Dilute acid	532	12	136	2010	0.67-0.82	Vaskan et al. (2018)

The range of minimum selling price for lignocellulosic ethanol is broad, and the differences can be attributed to feedstock price, plant capacity, process parameters, and co-products production. The most conservative studies assume higher costs for the feedstock and smaller yields, leading to higher ethanol selling prices (Kazi et al., 2010), while studies with low feedstock cost and high yields (even for technology at a low TRL) show the lowest selling prices (Laser & College, 2009a).

There are some techno-economic analyses available of bioethanol production from switchgrass. Huang et al. (2009) compared switchgrass with three other feedstocks (aspen, hybrid poplar and corn stover), basing the operating conditions for all feedstocks on the previous work developed by Humbird et al. (2011). The authors focused on the effect that plant size has on several production results, including ethanol selling price. They found that as the plant size

increases from 1000 to 4000 dry t per day the ethanol production costs decrease due to the economies of scale. Pfromm et al. (2010) compared the production of butanol to ethanol using switchgrass. The ethanol model was based on an ethanol yield of 0.41 kg ethanol per kg of C5 and C6 sugars (obtained for corn fiber and corn stover hydrolysates). The analysis method relied on carbon mass balances, lower heating value (LHV), and dynamic economic modeling. Laser & College (2009a) compared fourteen different biorefining technologies (bioethanol+Rankine power, bioethanol+H_2, etc.) and selected performance parameters according to a "knowledgeable optimist's most likely estimate". For the bioethanol production process, ammonium fiber expansion (AFEX) pretreatment and consolidated bioprocessing (CBP) were used, selected based on previous studies from the same authors (Laser & College, 2009b). Other analysis for switchgrass used hydrolysis and fermentation yields directly from those determined for corn stover (Gnansounou & Dauriat, 2010).

The most complete analysis to date concerning switchgrass is the one performed by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) 3 project. It includes techno-economic analysis comparing different pretreatment technologies for switchgrass (Tao et al., 2011). This work was based on experimental assays performed to determine the yield of each pretreatment (Garlock et al., 2011), although the hydrolysis yields were obtained experimentally at low solids concentration (1% glucan loading) and later used on the simulations at 20% solids content.

None of the studies found for switchgrass performed the sensitivity analyses of operating parameters chosen here, nor base their results on hydrolysis yields obtained experimentally at the conditions simulated, as developed in Chapter 4 of this work. Some studies include sensitivity to feedstock cost and plant size analysis but those were developed on conditions that differ greatly from those expected for a production facility located in Uruguay.

3.2. Description of the developed models and the simulated scenarios

Two models were created to simulate different scenarios. The first model represents a facility that produces ethanol from switchgrass with surplus electricity obtained from burning the lignin as a co-product, and will be referred to as the "Ethanol and electricity" facility or process. The second model shares most of the design assumptions of the first one. It represents a biorefinery facility that produces (i) ethanol from the solids fraction of pretreated switchgrass; (ii) furfural, acetic acid, and formic acid from the liquid fraction, and (iii) surplus electricity obtained from burning the lignin as a co-product. This will be referred to as the "Biorefinery" facility or process.

3.2.1. Ethanol and electricity production process

Plant capacity

Biomass logistic studies such as the one from Acharya et al. (2009) are fundamental to accurately determine the optimal size of a production facility. However, these studies rely on information of already established crops, or prospective studies about crop implementation that exceed the scope of this work. It is known that as the size of the industrial plant increases the cost of the production decreases (Gnansounou & Dauriat, 2010; Laser & College, 2009b). However, switchgrass is an experimental crop in Uruguay and there is no information on its industrial production at this location. Dr. Guillermo Siri-Prieto, an expert from the research institution that supplies the material, was consulted on the switchgrass supply that could be available to keep a facility operating year-round. In his expert opinion, it would be reasonable to start with a 250 dry t/day facility (Siri-Prieto - personal communication 2015), which could be increased after a few years. To take a conservative approach, this was considered as the base case scenario for the rest of the study.

The facility operates 8400 hours (350 days) per year.

Switchgrass composition

The switchgrass composition used for the simulation is shown in Table 3.2 and was based on the composition determined experimentally on Chapter 4 for the switchgrass (Alamo variety) produced in Uruguay.

Table 3.2. Switchgrass composition used for process simulation.

Component	Percentage (%) dry basis
Glucan	42
Xylan	16.5
Lignin	24
Arabinan	2.5
Galactan	0
Mannan	0
Acetate	2.5
Extractives	8.5
Ash	4

Modeling the bioethanol production facility

The model for the bioethanol production plant includes all operations from reception of the feedstock to purified ethanol production. It comprises feedstock pretreatment (LHW), enzymatic hydrolysis, fermentation, and ethanol recovery as well as other auxiliary processes such as wastewater treatment, combustion for heat and Rankine power generation, utilities and storage. The model and the simulations to obtain mass and energy balances were developed using Aspen Plus® (Aspen Technology, Inc.) V8.8, following the conceptual design basis developed by NREL. Its model for bioethanol production from corn stover was used as a starting point and the main model modifications are summarized in Table 3.3

Table 3.3. Summary of the main changes made to the NREL corn stover to bioethanol model Humbird et al. (2011).

Production stage		Changes
Overall process	-	Plant capacity changed to 250 dry t/day.
	-	Equipment sizing changed accordingly.
Feedstock	-	Feedstock changed to switchgrass.
	-	Included cost for handling area.
Pretreatment	-	Changed to LHW, changing yields.
Enzymatic hydrolysis	-	Enzyme purchased, not produced on site.
	-	Hydrolysis yields were modified.
Inoculum	-	Beer stillage used as fermentation broth.
Co-products	-	In the biorefinery case modifications were made to contemplate new processes (details in section 3.2.3).
Product storage	-	Eliminated cellulase storage.
Wastewater treatment	-	Only anaerobic, clarification and reverse osmosis.

Several results are presented as a function of ethanol volume, which was calculated from mass balances considering a density of 0.789 kg/L at 20 ºC. Conversion was defined as the percentage of a reactant that is converted with a given stoichiometry of reaction.

Detailed process description

•Feedstock reception and handling (Area 100)

Switchgrass arrives at the facility with a water content of 9% (achieved by field drying) and ground to an average particle size of 1 mm. Size reduction is assumed to take place at the switchgrass production facility as logistics studies suggest that this could lead to lower transportation and overall feedstock costs for switchgrass (Sokhansanj et al., 2009). According to the plant capacity of 250 dry t/day, it is necessary to receive 11 trucks with a loading capacity of 25 t each (those are currently used to transport sorghum to bioethanol producing facilities in Uruguay). The contents of these trucks are weighed in an electronic scale with a whole truck dumper and discharged into hoppers that distribute the biomass to a series of conveyors, carrying the biomass to the storage section, where it is stored in one of two concrete domes. Assuming that the reception of the biomass only occurs for 8 hours a day, it would be necessary to receive 31.25 dry t/h. Conveyors are designed to support a higher load because material arrival is not a continuous process. Another set of conveyor belts transports the material to the receiving bin of the pretreatment reactor at the pretreatment area. The model integrates a dust collection system to the conveyors and domes; therefore, it was assumed that no dry matter was lost in this area.

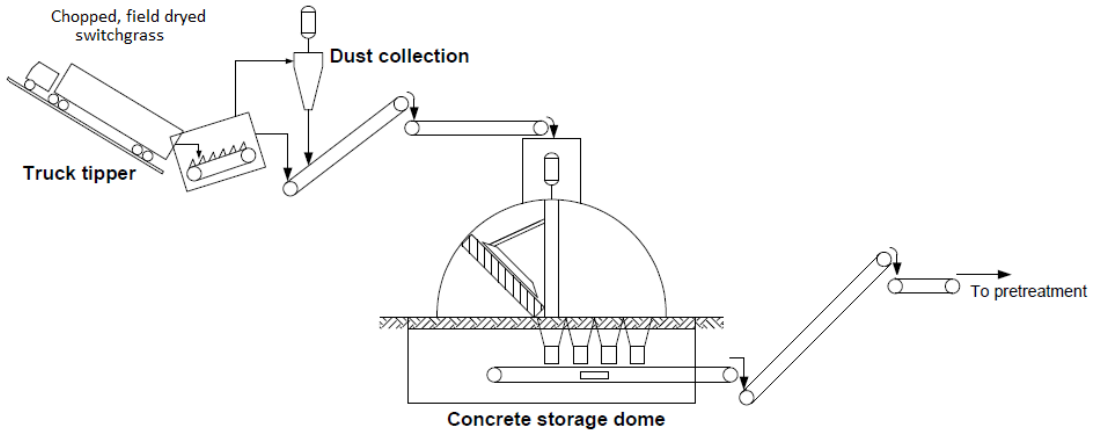


Figure 3.1. Simplified flow diagram of the feedstock handling area, adapted from Humbird et al. (2011).

•Pretreatment (Area 200)

Liquid hot water pretreatment was selected due to the favorable characteristics of this technology for a biorefinery approach mentioned in the state of the art chapter. LHW is a good pretreatment for switchgrass as shown by the techno-economical comparison of pretreatments made by Tao et al. (2011) (ammonia fiber expansion, diluted acid, lime, soaking in aqueous ammonia, steam explosion pretreatment with SO_2 and liquid hot water). This work was based on experimental yields and found that LHW had the best performance (considering oligomers).

In this area, biomass is treated with high-temperature water for a short period of time in order to prepare the material for the enzymatic hydrolysis. The carbohydrates of the hemicellulosic fraction are hydrolyzed to oligomers and a small amount of monomers (that could degrade to fermentation inhibitors). Acetyl groups are released as acetic acid. The structure of the cell wall is broken, partially solubilizing lignin and reducing crystallinity and length of the cellulose chain.

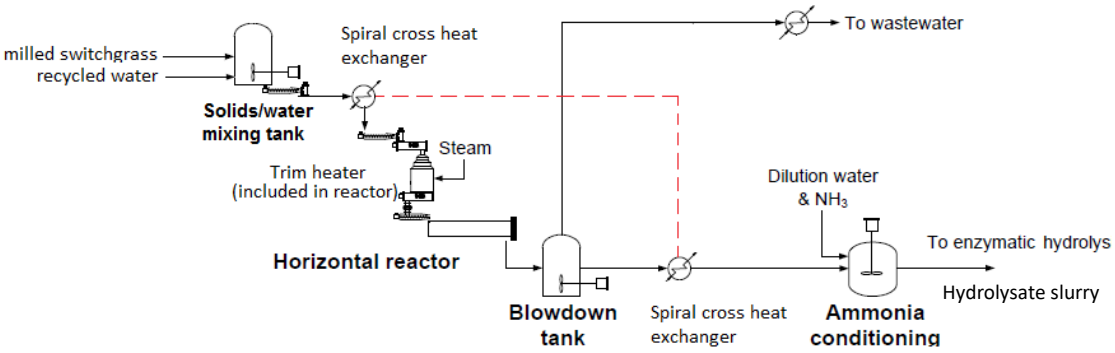


Figure 3.2. Simplified flow diagram of the pretreatment area, adapted from Humbird et al. (2011).

Figure 3.2 shows the main units used in the pretreatment area. Switchgrass is mixed with recycled water from the bottoms of the rectification column (A500) to obtain a 20 % solids content, in a tank. Then it is heated in a cross-heat exchanger, similar to a spiral exchanger with multiple shells and transported to a trim heater at the entrance of the pretreatment reactor, where a temperature of 200 ºC is reached using indirect steam heating. At the tubular section of the reactor, the temperature is maintained at 200ºC for a residence time of 5 min. Pretreatment conditions are summarized in Table 3.4. Operating pressure is higher than the saturation vapor pressure at this temperature, to avoid flashing. The reactor is similar to the U-tubes used for starch cooking (Tao et al., 2011). After pretreatment, the hydrolyzed material is cooled by cross exchange with the feed and held in a flash tank that operates at atmospheric pressure for venting non-condensable gases. The hydrolysate slurry is sent to a conditioning tank

where ammonia gas is used to adjust the pH to 5 (if necessary), with a residence time of 30 minutes.

Table 3.4. LHW pretreatment conditions.

Catalyst	Water
Residence time	5 min
Temperature	200ºC
Pressure	15 atm
Total solids loading	20 % (w/w)

The reactions that occur during pretreatment are summarized in Table 3.5. The conversions for oligomeric and monomeric sugars from cellulose and hemicellulose were calculated based on yields from the material balances reported by Garlock et al. (2011), while yields of other reactions not reported were taken from the NREL design. Lignin solubilization was taken from NREL as it is closer to the low removal observed on the experimental data shown in Chapter 4.

Table 3.5. Reactions and yields considered for LHW pretreatment.

Pretreatment reactions	Reactant	Conversion (%)	Source
$(\mathbf{Glucan})_n + n\ H_2O \rightarrow n\ \mathbf{Glucose\ oligomer}$	Glucan	8	Garlock et al. (2011)
$(\mathbf{Glucan})_n + n\ H_2O \rightarrow n\ \mathbf{Glucose}$	Glucan	0.5	Garlock et al. (2011)
$(\mathbf{Glucan})_n \rightarrow n\ \mathbf{HMF} + 2n\ H_2O$	Glucan	0.3	Humbird et al. (2011)
$(\mathbf{Xylan})_n + n\ H_2O \rightarrow n\ \mathbf{Xylose\ oligomer}$	Xylan	55	Garlock et al. (2011)
$(\mathbf{Xylan})_n + n\ H_2O \rightarrow n\ \mathbf{Xylose}$	Xylan	19.5	Garlock et al. (2011)
$(\mathbf{Xylan})_n \rightarrow n\ \mathbf{Furfural} + 2n\ H_2O$	Xylan	5	Humbird et al. (2011)
$(\mathbf{Arabinan})_n + n\ H_2O \rightarrow n\ \mathbf{Arabinose}$	Arabinan	25.5	Garlock et al. (2011)
$(\mathbf{Arabinan})_n \rightarrow n\ \mathbf{Furfural} + 2n\ H_2O$	Arabinan	5	Humbird et al. (2011)
$\mathbf{Acetate} \rightarrow \mathbf{Acetic}$	Acetate	100	Humbird et al. (2011)
$(\mathbf{Lignin})_n \rightarrow n\ \mathbf{Soluble\ lignin}$	Lignin	5	Humbird et al. (2011)

• Enzymatic hydrolysis and fermentation (Area 300)

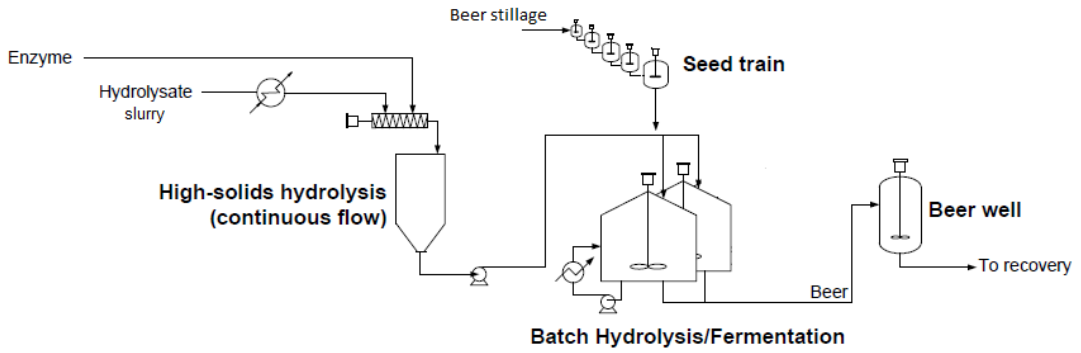


Figure 3.3. Simplified flow diagram of the hydrolysis and fermentation area, adapted from Humbird et al. (2011).

Enzymatic hydrolysis occurs in two stages. The first stage takes place in a continuous reactor designed to work with high solids content and great viscosity, where the slurry from pretreatment with a solids content of 20% is mixed with the enzymes. For this design, enzymes are purchased, not produced on site. After the material is partially hydrolyzed and its viscosity has decreased, it is sent to a series of parallel 1500 m^3 batch reactors where hydrolysis is completed. The number of continuous and batch parallel reactors was calculated to assure a continuous downstream process considering the residence times required. The enzyme used for the simulation (for comparison with experimental results) was Cellic® CTec2 from Novozymes, a mix of cellulases, β-glucosidases and hemicellulases (Novozymes, 2010). Hydrolysis conditions and reactions are summarized in Table 3.6 and Table 3.7 respectively.

Table 3.6. Enzymatic hydrolysis conditions.

Temperature	48 ºC
Solids loading	20 %
Residence time	84 h (24 h continuous+60 h batch)
Number and size of continuous reactors	2 continuous reactors of 425 m^3
Number and size of batch reactors	2 batch reactors of 1500 m^3
Cellulase loading	27 mg protein/g glucan (20 FPU/g glucan)

Table 3.7. Reactions and yields considered for the enzymatic hydrolysis.

Enzymatic hydrolysis reactions	Reactant	Conversion (%)	Source
$(\mathbf{Glucan})_n + \mathbf{n\ H_2O} \rightarrow \mathbf{n\ Glucose}$	Glucan	87	Garlock et al. (2011)
$(\mathbf{Xylan})_n + \mathbf{n\ H_2O} \rightarrow \mathbf{n\ Xylose}$	Xylan	90	Garlock et al. (2011)
$(\mathbf{Xylose\ oligomer})_n + \mathbf{n\ H_2O} \rightarrow \mathbf{Xylose}$	Xylan	79	Garlock et al. (2011)
$(\mathbf{Arabinan})_n + \mathbf{n\ H_2O} \rightarrow \mathbf{n\ Arabinose}$	Arabinan	100	Garlock et al. (2011)

Stirring and temperature control in the reactors is done through a centrifugal pump and an external heat exchanger. This system is also used to cool the hydrolysate to 32ºC, the temperature at which the inoculum is added to start the fermentation.

Inoculum production is also modeled and simulated in this process. The inoculum is done with 10% of the volume of the fermentation broth, added directly without a separation step. An inoculum of engineered *Saccharomyces cereviciae* yeast, like the *PE-2* MEC1121 capable to ferment glucose and xylose (Romaní et al., 2015), is generated using around 10% of the beer stillage from the recovery area, based on findings shared by ICM Inc (Spooner et al., 2017). The percentage of substrate converted to biomass on the seed train is low and most of it is converted to ethanol that will be added to the fermentation reactor with the inoculum. Inoculum production system consists of two seed trains with five sequential reactors each, operating in batch mode with 24 hours batch time and 12 hours turnaround time. Reactors have

an agitation system to ensure some oxygen diffusion (to improve biomass generation). Seed tanks are cooled with chilled water to maintain the temperature at 32 ºC. The first tank has a volume of 9 liters and is inoculated with a seed culture obtained from the lab. After 24 hours, this inoculates the second reactor. This process continues until the last tank. Inoculum is then pumped to a seed holding tank and later to the fermentation tank using a high capacity pump. Seed train conditions and reactions can be seen in Table 3.8 and Table 3.9.

Table 3.8. Seed train conditions.

Inoculum level	10 % of fermenter volume
Batch time	24 h
Reactor turnaround time	12 h
Number of trains	2
Number of inoculum stages	5
Volume of largest reactor	90 m^3
Corn steep liquor (CSL) load	0.5%w/w
Diammonium phosphate (DAP) load	0.67 g/L of hydrolysate slurry
Sorbitol	0.1% of the sugars at last reactor

Table 3.9. Reactions and yields considered for the seed train.

Seed train reactions	Reactant	Conversion (%)
$\mathbf{Glucose \rightarrow 2\ Ethanol + 2\ CO_2}$	Glucose	80
$\mathbf{Glucose + 0.047\ CSL + 0.018\ DAP \rightarrow 6\ (PE-2)\ + 2.4\ H_2O}$ *	Glucose	16
$\mathbf{Glucose\ + 2\ H_2O \rightarrow 2\ Glycerol + O_2}$	Glucose	0.4
$\mathbf{Glucose\ + 2\ CO_2 \rightarrow 2\ Succinic\ acid + O_2}$	Glucose	0.6
$\mathbf{3\ Xylose \rightarrow 5\ Ethanol + 5\ CO_2}$	Xylose	80
$\mathbf{Xylose + 0.039CSL + 0.015\ DAP \rightarrow 5\ (PE-2) + 2\ H_2O}$ *	Xylose	4
$\mathbf{Xylose\ + 5\ H_2O \rightarrow 5\ Glycerol + 2.5\ O_2}$	Xylose	0.3
$\mathbf{Xylose\ + H_2O \rightarrow Xylitol + 0.5\ O_2}$	Xylose	4.6
$\mathbf{3\ Xylose\ + 5\ CO_2 \rightarrow 5\ Succinic\ acid + 2.5\ O_2}$	Xylose	0.9

*Stoichiometry of this reaction is only to balance cell mass composition of PE-2.

Fermentation takes place in the same batch reactors used for the last stage of hydrolysis, agitated at 6 W/m^3, considered appropriate for the hydrolysate with viscosity reduced and for an anaerobic fermentation process. Diammonium phosphate and corn steep liquor are used as nutrients at both the seed and fermentation process. Even though CSL is less attractive in Uruguay than in the US it is still potentially better than other sources like yeast extract from an economic point of view. The use of a co-product of a local industry with high nutrients level would be more desirable. Fermentation conditions are summarized in Table 3.10.

Table 3.10. Fermentation conditions.

Microorganism	*PE-2 recombinant*
Temperature	32ºC
Solids content	20 %
Fermentation time	36 h
Inoculum level	10 % v/v
Number and size of batch reactors	2 batch reactors of 1500 m^3
Corn steep liquor (CSL) load	0.25 % w/w
Diammonium phosphate (DAP) load	0.33 g/L of hydrolysate slurry

Reactions and conversion percentages for the fermentation stage were considered the same as for the corn stover design, except for arabinose fermentation, for which a more conservative value was considered, as arabinose fermentation to ethanol for the strain was not available. The rest of the arabinose would transform to arabitol which was not considered in the simulation. Inhibition effects from furans and acetate were not modeled. However, conversions were based on fermentation experiments carried out in the presence of inhibitors for corn stover. Table 3.11 shows reactions and yields for this stage, including reactions that represent possible contamination losses, assuming that 3% of all sugars are converted to lactic acid by other microorganisms.

Table 3.11. Fermentation and contamination reactions.

Fermentation and contamination losses reactions	Reactant	Conversion (%)
$\mathbf{Glucose \rightarrow 2\ Ethanol + 2\ CO_2}$	Glucose	92
$\mathbf{Glucose + 0.047\ CSL + 0.018\ DAP \rightarrow 6\ (PE-2) + 2.4\ H_2O}$ *	Glucose	2
$\mathbf{Glucose + 2\ H_2O \rightarrow 2\ Glycerol + O_2}$	Glucose	0.4
$\mathbf{Glucose + 2\ CO_2 \rightarrow 2\ Succinic\ acid + O_2}$	Glucose	0.6
$\mathbf{Glucose \rightarrow 2\ Lactic\ acid}$	Glucose	3
$\mathbf{3\ Xylose \rightarrow 5\ Ethanol + 5\ CO_2}$	Xylose	82.5
$\mathbf{Xylose + 0.039\ CSL + 0.015\ DAP \rightarrow 5\ (PE-2) + 2\ H_2O}$ *	Xylose	1.8
$\mathbf{Xylose + 5\ H_2O \rightarrow 5\ Glycerol + 2.5\ O_2}$	Xylose	0.3
$\mathbf{Xylose + H_2O \rightarrow Xylitol + 0.5\ O_2}$	Xylose	4.5
$\mathbf{3\ Xylose + 5\ CO_2 \rightarrow 5\ Succinic\ acid + 2.5\ O_2}$	Xylose	0.9
$\mathbf{3\ Xylose \rightarrow 5\ Lactic\ acid}$	Xylose	3
$\mathbf{3\ Arabinose \rightarrow 5\ Ethanol + 5\ CO_2}$	Arabinose	20
$\mathbf{Arabinose + 0.039CSL + 0.015\ DAP \rightarrow 5\ (PE-2) + 2\ H_2O}$ *	Arabinose	1.8
$\mathbf{Arabinose + 5\ H_2O \rightarrow 5\ Glycerol + 2.5\ O_2}$	Arabinose	0.3
$\mathbf{3\ Arabinose + 5\ CO_2 \rightarrow 5\ Succinic\ acid + 2.5\ O_2}$	Arabinose	0.9
$\mathbf{3\ Arabinose \rightarrow 5\ Lactic\ acid}$	Arabinose	3

*Stoichiometry of this reaction is only to balance cell mass composition of PE-2 recombinant.

After fermentation time is completed, the fermentation broth is sent to the recovery area (Area 500).

•Product, solids and water recovery (A500)

In this stage, fermentation beer is separated into ethanol, water, and residual solids through distillation and solid-liquid separation.

Beer is sent to a distiller column (beer column), that removes CO_2 and about 90% of the water, followed by a rectification column that concentrates the ethanol to a near azeotrope composition of 92.5% ethanol. Both columns were modeled using Aspen's RADFRAC model that performs rigorous vapor-liquid calculations, column sizing, and rating. A summary of the columns characteristics is shown in Table 3.12. The bottom fraction of the beer column is cooled to 47 ºC and then separated with a pressure filter into two streams. The stream with the solids rich in lignin is dried to 35% moisture with air. Both solids and air are sent to the combustor in Area 800, where solids are burned to generate heat and power. Part of the stillage is used in the inoculum production, and the remaining fraction goes to wastewater treatment (Area 600). Around 0.8% of the ethanol that enters to the beer column is lost in this stream.

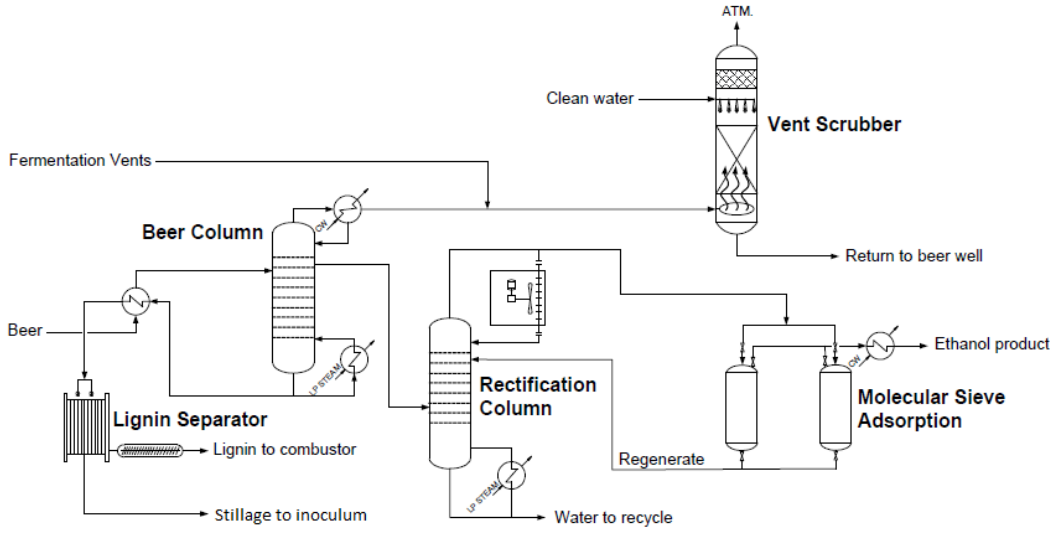


Figure 3.4. Simplified flow diagram of the recovery area, adapted from Humbird et al. (2011).

The overhead stream of the beer column (containing mostly CO_2 and ethanol) is sent to a water scrubber that also receives the fermentation vents. The scrubber recovers most of the ethanol on an effluent that is recycled to the beer well. Rectification column bottoms are recycled to the pretreatment reactor as dilution water.

Table 3.12. Beer and rectification column specifications.

	Beer column	Rectification column
Stages	16	35
Feed stage (from the top)	2	20 for main feed. 14 for molecular sieve recycle.
Pressure (overhead)	2.04 atm	1.6 atm
Molar reflux ratio	3:1	3.6:1
Design specifications	>99% of the ethanol in the feed is removed as a vapor side-draw at Tray 3 at 40 % w/w.	Overhead mixture of 92.5% w/w ethanol, bottoms composition of 0.05% w/w ethanol.

The azeotrope mix obtained from the rectification column is separated in a vapor phase molecular sieve to achieve a 99.5 % of ethanol. The molecular sieve consists of two columns packed with beds of adsorbent that selectively absorb water, from the superheated vapor that flows through them. The purified ethanol obtained from the molecular sieve is cooled by heat exchange with the sieve regeneration condensate, condensed with cooling water and sent to the storage area. One adsorption column operates while the other regenerates, using a stream of pure ethanol under vacuum conditions. Regeneration process generates a stream of 70% ethanol that is recycled back to the rectification column for recovery.

• Wastewater treatment (Area 600)

Effluents from the process (cooling tower and boiler blowdown, condensed pretreatment flash vapor, and beer column stillage) are mixed and cooled to 35ºC in a heat exchanger prior to anaerobic digestion treatment and reverse osmosis (RO) regeneration as shown in Figure 3.5.

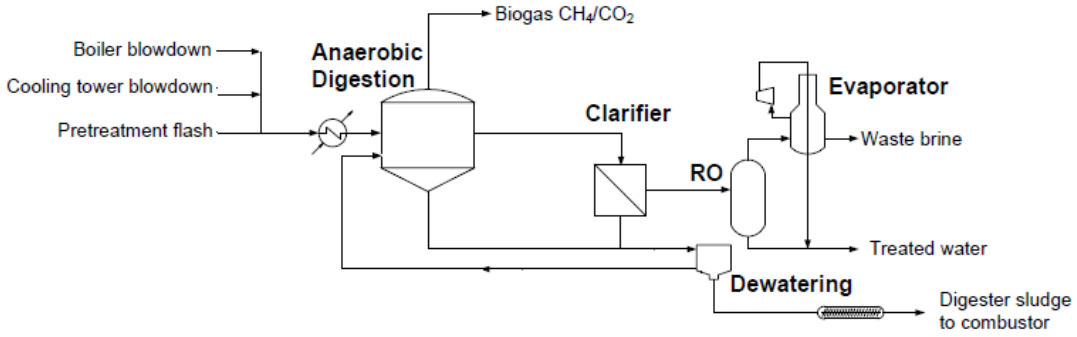


Figure 3.5. Simplified flow diagram of the water treatment area, adapted from Humbird et al. (2011).

Humbird et al. (2011) used anaerobic and aerobic pretreatment for wastewater treatment. Using switchgrass and sending part of the beer stillage for inoculum production lowers the amount of chemical oxygen demand (COD) that needs to be removed (from 87 g/L for corn stover to 41 g/L in this work). Consequently, the use of only an anaerobic treatment is sufficient, and consistent with previous reports (Spooner et al., 2017). In the anaerobic reactor, 95% of the organic compounds are converted to biogas and a 5% is converted to cell mass. The biogas, a mix of 51% CH_4 and 49% CO_2 (in molar basis), is produced at a rate of 228 g CH_4/kg COD and sent to the combustor area to be converted into heat and energy. The digested water stream is transferred to a membrane bioreactor for clarification. The membrane unit removes additional COD and colloidal particles. After clarification, the treated water is sent to a reverse osmosis membrane system where salts are removed, generating a stream of pure water that is mixed with makeup water and recycled to the process. The brine obtained from RO is concentrated in an evaporator to 50 % w/w solids and considered a waste material. The condensate from the evaporator is recycled to the process. Cell mass produced (45 g/ kg COD), is sent to a holding tank, where it is mixed with the sludge from the clarification step and dewatered on a centrifuge. Centrifuged solids are conveyed to the combustor for burning, and the centrifuged water is recycled to the wastewater treatment reactor.

•Storage (Area 700)

Chemicals used and produced through the process need to be stored for different periods of time. Table 3.13 shows the chemicals stored, the capacity and number of storage tanks. DAP is assumed to be received as a solid on big sacks, requiring a solid feeder, an unloading blower and a vent baghouse. DAP solution is prepared in a tank and is pumped to fermentation and inoculum reactors. Pumps to the liquid storage tanks are sized for quick loading and unloading of trucks. The fire water pump is sized for 10 m^3/min.

Table 3.13. Specification for storage tanks.

Product/chemical	Capacity	Quantity	Construction material
Ethanol	7 days of production.	2	A285C carbon steel.
Denaturant	7 days blend stock.	1	Carbon steel.
Ammonia	5 days storage.	2	SA-516-70.
Cellulase	5 days storage.	1	Glass lined carbon steel.
Fire water	4 hours fire suppression.	1	Glass lined carbon steel.
CSL	5 days storage.	1	Glass lined carbon steel.
DAP	7 days storage.	1	SS304.

• Steam and electricity generation (Area 800)

The solids from distillation (containing the lignin, unconverted cellulose and hemicellulose from the feedstock and other components of biomass), solids from wastewater treatment and the biogas from anaerobic digestion are combusted to produce high-pressure steam for electricity production and process heat.

The combustor is designed to handle wet solids. Air from the solids drying process (A500) is used in the combustion chamber. Treated water is boiled and superheated to high-pressure steam (62 atm) inside the heat exchanger circuit. The superheated steam goes to a multistage turbine and generator that produces low-pressure (9.5 atm) and high-pressure (13 atm) steam, and electricity to satisfy process needs. Remaining steam is condensed with cooling water in vacuum conditions (0.1 atm) and is recycled to the boiler feed with the condensates from other heat exchangers of the process. The surplus in electricity is sold to the grid, so electricity is considered as a co-product of the ethanol production process. Flue gas from the combustor is used to heat the air for the combustion chamber. Flue gas desulfurization is not necessary for this process.

• Utilities (Area 900)

This area includes a cooling water system, chilled water system, process water system, Cleaning in place (CIP) system, plant and instrument compressed air as well as tracking of the electricity usage through the plant.

Process water, a mix of fresh makeup water with treated wastewater provided a constant pressure to the facility by the water process manifold. Fresh water is also added to some internally-recycled water streams for dilution before pretreatment and enzymatic hydrolysis. Fresh water enters the facility at a temperature of 20 ºC (Uruguay has an average annual temperature of 18ºC) and goes through a heat exchanger to cool the streams entering the wastewater area before going to the process water tank. The process water tank is designed for a residence time of 8 hours. Process water goes to the boiler and cooling tower makeup, the CIP system, and the vent scrubbers. The CIP system consists of hot cleaning and sterilization chemicals for hydrolysis, fermentation and distillation equipment.

Air systems provide compressed air for pneumatic tools, clean-up, and instrument operation. The system includes a compressor sized for 11 m^3/min at 862 kPa, an instrument air dryer and surge tank.

The cooling water system is designed for water supplied at 28 °C, assuming an average 9°C temperature rise in coolers throughout the facility. It was assumed that cooling windage

losses would be 0.005 % of the total flow to the tower. The demand for the chiller system was assumed to be equal to the heat removed in the chilled-water loop that provides cooling to the fermentation reactors.

3.2.2. Biorefinery process

This process represents a biorefinery scenario in which the liquid fraction (mostly hemicellulose) is used to obtain higher value products instead of being fermented to ethanol. The high-value products obtained are furfural, acetic acid, and formic acid. Furfural was selected on the basis of available process information, and on the economic, technical and commercial potential of this biochemical in a biorefinery (Biddy et al., 2016; Gnansounou & Pandey, 2017). Acetic and formic acid are co-products of the selected production process (Xing et al., 2011). This involves new production stages and equipment. New processes and differences with the ethanol and electricity process are described below.

- **Pretreatment Area (A200)**

After LHW pretreatment, the slurry is sent to a hydrolysate tank, where it is mixed with water from the wash filtrate tank to assure that it can be pumped to the liquid/solid separation step (approx. 10% suspended solids). Liquid and solid fractions are separated in a Pneumapress® pressure filter (Aden et al., 2002). The liquid fraction is sent to a hydrolysate tank in Area 400. Solids are washed first with water from the filtrate tank and then with recycled process water. A total of two grams of water per gram of liquor remaining in the solid cake are used. Air is blown through the solid cake to displace the liquid. The filtrate from the washing step goes to the filtrate tank, and later to wastewater treatment. Solids are transported in a conveyor belt to the conditioning reactor where water is added to meet solids content specifications for enzymatic hydrolysis and fermentation. Figure 3.6 shows a simplified flow diagram for these processes. The rest of the process for the solid fraction is the same as described for the ethanol and electricity process (section3.2.1).

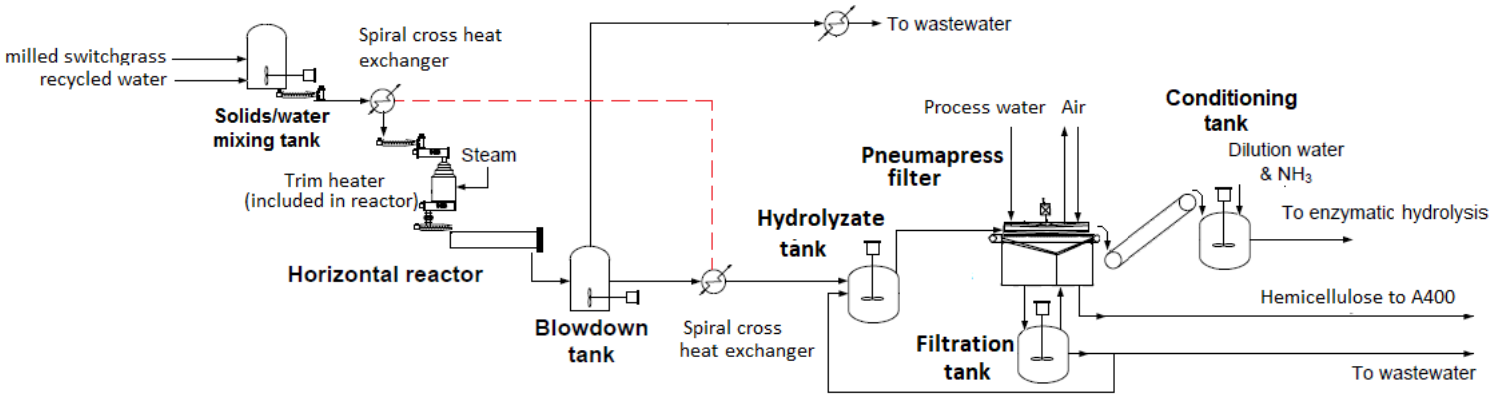


Figure 3.6. Simplified flow diagram for pretreatment, solids washing, solid/liquid separation and conditioning (A200) for the biorefinery scenario.

No extra raw materials are needed for this stage. New equipment in this area includes hydrolysate tank, filtration tank, Pneumapress® pressure filter, solid cake conveyor, and transfer pumps.

Furfural, acetic acid and formic acid production area (A400)

The liquid fraction from the pretreatment area has approximately 2% weight of xylose and xylose oligomers. To be further processed a concentration step is necessary. Concentration takes place in a three effects evaporator, operating at 0.6, 0.3 and 0.2 atm. New equipment was added to the equipment mentioned in the ethanol and electricity process (section 3.2.1). Figure 3.7 shows a simplified flow diagram from the hemicellulosic tank to the production and separation of the co-products.

The concentrated hemicellulose stream with 10.7% xylose and xylose oligomer, is converted to furfural, acetic acid, and formic acid. The transformation process was modeled with data obtained by Xing et al. (2011), combining experimental results with Aspen simulation of the production process. Some heat integration was done between Area 200 and Area 400, using waste vapor from A200 as partial energy input to the evaporator.

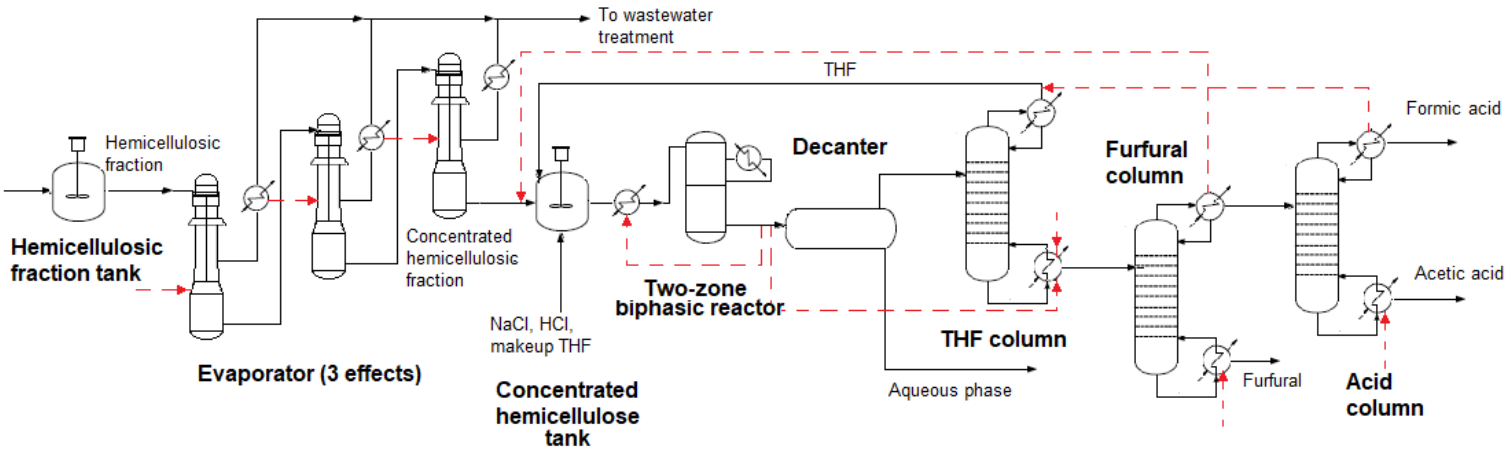


Figure 3.7. Diagram flow for the production of furfural, formic and acetic acids from waste aqueous hemicelluloses.

The transformation process takes place in a two-zone biphasic reactor. In the first zone, xylose oligomers are depolymerized into xylose monomers, and formylated and acetylated xylose oligomers are depolymerized into xylose and formic acid, and xylose and acetic acid respectively. In the second zone of the reactor, xylose monomers are dehydrated to furfural. The phases included in the reactor are: an **aqueous phase,** with the hemicellulose stream saturated on sodium chloride (NaCl) and hydrochloric acid (HCl) added as catalyzer (0.44 M), and an **organic phase** with NaCl pretreated tetrahydrofuran (THF), selected due to its great affinity for furfural, low-boiling point, and ease of separation from water. In the biphasic reactor, all the xylose is consumed, 90% forms furfural and 10% form humins (decomposition products). The distillation column system was designed to remove the most plentiful component (THF) first and to leave the most difficult separation for the last step. The amount of raw materials needed in this process were calculated as 46 g NaCl, 41 g HCl, and 2.2 g makeup THF per kg of the concentrated hemicellulosic stream. The heat that needs to be supplied by steam to the separation process was determined through Aspen simulation as 1.05 MJ/kg of the concentrated hemicellulosic stream that enters the process (Xing et al., 2011). New equipment for this area includes: hemicellulose tank, concentrated hemicellulose tank, three effects evaporator, biphasic reactor, decanter, distillation columns for THF, furfural and acids, heat exchangers and transfer pumps.

- **Storage area (Area 700)**

New tanks and pumps are necessary to store and distribute the reagents (hydrochloric acid, makeup THF) and the new products (furfural, acetic acid, formic acid). See Appendix A for further detail.

3.2.3. Conditions and basis for the other scenarios studied

This section describes all the scenarios and conditions simulated, briefly summarized in Table 3.14. Case 1 is the base model for the ethanol and electricity process previously described in section 3.2.1. Cases 2 to 4 are different scenarios for that model. Case 5 is the base model of the biorefinery process previously described. Cases 6-71 are different scenarios in the biorefinery model. Differences with the biorefinery base case (case 5) for each case are identified with a pink shade. The differences for each scenario will be further described, case number assigned will be maintained through all the Chapters of this work and the Appendix sections.

Table 3.14. Summary of parameters and cases analyzed.

Case	Size (dry t /day)	Co products	Feedstock cost ($/ dry t)	Enzyme cost ($/kg$_{protein}$)	Nutrient addition	Surfactant (g/g$_{solids}$)	Enzyme dosage (mg$_{protein}$ /g$_{glucan}$)	Hydrolysis time (h)	Hydrolysis efficiency (%)	Fermentation time (h)	Fermentation efficiency (%)	Solids content (%)	Glucan content (%)	Xylan content (%)	Lignin content (%)
1	250	Electricity	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
2	125	Electricity	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
3	500	Electricity	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
4	250	Electricity	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
5	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
6	250	Electricity, furfural acetic and formic acid	20	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
7	250	Electricity, furfural acetic and formic acid	60	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
8	250	Electricity, furfural acetic and formic acid	30	3	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
9	250	Electricity, furfural acetic and formic acid	30	11	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
10	250	Electricity, furfural acetic and formic acid	30	4.24	No nutrients at fermentation	0	27	84	90	36	92	20	42	16.5	24
11	250	Electricity, furfural acetic and formic acid	30	4.24	1.0 w/w CSL, 1.34 g/L DAP	0	27	84	90	36	92	20	42	16.5	24
12	250	Electricity, furfural acetic and formic acid	30	4.24	No nutrients at fermentation	0	27	84	90	36	70	20	42	16.5	24
13	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0.05	27	84	90	36	92	20	42	16.5	24
14	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0.05	6.75	84	90	36	92	20	42	16.5	24
15	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	6.75	84	90	36	92	20	42	16.5	**24**
16	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	13.5	84	90	36	92	20	42	16.5	24
17	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	20.25	84	90	36	92	20	42	16.5	24
18	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	33.75	84	90	36	92	20	42	16.5	24
19	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	40.5	84	90	36	92	20	42	16.5	24
20	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	24	90	36	92	20	42	16.5	24
21	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	48	90	36	92	20	42	16.5	24
22	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	72	90	36	92	20	42	16.5	24
23	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	96	90	36	92	20	42	16.5	24
24	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	120	90	36	92	20	42	16.5	24
25	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	168	90	36	92	20	42	16.5	24
26	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	95	36	92	20	42	16.5	24
27	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	92	20	42	16.5	24
28	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	75	36	92	20	42	16.5	24
29	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	65	36	92	20	42	16.5	24
30	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	24	92	20	42	16.5	24
31	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	48	92	20	42	16.5	24
32	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	72	92	20	42	16.5	24
33	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	96	92	20	42	16.5	24
34	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	87	20	42	16.5	24
35	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	82.5	20	42	16.5	24
36	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	73	20	42	16.5	24
37	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	63	20	42	16.5	24

Case	Size (dry t /day)	Co products	Feedstock cost ($/ dry t)	Enzyme cost ($/$kg_{protein}$)	Nutrient addition	Surfactant (g/g_{solids})	Enzyme dosage ($mg_{protein}/g_{glucan}$)	Hydrolysis time (h)	Hydrolysis efficiency (%)	Fermentation time (h)	Fermentation efficiency (%)	Solids content (%)	Glucan content (%)	Xylan content (%)	Lignin content (%)
38	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	25	42	16.5	24
39	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	22.5	42	16.5	24
40	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	17.5	42	16.5	24
41	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	15	42	16.5	24
42	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	12.5	42	16.5	24
43	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	10	42	16.5	24
44	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	92	25	42	16.5	24
45	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	92	22.5	42	16.5	24
46	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	92	17.5	42	16.5	24
47	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	92	15	42	16.5	24
48	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	92	12.5	42	16.5	24
49	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	75	36	92	25	42	16.5	24
50	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	75	36	92	22.5	42	16.5	24
51	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	75	36	92	17.5	42	16.5	24
52	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	75	36	92	15	42	16.5	24
53	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	75	36	92	12.5	42	16.5	24
54	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	65	36	92	25	42	16.5	24
55	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	65	36	92	22.5	42	16.5	24
56	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	65	36	92	17.5	42	16.5	24
57	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	65	36	92	15	42	16.5	24
58	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	65	36	92	12.5	42	16.5	24
59	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	80	36	78	25	42	16.5	24
60	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	95	36	92	25	42	16.5	24
61	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	95	36	92	22.5	42	16.5	24
62	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	95	36	92	17.5	42	16.5	24
63	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	95	36	92	15	42	16.5	24
64	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	95	36	92	12.5	42	16.5	24
65	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	37	16.5	24
66	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	47	16.5	24
67	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	11.5	24
68	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	21.5	24
69	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	19
70	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	42	16.5	29
71	250	Electricity, furfural acetic and formic acid	30	4.24	0.5 w/w CSL, 0.67 g/L DAP	0	27	84	90	36	92	20	37	21.5	24

Economy of scale (case 2 and case 3)

As it was stated before plant size was selected considering that larger scale leads to smaller costs and the limitations on switchgrass supply. Nevertheless, a plant size of 125 t/day (case 2) and 500 t/day (case 3) were analyzed to estimate the effects that these scale changes would have on the economic aspects of the process. It was assumed that the switchgrass recollection radius, and therefore switchgrass delivery cost, remained constant in the production range studied.

No fermentation of the hemicellulosic fraction (case 4)

In case 1, the hemicellulose fraction of the pretreated slurry is hydrolyzed and fermented along with the cellulosic fraction by a microorganism capable of fermenting pentoses and hexoses. Robust microorganisms capable of working under industrial conditions and with high xylose-glucose fermenting capacity and viability are still under development (Nielsen et al., 2017). Case 4 does not consider the xylose fermentation; therefore, it shows the effect that the loss of xylose fermentation ability would have on process economics.

Sensitivity to enzyme and feedstock costs (Cases 6-9)

Feedstock cost can comprise 40-70% of total production costs, and this percentage could increase over time due to technology development, decreasing the capital costs of the overall process (IRENA, 2016). There is great uncertainty about feedstock price since it is currently an experimental crop in Uruguay.

The cost of enzymes is a major contributor to ethanol selling price and is the subject of great debate (Klein-Marcuschamer et al., 2012).

To contemplate the uncertainties in these important costs, feedstock cost was varied between 20 $/ dry t (case 6) and 60 $/ dry t (case 7) and enzyme cost was varied between 3 $/kg of protein (case 8) and 11 $/kg of protein (case 9), according to what was reported by Klein-Marcuschamer et al. (2012).

Sensitivity to feedstock composition (Cases 65-71)

Feedstock composition in a biorefinery in which products with different value are obtained from different feedstock fraction can have an important effect on productivity and overall process economics. Switchgrass composition reported by different authors show differences amongst their results, and with the experimental results obtained in this work. Therefore, variations on the main components of lignocellulosic material were studied. Cases 65 and 66 simulate a variation of $\pm$ 5 % in glucan composition. Cases 67 and 68 simulate a variation

of ± 5 % in xylan composition. Cases 69 and 70 simulate a variation of ± 5 % in lignin composition. The changes in these components were compensated by the correspondent increase or decrease on extractive content. Case 71 simulates a 5% increase in xylan composition at the expense of a 5% reduction in glucose composition. This composition is within the reported range (Cybulska et al., 2013; Esteghlalian et al., 1997; Garlock et al., 2011; Li et al., 2013a; Li et al, 2013b; Yan et al., 2010).

Sensitivity to different process parameters (cases 10-64)

Based on case 5, different scenarios were simulated to estimate the sensitivity of minimum selling price to parameters associated to: the enzymatic hydrolysis of cellulose (addition of surfactant, enzyme dosage, hydrolysis time, hydrolysis efficiency, solids content), and to the glucose fermentation (nutrient addition, fermentation time and efficiency), summarized in Table 3.15, Table 3.16 and Table 3.17. For each case, only the parameters specified on the tables were modified, while the rest of the process parameters remained the same as the base case scenario (case 5).

Parameters associated with enzymatic hydrolysis of cellulose

Maximum cellulose hydrolysis corresponds to all the cellulose being converted to glucose($(\text{Glucan})_n + n\ H_2O \rightarrow n\ \text{Glucose}$), hydrolysis efficiency (H, %) is defined as the percentage of cellulose that is converted to glucose with this reaction.

The Surfactant addition conditions were selected based on the work of Camesasca et al. (2015). In these assays, the enzyme dosage varied from 6.75 - 40.5 mg $_{protein}$ /g $_{glucan}$, the hydrolysis time from 24 to 168 h, and the hydrolysis efficiency from 65 to 90%. Price of PEG was considered as 1.92 $/kg PEG.

Table 3.15. Summary of simulation scenarios for sensitivity analysis of process parameters associated with hydrolysis.

Case	Surfactant addition (g_{PEG}/g_{solids})	Enzyme dosage ($mg_{protein}/g_{glucan}$)	Hydrolysis time (h)	Hydrolysis efficiency (%)
13	0.05	27	84	90
14	0.05	6.75	84	90
15	0	6.75	84	90
16	0	13.5	84	90
17	0	20.25	84	90
18	0	33.75	84	90
19	0	40.5	84	90
20	0	27	24	90
21	0	27	48	90
22	0	27	72	90
23	0	27	96	90
24	0	27	120	90
25	0	27	168	90
26	0	27	84	95
27	0	27	84	80
28	0	27	84	75

29	0	27	84	65

Parameters associated with glucose fermentation

Maximum glucose fermentation corresponds to all the glucose being converted to glucose ($\text{Glucose} \rightarrow 2\ \text{Ethanol} + 2\ CO_2$), fermentation efficiency (F, %) is defined as the percentage of glucose that is converted to ethanol with this reaction.

The fermentation time was varied in the range 24 - 96 h and the fermentation efficiency in the range 63-92%. Also, the effect of nutrient addition was studied.

Table 3.16. Summary of simulation scenarios for sensitivity analysis of process parameters associated with fermentation.

Case	Nutrient addition	Fermentation time (h)	Fermentation efficiency (%)
10	No nutrients at fermentation	36	92
11	1.0 w/w CSL 1.34 g/L DAP	36	92
12	No nutrients at fermentation	36	70
30	0.5 w/w CSL 0.67 g/L DAP	24	92
31	0.5 w/w CSL 0.67 g/L DAP	48	92
32	0.5 w/w CSL 0.67 g/L DAP	72	92
33	0.5 w/w CSL 0.67 g/L DAP	96	92
34	0.5 w/w CSL 0.67 g/L DAP	36	87
35	0.5 w/w CSL 0.67 g/L DAP	36	82.5
36	0.5 w/w CSL 0.67 g/L DAP	36	73
37	0.5 w/w CSL 0.67 g/L DAP	36	63

Solids content

Solids content was defined as the weight-based solids percentage in the slurry (in dry basis), at the beginning of the hydrolysis stage. It was varied from 12.5 to 25% w/w. In these assays, the hydrolysis efficiency varied from 65 to 90%.

Table 3.17. Summary of simulation scenarios for sensitivity analysis of solids content and hydrolysis efficiency.

Case	Hydrolysis efficiency (%)	Solids content (%, w/w)	Fermentation efficiency (%)
38	90	25	92
39	90	22.5	92
40	90	17.5	92
41	90	15	92
42	90	12.5	92
43	90	10	92
44	80	25	92
45	80	22.5	92
46	80	17.5	92
47	80	15	92
48	80	12.5	92
49	75	25	92
50	75	22.5	92
51	75	17.5	92
52	75	15	92
53	75	12.5	92
54	65	25	92
55	65	22.5	92
56	65	17.5	92
57	65	15	92
58	65	12.5	92
59	80	25	78
60	95	25	92
61	95	22.5	92
62	95	17.5	92

63	95	15	92
64	95	12.5	92

3.2.4. Economic parameters and considerations

Results from material and energy balances executed on the different simulation cases were used to determine the number and size of equipment, as well as the amount of chemicals and feedstocks used and how this affects the capital and operative costs of the process.

Capital costs

As operating conditions change the cost of equipment, this was calculated considering the base cost and size of the equipment, a scaling factor (see Appendix A, Table A.2) and the new size obtained from simulations results, following the formula:

$$New\ cost = Base\ cost\left(\frac{New\ size}{Base\ size}\right)^{n} \quad (3.1)$$

The base costs for most equipment were obtained by Humbird et al. (2011). The cost of the LHW pretreatment reactor was taken from Tao et al. (2011). Equipment costs for A200 of the biorefinery process were obtained from the work of Aden et al. (2002). These costs can be found in Appendix A (Table A.2).

All costs are converted to 2015 US dollars, applying plant cost indexes from the Chemical Engineering magazine and the following formula:

$$2015\ cost = Base\ cost\left(\frac{2015\ cost\ index}{base\ year\ index}\right) \quad (3.2)$$

A location factor of 1.25 was used to consider that the facility is located in Uruguay, instead of the US as the quotations consider. This was based on Richardson (2008) location factors. Installation factors were also taken into account. Indirect and direct overhead costs were determined by applying factors to the total equipment capital cost. Base costs, specifications, scaling and installation factors quotation year and the source of the data can be found in Appendix A.

Location and scale factor application were validated by comparing the equipment cost obtained by this method with the actual cost of equipment bought and installed in Uruguay, provided by ALUR (Alcoholes del Uruguay) for a few key equipments (data not shown). The values obtained in this work were similar but higher, indicating a more conservative TEA.

Direct costs associated with the warehouse, site development and pipping are considered as 17.5% of total equipment cost. These costs add up to the total direct cost (TDC). The costs related to the construction stages, including proratable costs, field expenses,

construction, project contingency, and others are considered as a percentage of the total direct costs, and they add up to 60% of the TDC. These percentages were taken from Humbird et al. (2011). The sum of these costs, added to the TDC, is the fixed capital investment (FCI).

The sum of FCI and the working capital for the project is the total capital investment (TCI).

It should be noted that this analysis is based on nth-plant assumption. Meaning that this analysis refers to a facility operating with proven technology and does not include the uncertainties and extra costs of developing innovative technology at an industrial scale.

Operating costs

The amount of raw materials required for the process was quantified through the material balances of the simulation. The cost for most chemicals that would be imported was multiplied by a factor of 1.1 considering that transportation accounts for a 10% of the value for the assumed origin of these materials (http://worldfreightrates.com/freight access 04/2018). Raw material prices can be found in Appendix A.

Feedstock cost was estimated using the excel spreadsheet created by researchers at the Pennsylvania State University (Jacobson & Helsel, 2014) for budgeting switchgrass production. Types and amount of fertilizers and herbicides were adjusted to meet the requirements of Uruguayan soil provided by Guillermo Siri-Prieto (Siri-Prieto personal communication, 2016). Grinding cost is included with the cost suggested by Sokhansanj et al. (2009). This led to an estimated low feedstock price of $ 30 /dry t, consistent with suggestions by Siri-Prieto (Siri-Prieto personal communication, 2016). The modified spreadsheet can be seen in Appendix A.

Enzyme cost was set at 4.24 $/kg$_{protein}$ (Humbird et al., 2011), assuming 160 mg $_{protein}$/g, 120 FPU/g (Hsieh et al., 2014). This translates to 0.68 $/kg$_{enzyme}$.

Co-product market values were fixed at: 1.80 $/kg for furfural, 0.85 $/kg for acetic acid, and 0.65 $/kg for formic acid (Biddy et al., 2016; TranTech Consultants Inc., 2014).

When excess electricity was generated in the process, it was sold as a co-product with a value of 0.092 $/kWh, as this was the price required by the state electricity company UTE for providers of electricity by 2014 (Elender, 2012).

Fixed operative costs include personals salary, which was estimated based on the number of people required for each position and the average salary for each position and a labor burden of 12.5%, as informed in a report about the cost of installing a company in Uruguay (Uruguay XXI, 2015). The list of workers and salaries is included in Appendix A.

Discounted cash flow analysis

A discounted cash flow analysis was used to estimate the minimum ethanol selling price. This price was determined by iterating the selling price of ethanol until a net present value of zero was obtained. The values specified for different parameters affecting the discounted cash flow analysis are shown in Table 3.18. These parameters were kept the same as those selected by Humbird et al. (2011), as they are widely used for TEAs. Taxes were not considered.

Table 3.18. Parameters for discounted cash flow analysis.

Parameter	Value
Plant life	30 years
Internal rate of return	10 %
Plant depreciation	200 % declining balance (DB)
Plant recovery period	7 years
Vapor plant depreciation	150 % DB
Vapor plant recovery period	20 years
Taxes	No taxes considered
Financing	40%
Loan terms	10 years, 8% interest
Construction time	3 years
First year expenditures	8%
Second year expenditures	60%
Third year expenditures	32%
Working capital	5% of FCI
Start-up time	3 months
Revenues during start-up	50%
Variable costs during start-up	75%
Fixed costs during start-up	100%

3.3. Results and discussion

Material and energy balances, as well as equipment and raw material costs, obtained for each simulation case, are the basis for all the analyses in this section. These results are shown in detail in Appendix A, and more complete process flow diagrams can be found in Appendix B.

3.3.1. Economics of the ethanol and electricity production process

The resulting production cost or minimum selling price for ethanol (MESP) in a facility producing only ethanol and electricity was 0.89 $/L. This is on the expected price range for advanced alcohol fuels (International Renewable Energy Agency, 2016). Fuels with this price range would have a tough time competing with oil at prices below 80 $/ barrel, but if oil prices exceed 100 $/ barrel, they should be able to compete effectively (International Renewable Energy Agency, 2016).

This value was higher than values found by Laser & College (2009b), Gnansounou & Dauriat (2010) and Huang et al. (2009) in TEA´s of bioethanol production from switchgrass, who reported prices of 0.16-0.22 $/L, 0.77 $/L, and 0.42-0.51 $/L respectively. The major differences with and between reported values can be explained by plant capacity and overall process efficiencies considered in the studies. Reported production capacity was 5000 dry t/day for Laser & College (2009b), 1800 dry t/day for Gnansounou & Dauriat, (2010) and 1000-4000 dry t/day for Huang et al. (2009), while this work assumes a 250 dry t/day capacity, leading to higher costs associated with a smaller scale (Gnansounou & Dauriat, 2010).

As shown in Figure 3.8, plant size had a big effect on MESP that could justify the high value obtained. Increasing plant capacity to twice the base case, could reduce MESP to 0.72 $/L, but more importantly, any problems with supply affecting plant capacity could increase MESP. This is consistent with previous findings showing that the cost of bioethanol obtained from switchgrass decreased from 0.51 to 0.42 $/L as a consequence of an increase in the production scale from 1000 to 4000 dry t /day (Huang et al., 2009) and from approximately 1.00 to 0.77 $/L increasing capacity from 450 to 1818 dry t/day (Gnansounou & Dauriat, 2010). Huang et al. (2009) also found that the optimal production size was between 2000 and 4000 dry t /day, but this is outside of the expected supply and logistics limitations, for a current facility in Uruguay. Therefore, the rest of the analysis was made for the maximum expected supply (250 dry t/day), as recommended by the agronomical researcher Guillermo Siri-Prieto (Siri-Prieto personal communication, 2015).

Considering these results, it is fundamental to develop crop production and logistics systems to satisfy the demand of the installed facility. An increased capacity by adding a second feedstock (e.g. agricultural residues) could be beneficial, but it requires the development of more flexible technological processes.

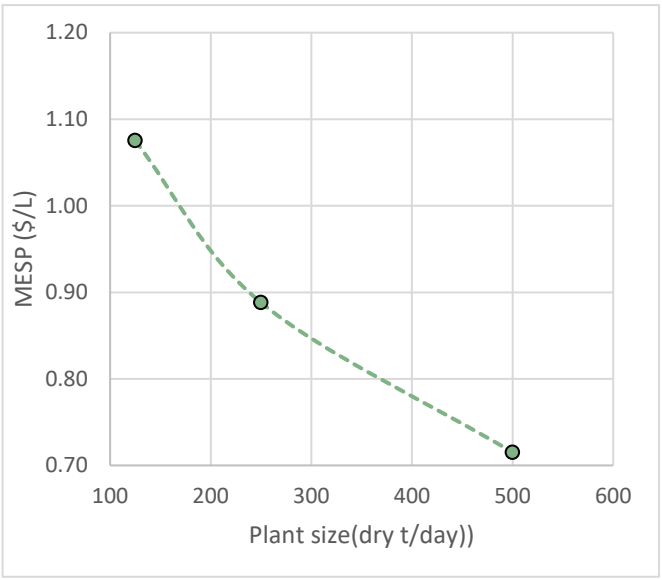


Figure 3.8. Minimum ethanol selling price (MESP) as a function of plant size for the ethanol and electricity scenario.

Minimum selling price for the first scenario can be further broken down into the cost of each process area. Figure 3.9 and Figure 3.10 illustrate the contribution to the overall cost by process area and capital, operations, and fixed costs.

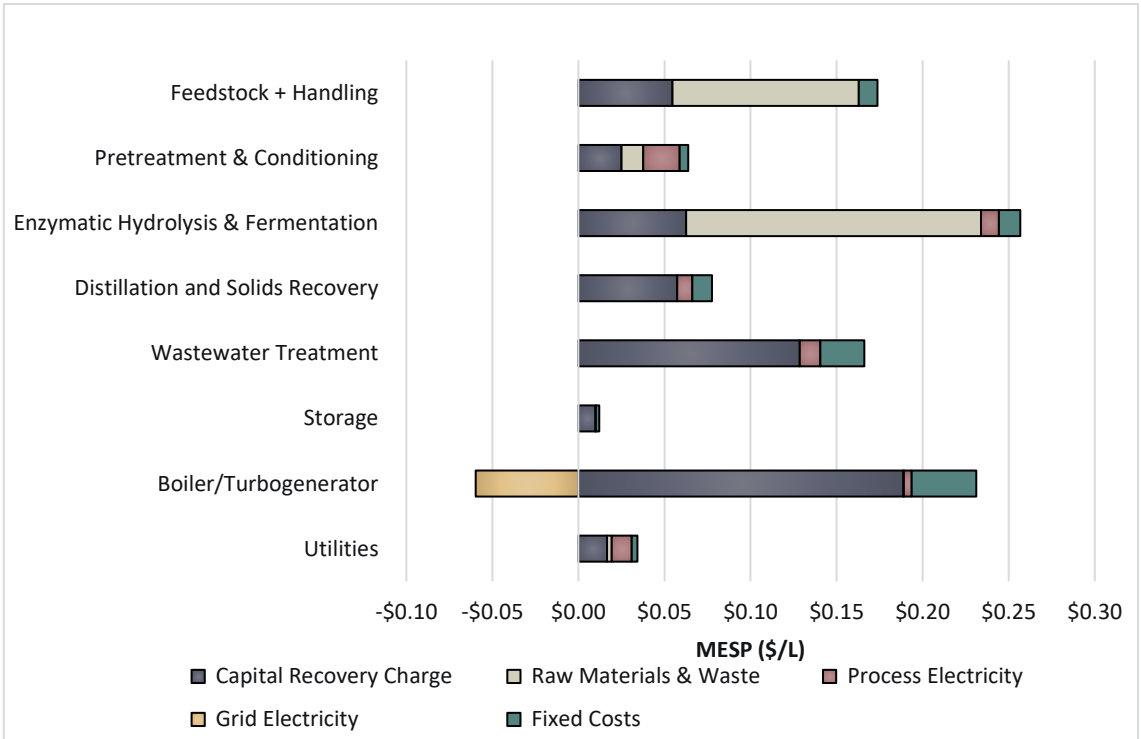


Figure 3.9. Cost contribution of various sections to the Minimum ethanol selling price (MESP) for the ethanol and electricity scenario.

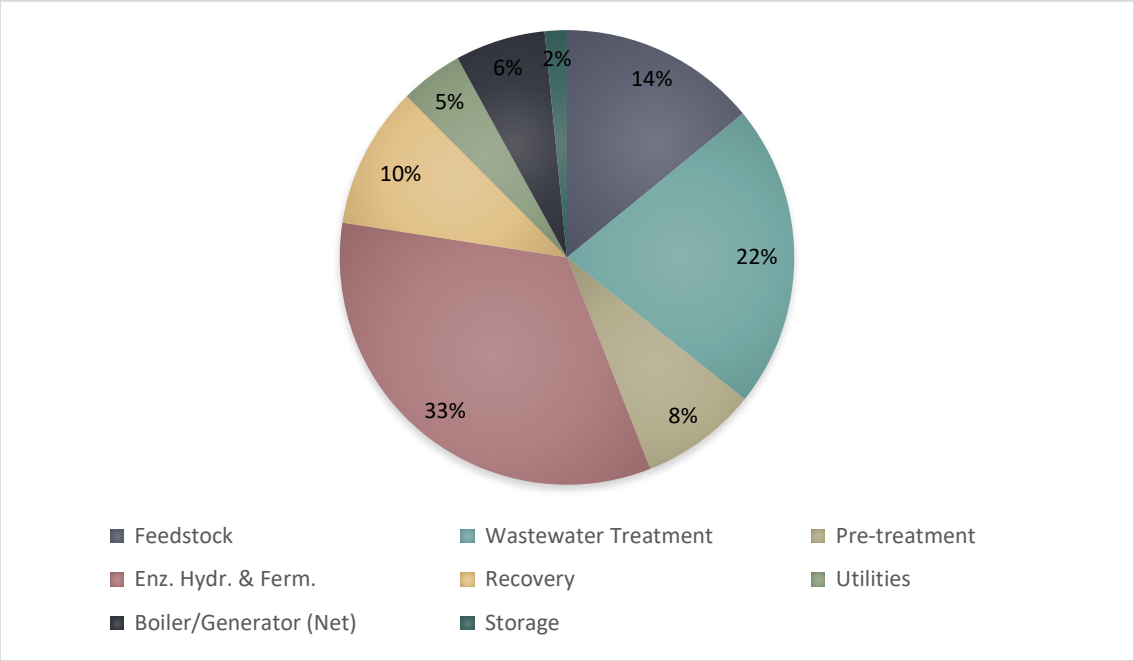


Figure 3.10. Percentage contribution of the different plant areas to the total cost for the ethanol and electricity scenario.

Compared with results obtained by Humbird et al. (2011), this process showed a greater impact of capital recovery charge on the overall process (61% of the costs vs 42%). This difference could be explained by lower variable costs associated with the LHW process (compared with the dilute acid pretreatment), by lower fixed costs for labor at the selected location and by plant capacity differences.

As expected the main contributors to variable cost were feedstock (14 cents/L) and enzymes (20 cents/L) (Sainz, 2009).

3.3.2. Effect of the use of hemicellulose

Figure 3.11 shows the MESP for different uses of the hemicellulose from the liquid fraction of the pretreatment. In the ethanol and electricity scenario, liquid and solids from pretreatment are both send to the fermentation process to produce ethanol. If both xylose and glucose are fermented to ethanol the MESP is considerably lower than a case where only glucose is fermented. If the plant is designed to ferment both xylose and glucose to ethanol, it is critical that the strain retains its xylose fermenting capability, as xylose not being fermented would increase the MESP from 0.89 to 1.14 $/L. Therefore, the development of robust microorganisms capable of working under industrial conditions and with high xylose-glucose fermenting capacity and viability is very important in a facility designed for the ethanol and electricity process.

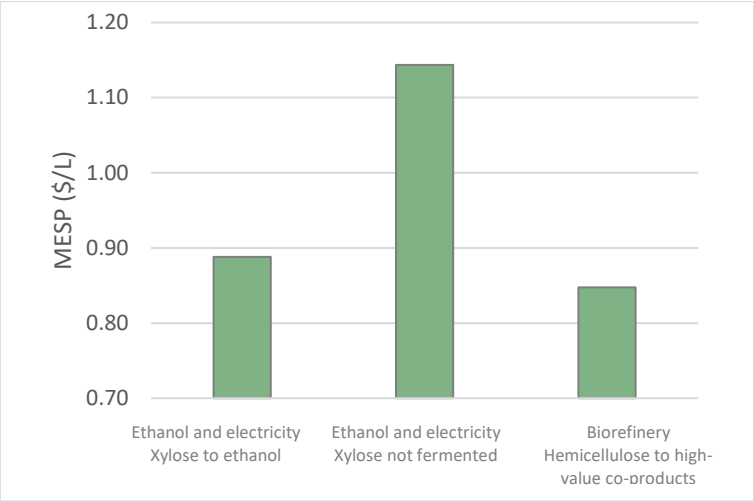


Figure 3.11. Minimum ethanol selling price (MESP) for different hemicellulose uses. Cases 1, 4 and 5.

In the biorefinery scenario (case 5), where the liquid fraction from pretreatment (containing most of the hemicellulose derivates) was used to obtain higher value products (furfural, acetic acid, and formic acid) instead of being fermented to ethanol, the MESP (0.85 $/L) was lower than for the ethanol and electricity process. These results indicate the success of the energy-driven biorefinery strategy, seeking to maximize the value obtained from co-products to sustain fuel/energy production (IEA, 2009).

Cost distribution for this scenario is shown on Figure 3.12, including costs for the area where the new co-products are produced. In this scenario, the increase in overall production cost was compensated by selling the co-products. As in all the previous cases, the steam and electricity generated by burning lignin were enough to satisfy process needs, and electricity surplus was sold to the grid.

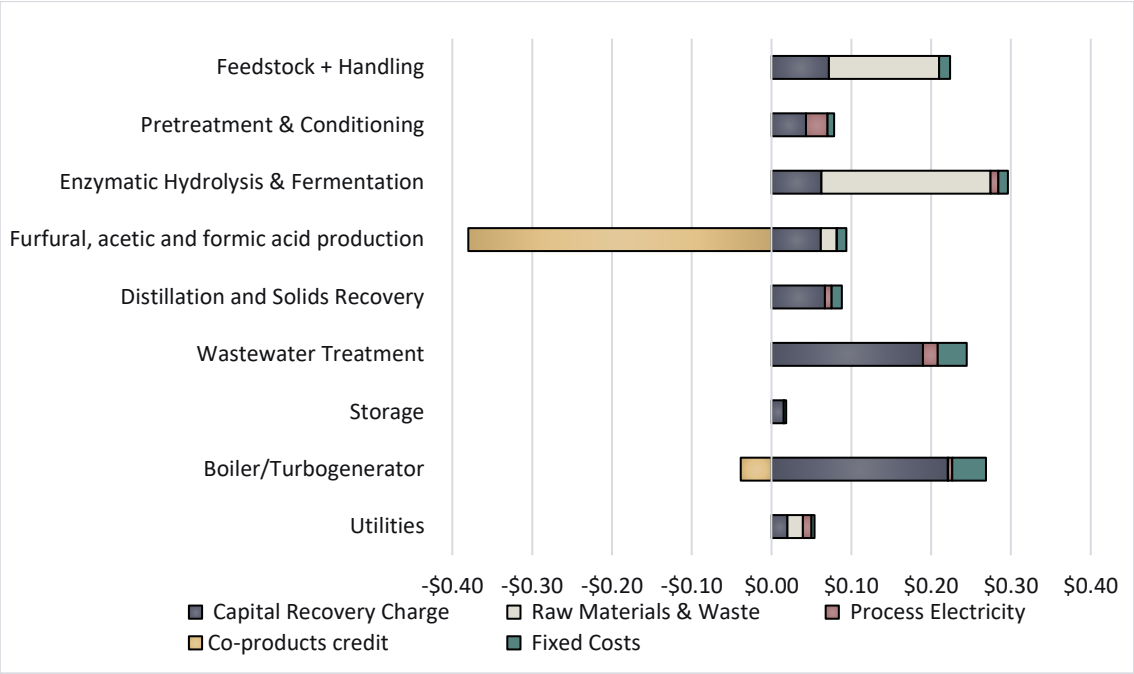


Figure 3.12. Cost contribution of various sections to the Minimum ethanol selling price (MESP) for the ethanol, furfural, acetic acid, formic acid and electricity base case scenario.

3.3.3. Effect of feedstock and enzyme costs

The main contributors to variable cost on the biorefinery scenario were also feedstock (0.14 $/L) and enzymes (0.20 $/L). Both materials have great uncertainties surrounding their price, and changes in their costs greatly affect MESP as shown in Figure 3.13.

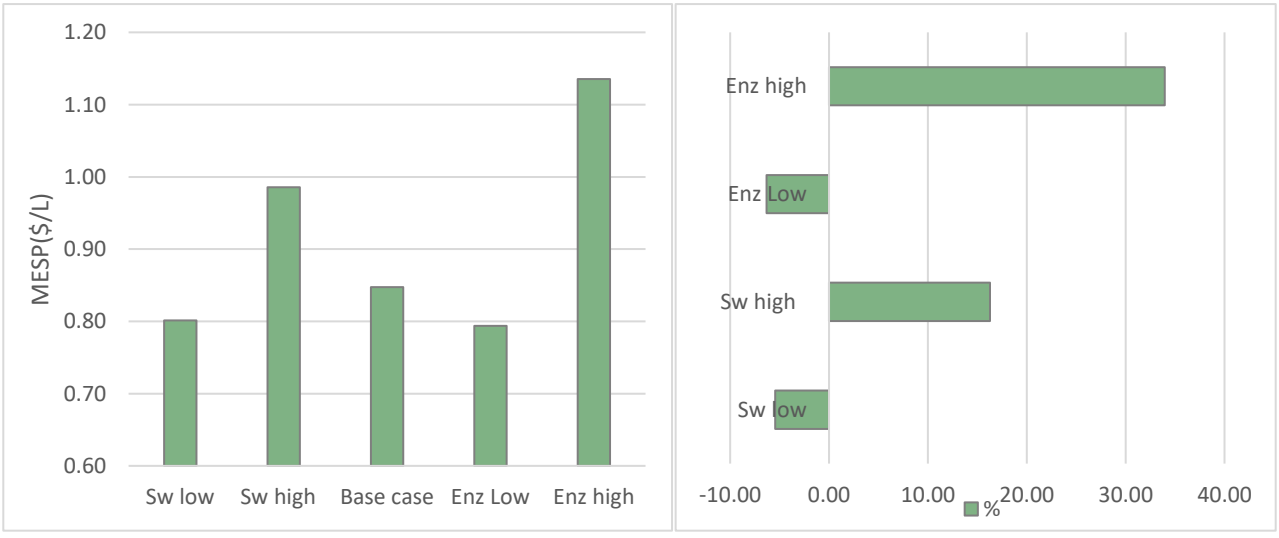


Figure 3.13. Minimum ethanol selling price (MESP) and MESP percental variations for different feedstock and enzyme costs. Sw low= 20$/dry ton of switchgrass; Sw high= 60$/dry ton of switchgrass, Enz low= 3$/kg protein; Enz high= 11 $/kg protein. Cases 5 and 6-9.

3.3.4. Effect of feedstock composition

As shown in Figure3.14 changes in glucan (related mostly to cellulose content) and xylan content (related to hemicellulose content) greatly affect MESP, while changes in lignin content do not have a significant effect. The impact on MESP of variations in the xylan content was greater than the impact of the same percentage of variation in the glucan content. This finding is consistent with the fact that hemicellulose is used to obtain high value co-products.

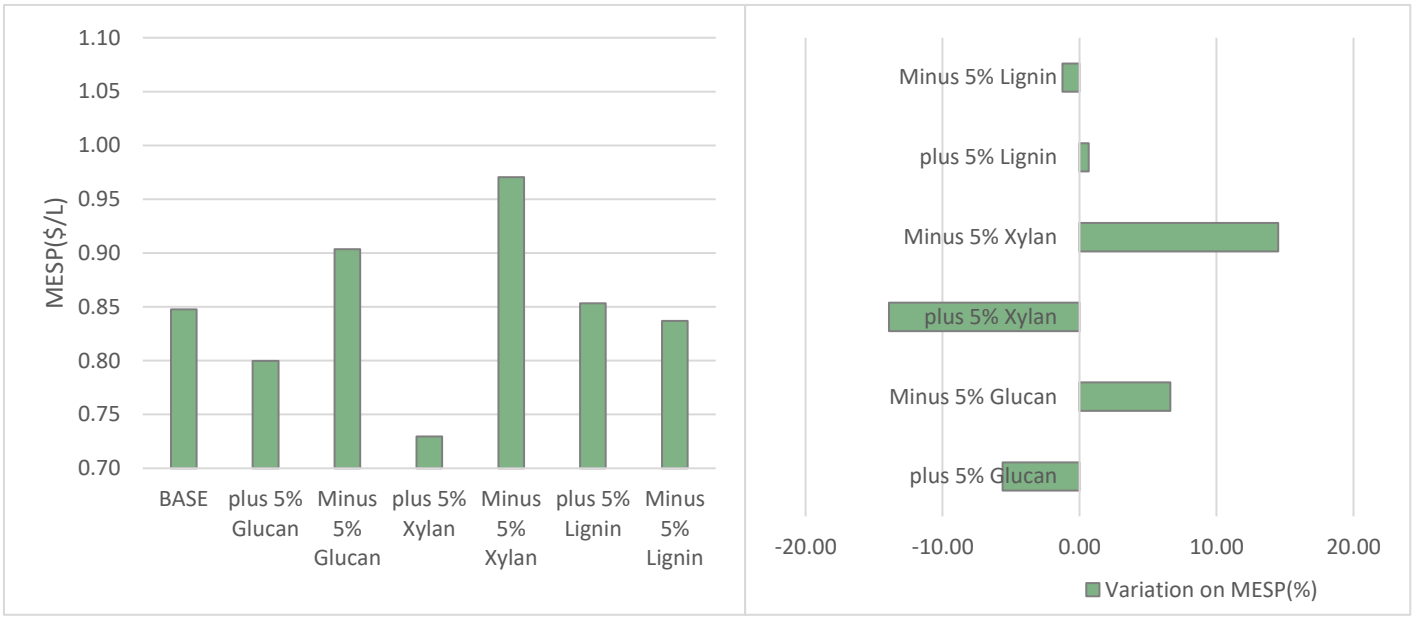


Figure 3.14. Minimum ethanol selling price (MESP) and MESP percental variations for different feedstock compositions. Cases 65 -70.

Figure 3.15 shows that even if the increase of xylan content is at the expense of glucan content, the economy of the process improves. This information is useful as a guideline for further research and development of crop enhancement, selection and field handling.

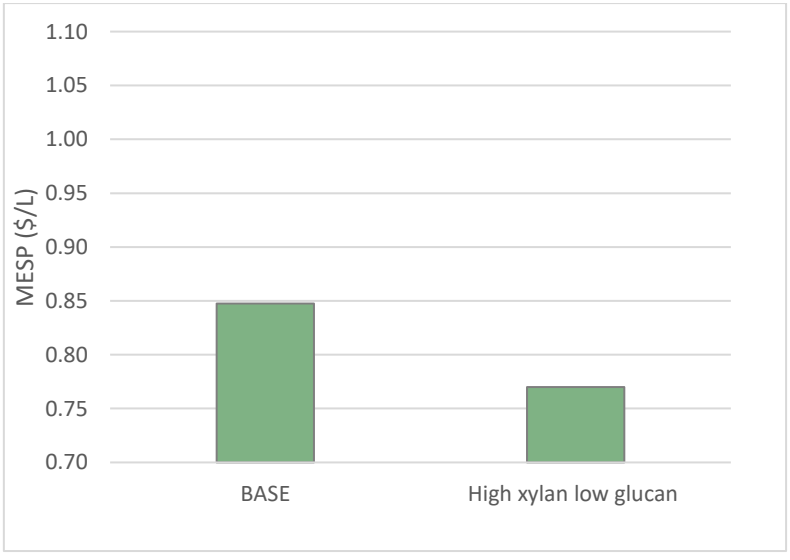


Figure 3.15. Minimum ethanol selling price (MESP) for two different feedstock compositions. Cases 5 and 71.

Degradation of xylan or glucan components during pretreatment into undesired molecules, would have a similar effect on process economics to the reduction in the content of said component in the feedstock. If the undesired molecules are inhibitors of the following process, yield reductions are added to the negative effect of less sugars available. Therefore, mild treatments like LHW in which monomer degradation is minimized and material losses are reduced, have an important effect on the economic aspects of a biorefinery, as previously reported (Alvira et al., 2010; Mosier et al., 2005).

3.3.5. Effect of hydrolysis parameters

Cellulose hydrolysis efficiency can greatly affect MESP as shown in Figure 3.16.

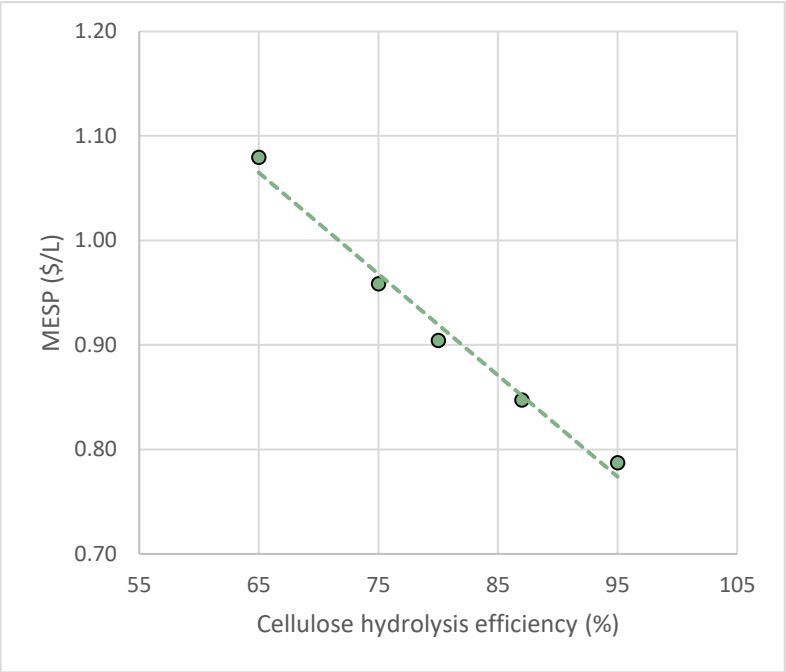


Figure 3.16. Minimum ethanol selling price (MESP) as a function of hydrolysis efficiency. Cases 5 and 26-29.

Due to the high-cost contribution of enzymes, a reduction of enzymes dosage while maintaining the same hydrolysis yield could lead to considerable savings in cost (Figure 3.17).

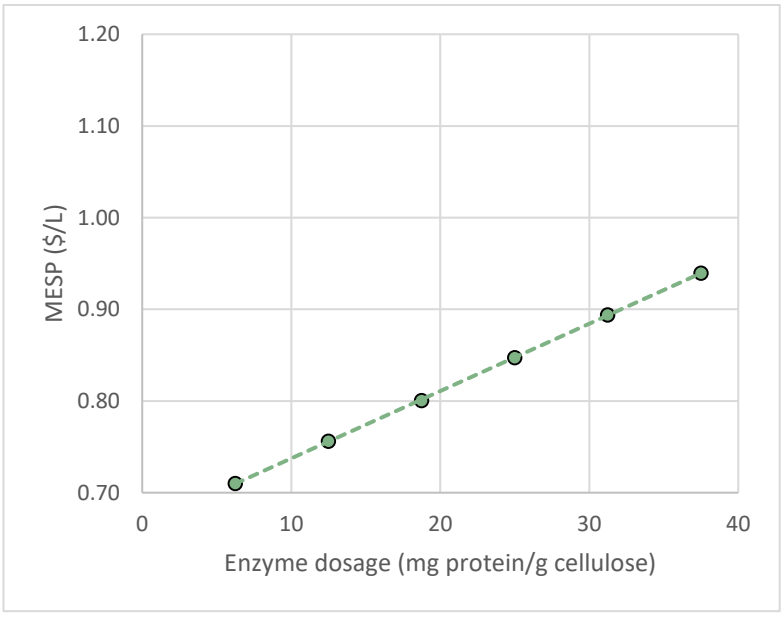


Figure 3.17. Minimum ethanol selling price (MESP) as a function of enzyme dosage. Cases 5 and 15-19.

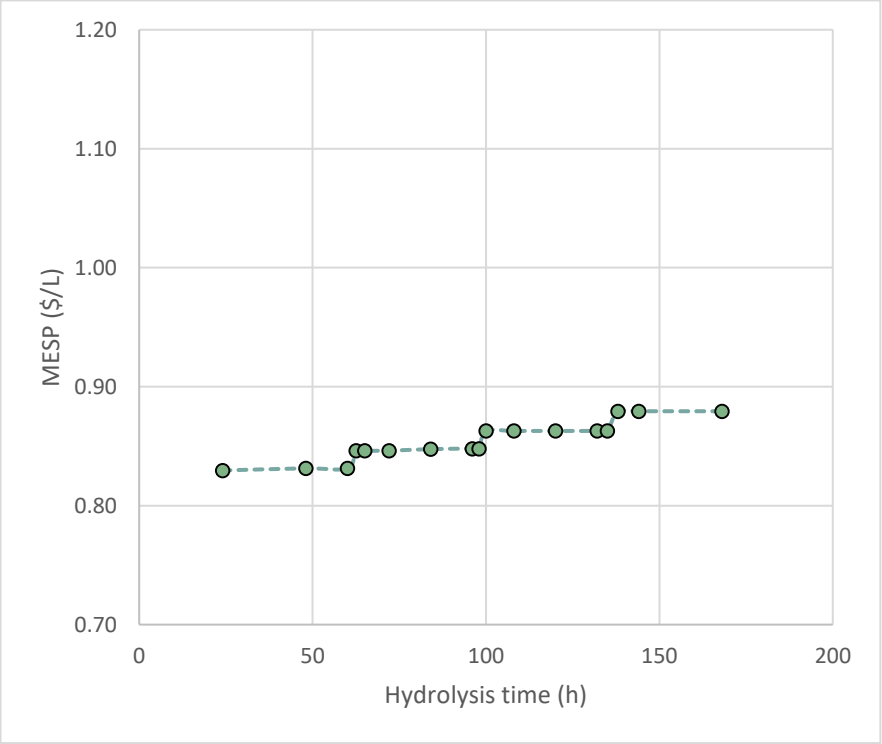


Figure 3.18. Minimum ethanol selling price (MESP) as a function of hydrolysis time. Cases 5 and 20-25.

As shown in Figure 3.18 changes in hydrolysis time had no effect in MESP for some periods of time, and sudden changes occurred around: 62, 100 and 138 h. This was due to the way in which hydrolysis and fermentation reactors were modelled. As hydrolysis and fermentation are batch processes, and the recovery stage is a continuous process, the number of reactors to assure the continuous downstream processing was calculated for the fixed reactor size. Changes in MESP were associated with a change in the number of vessels needed.

Surfactant addition could contribute to a higher enzymatic hydrolysis efficiency (Sipos et al., 2010). A polyethylene glycol (PEG) dosage of 0.05 g of PEG 6000/g of solids could lead to a 10% increase in hydrolysis efficiency according to Camesasca et al. (2015). With a price of 1.92 $/kg PEG, surfactant addition considerably increased the MESP. The high surfactant cost means that even if its addition increases hydrolysis efficiency from 65 to 87%, or if it decreases enzyme usage from 25 to 6.25 $mg_{protein}/g_{glucan}$, its use would not be beneficial from an economical point of view (as shown in Figure 3.19). Therefore, experimental studies on the use of surfactants are justified if the surfactant has a low cost, is applied in low dosages and achieves drastic changes in hydrolysis efficiency.

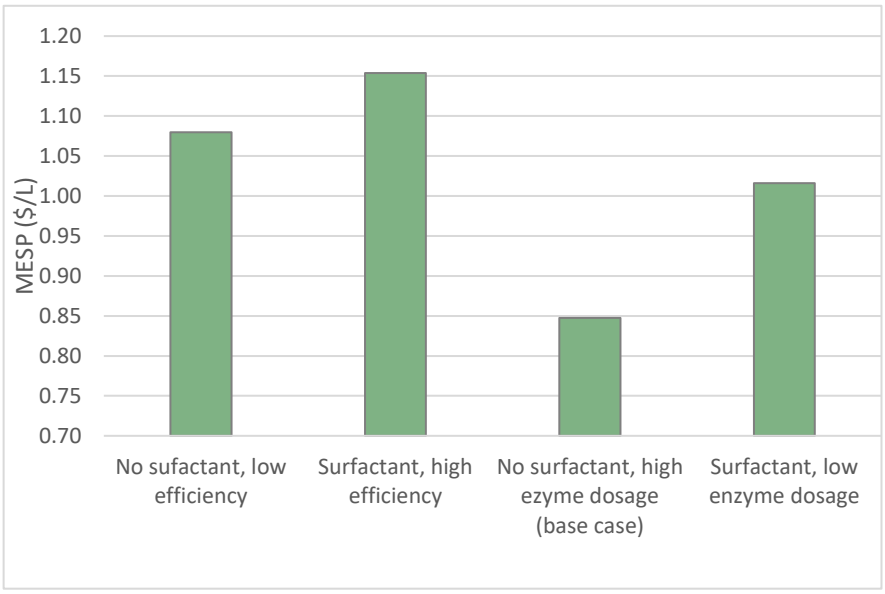


Figure 3.19. Minimum ethanol selling price (MESP) for cases relating to surfactant addition and hydrolysis. No surfactant, low efficiency = 65% efficiency (case 29); Surfactant, high efficiency = 87% (case 13); No surfactant, high enzyme dosage = 25 mg protein/g cellulose (case 5); Surfactant, low enzyme dosage = 6.25 mg protein/g cellulose (case 14).

3.3.6. Effect of fermentation parameters

Fermentation efficiency has a considerable impact on MESP (see Figure 3.20).

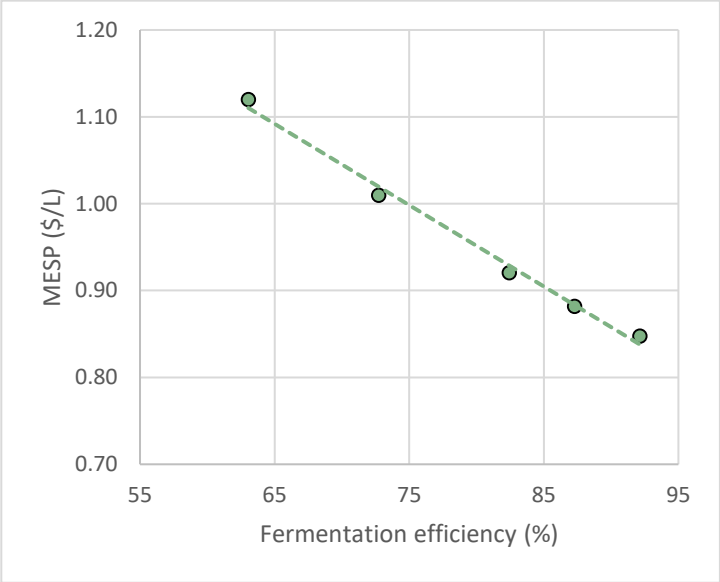


Figure 3.20. Minimum ethanol selling price (MESP) as a function of fermentation efficiency. Cases 5 and 34-37.

Nutrient addition can affect fermentation efficiency. As shown in Figure 3.21 the cost of adding a high amount of nutrients (twice the amount of the base case) would be compensated by the higher ethanol production resultant of increasing fermentation efficiency from 70 to 95%. Therefore, experimental studies to optimize fermentation efficiency and nutrient usage are desirable.

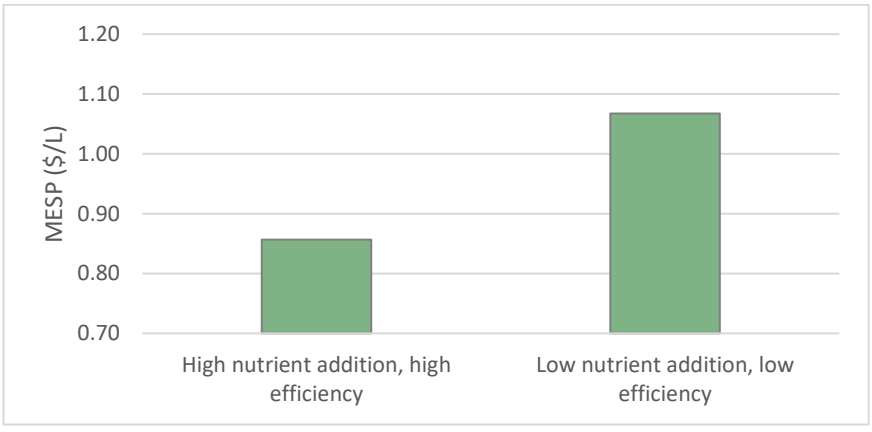


Figure 3.21. Minimum ethanol selling price (MESP) for cases relating to nutrient addition and fermentation. High nutrient addition (twice the base case amount for both inoculum and fermentation), high efficiency = 95% (case 11); Low nutrient addition (half the base case amount for inoculum and no addition in fermentation), low efficiency=70% (case 12).

Fermentation time had a similar effect to hydrolysis time on MESP. Changes occurred around: 50 and, 90 h (see Figure 3.22).

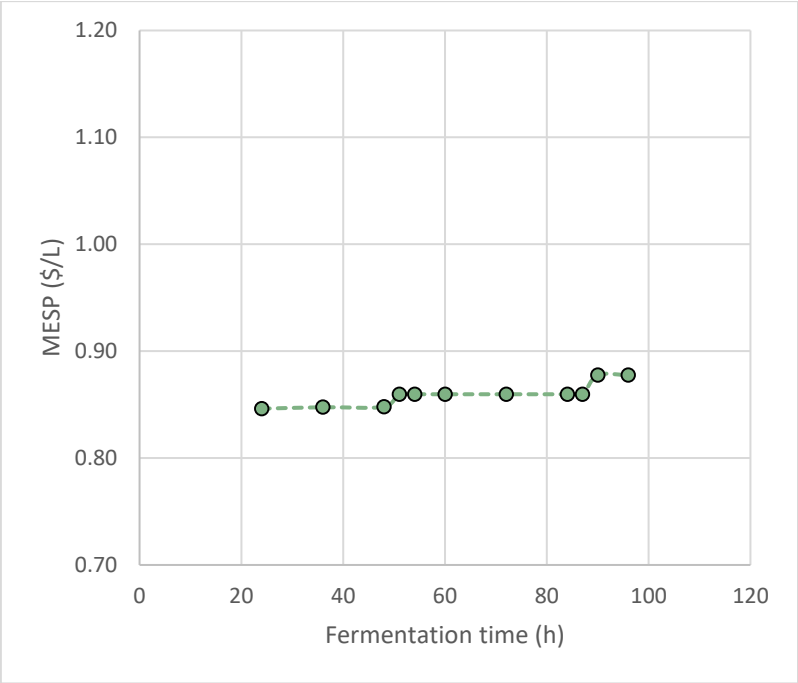


Figure 3.22. Minimum ethanol selling price (MESP) as a function of fermentation time.

3.3.7. Effect of solids content

As shown on Figure 3.23, solids content had an important effect on MESP values, the effect was higher at solids contents below 20%.

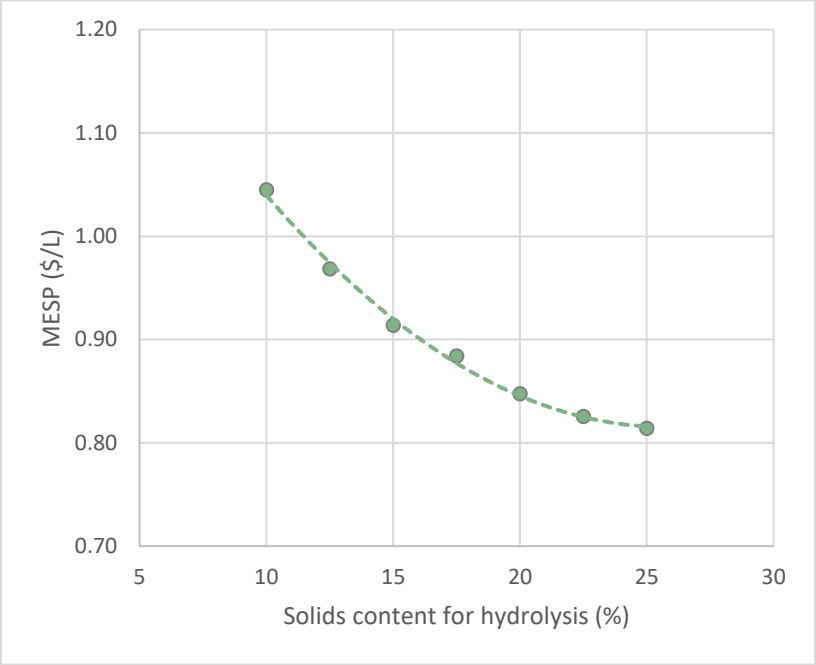


Figure 3.23. Minimum ethanol selling price (MESP) as a function of solids content at the beginning of enzymatic hydrolysis. Cases 38-42.

The parameters with higher impact on MESP were hydrolysis efficiency, fermentation efficiency, solids content and enzyme dosage.

Hydrolysis and fermentation efficiencies and solids content affected the ethanol concentration achieved after fermentation, and therefore, the energy and equipment needed for the recovery steps. This is consistent with results found by Humbird et al. (2010) for ethanol from corn stover with dilute acid pretreatment. Figure 3.24 shows the MESP values that would be obtained for different combinations of cellulose hydrolysis efficiency (H, %) and solids content (S, % w/w).

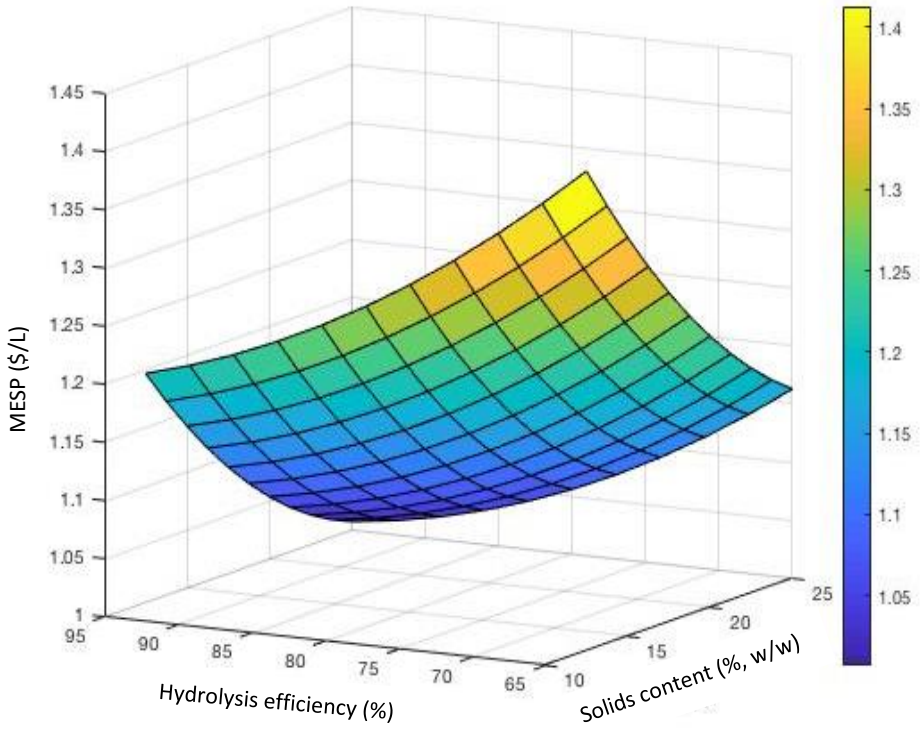


Figure 3.24. Minimum ethanol selling price (MESP) as a function of initial solids content and efficiency of the enzymatic hydrolysis stage. Cases 38-42, 44-58 and 60-64.

Solids content can affect both hydrolysis and fermentation efficiencies (Kristensen et al., 2009; Zhang et al., 2010), so these are not independent variables. As

Figure 3.25 shows working at a higher solids content (25% vs 10%) could be better from an economic point of view, even if it leads to low efficiencies (overall glucan to ethanol efficiency of 62%, vs 80% respectively). Therefore, it is important to have experimental data that relates hydrolysis and fermentation efficiencies with solids content, especially at high solids content (>15 %), for a more accurate economic and environmental evaluation.

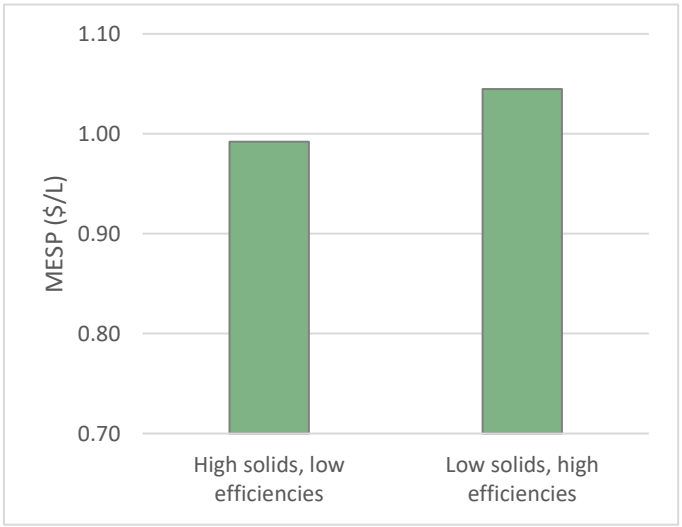


Figure 3.25. Minimum ethanol selling price (MESP) for different scenarios. High solids content (25%), low efficiencies (80% for hydrolysis and 78% fermentation) (case 59); Low solids content (10%), high efficiencies (87% for hydrolysis and 92% fermentation) (case 43).

3.3.8. Process variables affecting carbon dioxide emissions and energy balance on the industrial stage

Carbon dioxide emissions and energy consumption/generation, as well as the use of chemicals and enzymes, are some of the aspects of the industrial stage that can affect the environmental impact of the entire process. The information on which operating conditions have an effect on these values contributes to select the conditions that should be studied on the environmental evaluation.

The material and energy balances obtained from the simulations for the aforementioned cases, include CO_2 equivalent emissions generated at the fermentation, wastewater treatment and heat and energy generation (boiler) processes. Net energy corresponds to the electric energy generated in Area 800 that was not used in the industrial process, and is therefore sold to the grid as a co-product. It does not consider the energy contained in the ethanol produced.

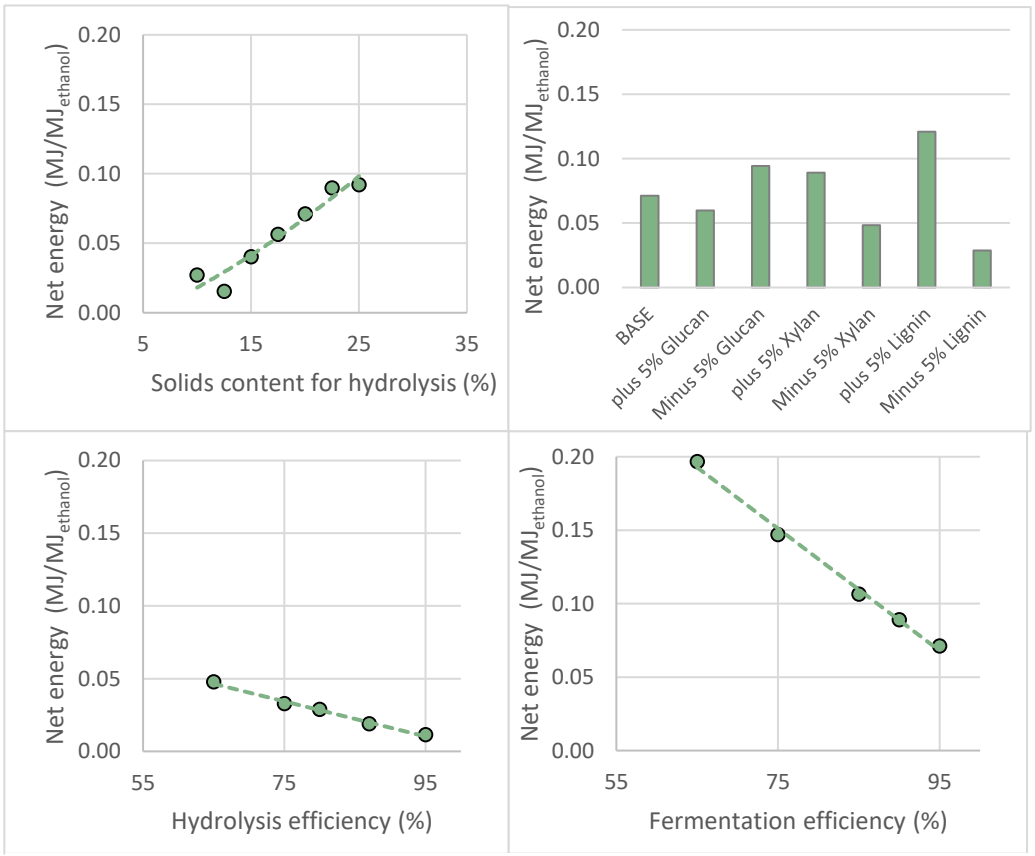


Figure 3.26. Net electric energy generated in the production process as a function of a) solids content for hydrolysis b) switchgrass composition, c) hydrolysis efficiency, d) fermentation efficiency.

Results show that all of the studied variables, solids content, hydrolysis and fermentation efficiency, and switchgrass composition affected the amount of net energy generated, which represents the electric energy generated after satisfying the needs of the industrial process (see Figure 3.26).

Carbon dioxide equivalent (CO_{2eq}) emissions were affected by hydrolysis and fermentation efficiencies, switchgrass composition, and by solids content only for low solids content (10-12.5 %) (see Figure 3.27).

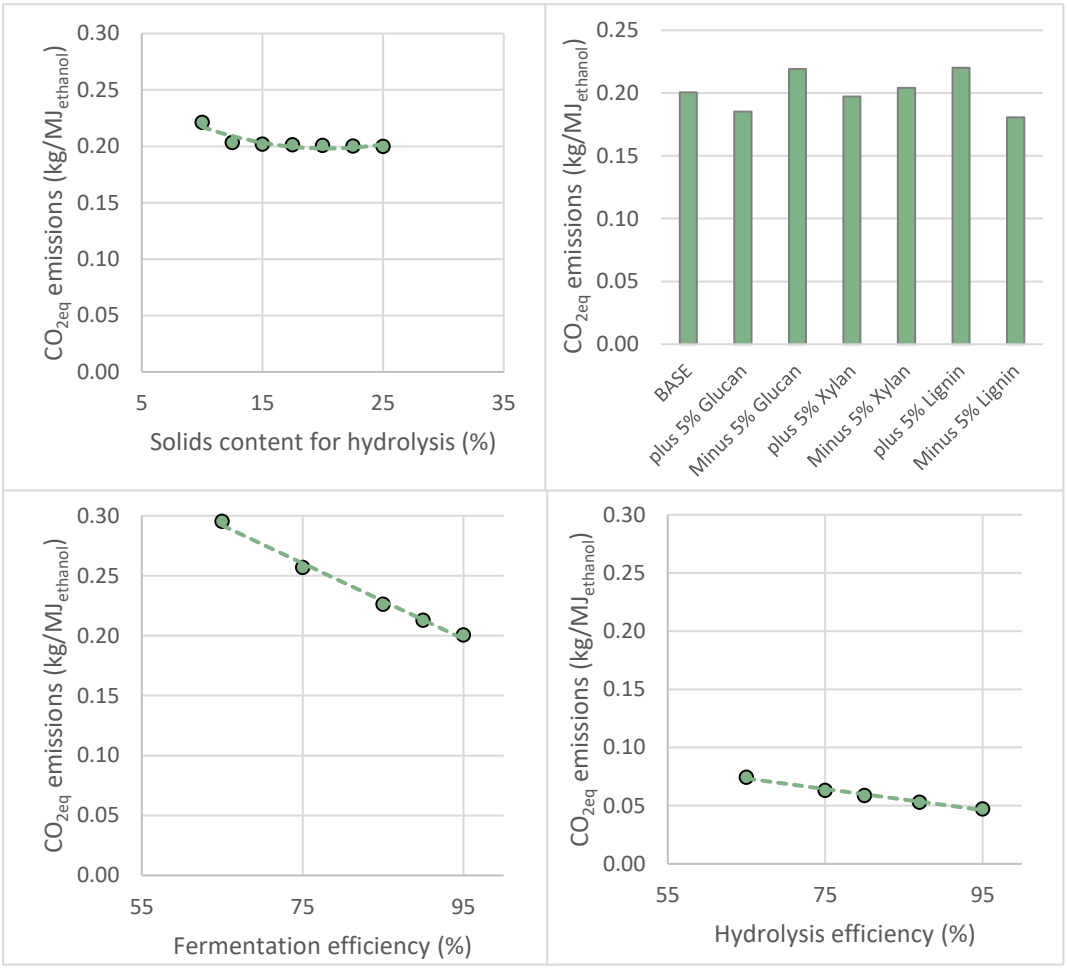


Figure 3.27. CO_{2eq} emissions as a function of a) solids content for hydrolysis, b) switchgrass composition, c) hydrolysis efficiency and d) fermentation efficiency.

Consequently, variations in solids content, hydrolysis and fermentation efficiency and switchgrass composition were taken into account on the environmental evaluation (see Chapter 5).

3.4. Chapter conclusions

The production cost obtained for ethanol (MESP) ($ 0.89/L) in a facility producing only ethanol and electricity was within the expected price range for advanced alcohol fuels and is not competitive with oil at prices below 80 $/ barrel but could compete with oil prices above 100 $/ barrel.

Several factors affect the MESP, which should be considered in order to minimize cost:

- When the hemicellulose from the liquid fraction of pretreatment was used to obtain high-value products (furfural, acetic acid and formic acid), the MESP was lower than when it was used to produce ethanol. This configuration constitutes an effective energy-driven biorefinery strategy that seeks to maximize the value obtained from co-products to sustain fuel/energy production.
- Variations of glucan and xylan content in switchgrass greatly affected MESP, while changes in lignin content did not have a significant effect. Xylan content variations had a higher effect, which is consistent with its beneficial conversion into high-value products. Therefore, crop improvement aimed to this biorefinery concept should aim to increase xylan content.
- Enzyme dosage, hydrolysis and fermentation efficiencies and solids content had a high impact on MESP. It is important to have experimental data that correlates hydrolysis and fermentation efficiencies with solids content, and enzyme dosage (for hydrolysis efficiency), to obtain more reliable economic and environmental conclusions.

The amount of net electric energy generated (electric energy generated minus energy consumed in the process) and GHG emissions (CO_{2eq}) are important aspects for the environmental evaluation of the production process. The amount of net energy generated was affected by solids content, hydrolysis and fermentation efficiency and switchgrass composition, while GHG emissions (CO_{2eq}) were affected only by hydrolysis and fermentation efficiency and switchgrass composition. Variations in these parameters should be analyzed in the life cycle assessment.

Chapter 4: Experimental study of the cellulose enzymatic hydrolysis at high solids content

"Look deep into nature, and then you will understand everything better"

Albert Einstein

4.1. Introduction

Enzymatic hydrolysis is a very important step in ethanol production. Fermentable sugars concentration achieved at this stage determines the maximum ethanol concentration that can be obtained by fermentation. The minimum ethanol concentration for an efficient distillation is 4% w/w. It can be reached for most lignocellulosic materials (considering current yields) for an initial hydrolysis solids content of 20% w/w. Solids loading higher than 15% w/w are referred to as high solids loading (Modenbach & Nokes, 2013). High solids content can lead to improved overall productivity, process intensification, smaller volumes transported, lower capital and operating costs, and less energy consumption on product separation (Chen & Liu, 2017; Dutta et al., 2009). Working at high solids contents also presents some challenges as: inhibition effects from pretreatment degradation products on hydrolysis and fermentation, sugar inhibition effects on hydrolysis, poor mass transfer due to low free water content (decreasing the conversion of biomass to ethanol) and process handling issues due to viscosity (Chen & Liu, 2017; Kristensen et al., 2009). Inhibition from degradation product can be minimized through detoxification or through the washing of the pretreated solids. The effects of sugar inhibition can be reduced by enzyme synergism (addition of xylanase to cellulase mix) or through a fed-batch feeding strategy. Mass transfer and high viscosity problems can be mitigated through novel reactor designs with a focus on the stirring methods (Chen & Liu, 2017). Roche et al. (2009) studied agitation methods for the enzymatic hydrolysis at high solids content (using corn stover). They found that roller bottle reactors (RBRs) are laboratory-scale reaction vessels that can provide adequate mixing for enzymatic saccharification at high-solids biomass loadings, showing considerably better results than orbital shakers for solids content up to 30%.

In order to obtain accurate results for ethanol production from biomass TEA and LCA studies, it is necessary to understand how cellulose conversion yields vary as a function of solids loading, enzyme dosage and other operating variables (Humbird et al., 2010).

Humbird et al. (2010) studied corn stover hydrolysis varying solids content (10-30%), enzyme loading (10-40 $mg_{protein}/g_{cellulose}$) and temperature (42-58ºC). They found that an optimal economic solids content could be found if there was a reduction of hydrolysis efficiency due to high solids content. This optimal value depends on enzyme cost and dosage. Kadhum et al. (2017) found that high solids content (45%) lead to high ethanol production for wheat straw and corn stover, but his TEA study used hydrolysis efficiencies obtained at different enzyme dosage, from other authors, and under different conditions.

The majority of hydrolysis studies involving switchgrass hydrolysis have been performed to evaluate the effect of different pretreatments or enzyme loadings, and have been conducted

at low solids content of 1% glucan (Frederick et al., 2016; Li et al., 2013; Li & Zheng, 2018; Soares Rodrigues et al., 2016; Wyman et al., 2011) following protocols suggested by NREL (Dowe & Mcmillan, 2008; Resch et al., 2015). Other works evaluated the effects of genetic modification of switchgrass, through simultaneous saccharification and fermentation at a solids content of 15% (Fu et al., 2011; Yee et al., 2012). Li et al. (2013) studied switchgrass hydrolysis working at 10% solids loading, and 60 $mg_{protein}/g_{glucan}$ in a 2 L fermenter, with ionic liquid pretreated switchgrass obtaining a hydrolysis efficiency of 99.8% at 72 hours. Ioelovich & Morag (2012) analyzed hydrolysis of dilute acid and hypochlorite-alkaline pretreatment at 5 and 20% solids content in a 2 L fermenter, with 5 FPU/g_{solids} of the enzyme complex (filter paper units [FPU] measure enzyme complex activity). They found that conversion and sugar concentration varied considerably between the two pretreatment methods.

In this chapter, some aspects of the cellulose enzymatic hydrolysis were studied for high solids content. A Response Surface Methodology with a Box-Behnken experimental design was used to analyze the effect of solids content, enzyme dosage, and xylanase substitution on the hydrolysis of the cellulosic fraction of LHW pretreated switchgrass. Empirical correlations representing hydrolysis efficiency and glucose concentration as a function of solids content, enzyme dosage and xylanase substitution were obtained. Experimental results were integrated with the biorefinery techno-economic model previously presented, to have a more realistic assessment of the impact of solids content on process economics. Results from this chapter were also used on the life cycle analysis model to evaluate the impact of solids content on environmental parameters (presented in Chapter 5).

4.2. Materials and Methods

4.2.1. Feedstock

Switchgrass (*Panicum virgatum)* (Alamo variety) was provided by the *Departamento de Producción Vegetal, EEMAC, Facultad de Agronomía de la Universidad de la República*, cultivated at INIA Glencoe, Paysandú, Uruguay (Siri Prieto et al., 2017). Switchgrass was received dried (8.8 ± 0.5 % water content) and ground. Characterization was performed using analytical techniques described below.

4.2.2. Pretreatment

Liquid hot water (LHW) pretreatment took place in a 2 L Parr reactor containing 1 kg of the material for pretreatment. The pretreatment experimental conditions, selected based on the work of Tao et al. (2011), were 15% w/w solids content at 200 ºC for 5 minutes. Liquid and solid fractions were separated by filtration. Characterization of the pretreated material was performed using analytical techniques described below. The material and the reactor can be observed in Figure 4.1.

Figure 4.1. Switchgrass inside the Parr reactor before pretreatment.

4.2.3. Enzymatic hydrolysis

Study of solids washing effect

Solid fraction of the pretreated switchgrass was washed by immersion in 10 g of distilled water per gram of solids and separated of the washing water through filtration.

This washing step was repeated up to 4 times in order to determine the number of washes necessary. Solids characterization was performed to determine structural carbohydrates, lignin, ash and water content using techniques described below.

Hydrolysis assays were performed for the solids with different numbers of washing steps, in 50 mL falcon tubes. The tubes were filled with 20 g of the hydrolysis medium containing 17.5% w/w washed solids. Cellulase complex was added in a ratio of 50 $mg_{protein}/g_{glucan}$, and the total mass was completed with buffer citrate at a pH of 4.8. Hydrolysis took place in a bottle roller (Thermo Fisher Scientific, Inc, MA, USA) operated at 5 rpm, located in an incubator set at 50 °C, as can be seen in Figure 4.2. The whole content of the tubes was taken after 72 hours and used for analysis. The enzymes complex used was Cellic® CTec2 donated by Novozymes with an activity of: 103 ± 10 FPU/mL and a protein content of 247 ± 3 mg/mL.

Figure 4.2. Bottle roller containing falcon tubes with hydrolysis mix.

Study of the effect of initial pH for different solids content

Solid fraction of the pretreated switchgrass was washed three times by immersion using 10 g of distilled water per gram of solids and separated of the washing water through filtration.

Hydrolysis assays were performed with the addition of different pH buffers for 3 different solids contents, in 50 mL falcon tubes. The tubes were filled with 20 g of the hydrolysis medium containing 15, 20 and 25 % w/w washed solids. Cellulase was added in a ratio of 50 $mg_{protein}/g_{glucan}$ (20 FPU/g_{glucan}), and the total mass was completed with a mix of buffer citrate

and sodium hydroxide at the selected pH values (see Table 4.1). Hydrolysis took place in a bottle roller (Thermo Fisher Scientific, Inc, MA, USA) operated at 5 rpm, located in an incubator set at 50ºC. The whole content of the tubes was taken after 72 hours and used for analysis.
The enzymes complex used was Cellic® CTec2 donated by Novozymes with an activity 103 ± 10 FPU/mL and a protein content of 247 ± 3 mg/mL.

Table 4.1. Values of pH of the buffer solutions added in the assays for different solids content.

Solids content (%)	pH
15	4, 4.8, 5, 6, 7
20	4, 5, 5.5, 6, 7
25	4, 5, 6, 7

Study of the effect of solids content, enzyme dosage and substitution of cellulase by xylanase

A Box-Behnken design was used to select the assays necessary to evaluate the effect of solids content, enzyme dosage and substitution of cellulase by xylanase. The ranges and values of these parameters were chosen based on TEA results and on the literature review.

Solid fraction of the pretreated switchgrass was washed three times by immersion in 10 g of distilled water per every gram of solids and separated of the washing water through filtration.

Hydrolysis assays were performed in 50 mL falcon tubes. The tubes were filled with 20 g of the hydrolysis medium, containing solids, cellulase and xylanase complex, and a mix of buffer citrate and sodium hydroxide at the pH values found optimal for each solids content (4.8 for 15% w/w and 6 for both 20 and 25% w/w). A summary of the conditions of the different assays can be found in Table 4.2. Hydrolysis took place in a bottle roller (Thermo Fisher Scientific, Inc, MA, USA) operated at 5 rpm, located in an incubator set at 50ºC. Assays were made by triplicate and the whole content of the tubes was taken after 24, 48 and 168 hours, and used for analysis. Figure 4.4 and Figure 4.3 show the falcon tubes containing switchgrass hydrolysate after 24 h of enzymatic hydrolysis, and the liquid and solid fractions obtained after 48 h of enzymatic hydrolysis, for different solids content and enzyme dosages, respectively. Enzyme dosage was calculated in terms of $mg_{protein}/g_{glucan}$. For xylanase replacement, the total dosage ($mg_{protein}/g_{glucan}$) remained the same but a percentage of the protein (weight based) was replaced by the xylanase complex (instead of the cellulase complex). A new batch of Cellic® Ctec2 cellulase complex, purchased from Sigma Aldrich with an enzyme activity of 148 ± 10 FPU/mL and a protein content of 344 ± 2 mg/mL, and Cellic® HTec2 xylanase complex, with a protein content of 320 ± 2 mg/mL, were used. Selected enzyme dosage for the assays were: 4, 17 and 30 FPU/g glucan, which

corresponded to enzyme contents of 10, 40 and 70 $mg_{protein}/g_{glucan}$ respectively. The final time was determined as the time at which hydrolysis efficiency was higher than 95 % or 168 hours when this value was not achieved. An extra assay was performed in 250 mL Nalgene® centrifuge bottle 3120 (Thermo Fisher Scientific, Inc, MA, USA) at 20 % solids content and 40 $mg_{protein}/g_{glucan}$, samples were taken regularly to follow the kinetics of the hydrolysis reaction.

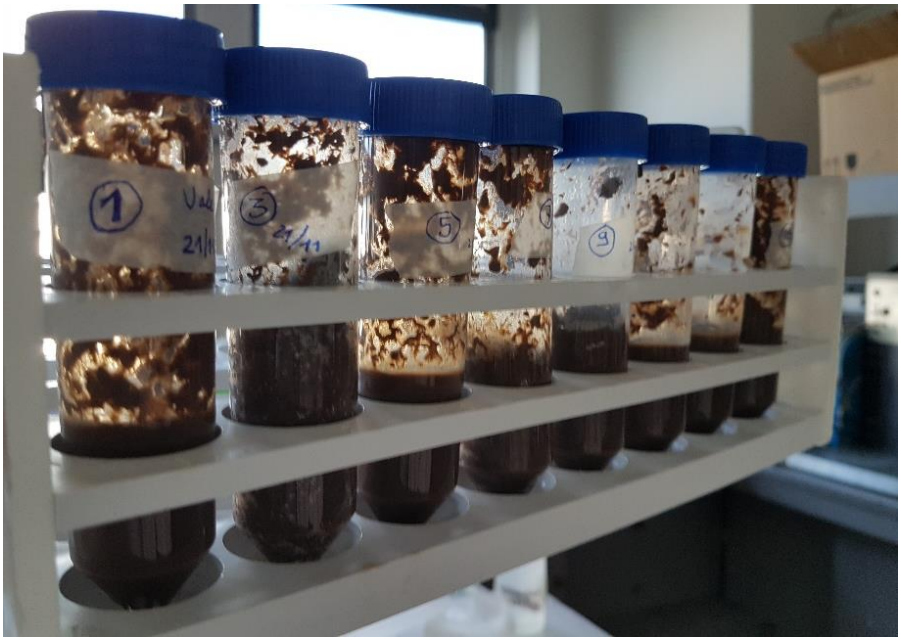


Figure 4.4. Falcon tubes containing switchgrass hydrolysate after 24 h of enzymatic hydrolysis for different solids content and enzyme dosages (Box-Behnken assays)

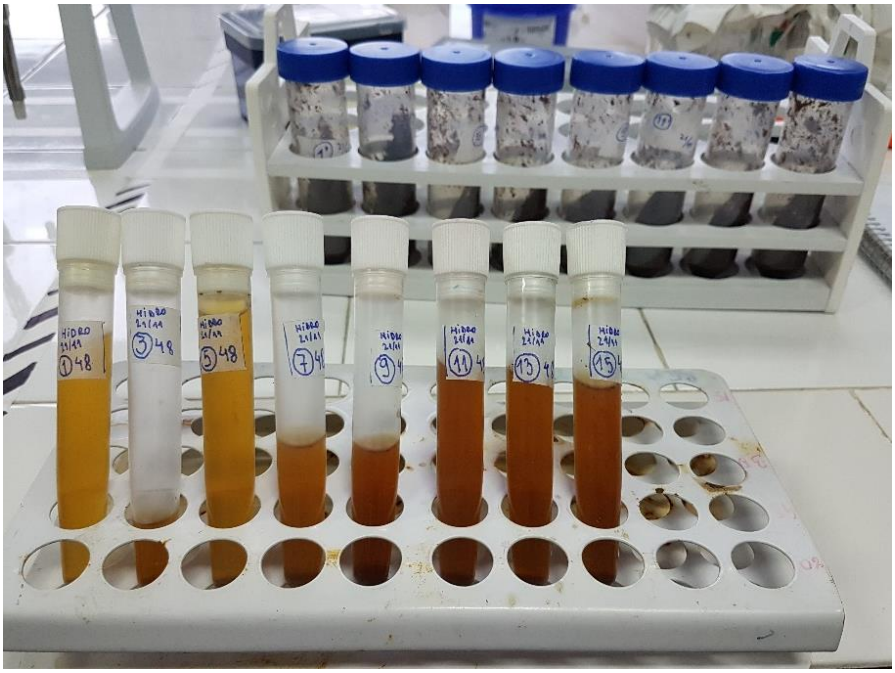


Figure 4.3. Liquid and solid fractions obtained after 48 h of enzymatic hydrolysis for different solids content and enzyme dosages (Box-Behnken assays)

The experimental results were analyzed applying a lineal regression analysis using the InfoStat software. ANOVA test was used to statistically evaluate the effects of the solids loading (S, % w/w), the enzyme dosage (E, $mg_{protein}/g_{glucan}$), and xylanase substitution (X, % $w/w_{protein}$) respectively, on glucose concentration (G, g/L), and on hydrolysis efficiency (H, %). Effects were considered significant when $p < 0.05$. The variables studied were normalized and coded in the following form:

$$x_1 = \frac{S-20}{5} \quad (4.1) \qquad x_2 = \frac{E-40}{30} \quad (4.2) \qquad x_3 = \frac{X-10}{10} \quad (4.3)$$

Table 4.2. Solids content, enzyme loading and xylanase substitution values for the different assays conducted.

Assay number	S (%)	x_1	E ($mg_{protein}/g_{glucan}$)	x_2	X (% $w/w_{protein}$)	x_3	pH
1	15	-1	10	-1	10	0	4.8
2	15	-1	70	1	10	0	4.8
3	25	1	10	-1	10	0	6
4	25	1	70	1	10	0	6
5	15	-1	40	0	0	-1	4.8
6	15	-1	40	0	20	1	4.8
7	25	1	40	0	0	-1	6
8	25	1	40	0	20	1	6
9	20	0	10	-1	0	-1	6
10	20	0	10	-1	20	1	6
11	20	0	70	1	0	-1	6
12	20	0	70	1	20	1	6
13	20	0	40	0	10	0	6
14	20	0	40	0	10	0	6
15	20	0	40	0	10	0	6

Experimental results were used to obtain empirical correlations that represent glucan hydrolysis yield and glucose concentration as a function of solids content, enzyme dosage, and xylanase substitution.

The selected parameters (solids content and total enzyme dosage) and the experimental results (hydrolysis efficiency and fermentation time), were used in the techno-economic model previously developed (case 5) to determine the minimum ethanol selling price for each assay. For these new models the protein content and specific activity of the enzyme were modified to match the characteristics of the Cellic® Ctec2 enzyme used. Consequently, a more realistic assessment of the impact of solids content on process economics was performed. The material

and energy balances and discount cash flow for these cases can be seen in Appendix A and they are identified as the case "BB" and the assay number (e.g. BB12).

4.2.4. Analytical methods

Particle size distribution of raw material

Dried and ground switchgrass was sieved through a set of mesh with opening size ranging from 0.074 and 1 mm. The fraction of switchgrass retained by each mesh was weighted on an analytical scale.

Water content

For the characterization of switchgrass before and after pretreatment (for the solid fraction) water content was determined according to NREL's protocol "Determination of Total Solids in Biomass" (Sluiter et al., 2008), by drying at 105 ºC until constant weight.

The water content determination prior to hydrolysis (in order to match the desired solids content in the assay) and after hydrolysis (in order to calculate the exact amount of hydrolyzed sugars) were determined using a Radwag MA 50.R moisture analyzer.

Ash

Ash content was determined according to NREL's protocol "Determination of Ash in Biomass" (Sluiter et al., 2004) by incinerating samples in a muffle furnace at 575 ºC until constant weight was obtained.

Extractives

Extractives were determined following NREL's protocol for "Determination of Extractives in Biomass" (Sluiter et al., 2008) with 8 and 16 hours of reflux with water and ethanol, respectively using a Soxhlet apparatus.

Determination of structural carbohydrates and lignin

Structural carbohydrates and lignin on switchgrass were determined for the extractive-free biomass according to NREL's protocol "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter et al., 2012). After LHW pretreatment, structural carbohydrates and lignin in the solid fraction were determined following the same protocol, while the liquid fraction was analyzed following NREL's "Determination of sugars by-products and degradation in liquid" protocol (Sluiter et al., 2008). The analysis involves a hydrolysis of the sugar with sulfuric acid at 121ºC. Acid soluble lignin is determined through UV spectroscopy at 320 nm and insoluble lignin by incineration at 575 ºC of the solid residue remaining after acid hydrolysis.

Determination of sugars, acids, and ethanol

Cellobiose, glucose, xylose, mannose, arabinose, glycerol, acetic acid, HMF, furfural, and ethanol were determined by high performance liquid chromatography (HPLC). A Shimadzu

chromatograph with a refraction index detector (RID-10A), and a Biorad Aminex HPX-87H column, was used. Method conditions selected were: 0.3-0.6 mL/min of 0.005 M sulfuric acid as mobile phase at 35ºC.

Samples were pretreated before being analyzed. When necessary, protein precipitation was done through the addition of 120 µL sulfosalicylic acid for 10 mL of sample. Samples were centrifuged, and the supernatant was separated. All samples were filtrated through a 0.22 µm Millipore filters. Dilutions were prepared using the mobile phase, and the pH on concentrated samples was previously adjusted to 1-3 value.

Determination of enzyme activity and protein content

Enzyme activity was determined according to NREL's protocol "Measurement of Cellulase Activities"(Adney et al., 2008).

Protein content was determined through the MicroBCA method, according to the manufacturer's directions (Micro BCA Protein Assay Kit, Thermo Scientific).

4.2.5. Calculations

Removed fraction (% w/w): total solids, glucan, xylan or lignin fraction removed from the solids by the pretreatment.

$$Component\ removal\ from\ solids\ (\%) = \frac{\mathrm{w}_1 \times \mathrm{f}_{x1} - \mathrm{w}_2 \times \mathrm{f}_{x2}}{\mathrm{w}_1 \times \mathrm{f}_{x1}} \times 100 \qquad (4.4)$$

$$Component\ recovery\ (\%) = \frac{w_2 \times f_{x2} + L_2 \times f_{Lx2}}{w_1 \times f_{x1}} \times 100 \qquad (4.5)$$

w_1, w_2: solids before and after pretreatment (solids residue) respectively (g)

f_{x1}, f_{x2}: fraction of the component of interest before and after pretreatment respectively. Includes the derived monomer (e.g. xylose for xylan)

L_2: liquid after pretreatment (g)

f_{Lx2}: fraction of the component of interest in the liquid after pretreatment.

All weights and fractions expressed on dry basis.

Cellulose hydrolysis efficiency (%): fraction of the initial amount of cellulose that was hydrolyzed to glucose

$$Hydrolysis\ efficiency(\%) = \frac{[Glucose]_t \times V_L - [Glucose]_0 \times V_L - m_{ge}}{m_s \times f_{glucan} \times 1.11} \times 100 \quad (4.6)$$

[Glucose]: glucose concentration in the supernatant obtained at initial (0) and final (t) hydrolysis time (g/L).

V_L: volume of liquid after hydrolysis (L), determined by weighing the supernatant and water content in the solids.

m_{ge}: glucose added with the enzymes (g)

m_s: initial total solids (g)

f_{glucan}: initial glucan fraction of the total solids.

1.11: conversion factor from glucan to glucose.

Glucose productivity:

$$Glucose\ productivity\left(\frac{\mathrm{g}}{\mathrm{Lh}}\right) = \frac{[Glucose]}{\mathrm{t}} \qquad (4.7)$$

[Glucose]: glucose concentration in the supernatant obtained from hydrolysis (g/L).

t: hydrolysis time (h), defined as the time at which hydrolysis yields were over 95%, or the final time of the experience if 95% yields were not achieved.

4.2.6. Statistical analyses

Analysis of variance (ANOVA) studies were performed to analyze if differences found were statistically significant ($p \leq 0.05$). Fisher's least significant difference (LSD) comparisons were used when assessing the difference between different levels of the same parameter.

For the Box-Behnken design assays, linear regression with a quadratic model was implemented. Non-significant parameters to the regression model were eliminated (see Appendix D for further details). Models were considered a good fit when they had significant regression and non-significant lack of fit.

The software used in these analyses was InfoStat/Estudiantil versión 2018e (Di Rienzo et al., 2011). Results are shown in Appendix D.

4.2.7. Function optimization

Optimization was performed using MATLAB and Statistics Toolbox Release 2018a The MathWorks, Inc., Natick, Massachusetts, United States. Constrained nonlinear minimization was done through the "fmincon" solver from the Optimization Tool. Scripts for functions and constraints used can be found in Appendix D.

4.3. Results and discussion

4.3.1. Switchgrass: particle size and composition

Particle size distribution of the switchgrass (Alamo variety) grown experimentally as an energy crop is shown in Figure 4.5. The average particle size of most of the particles (almost 80%) was between 0.297 and 1.000 mm.

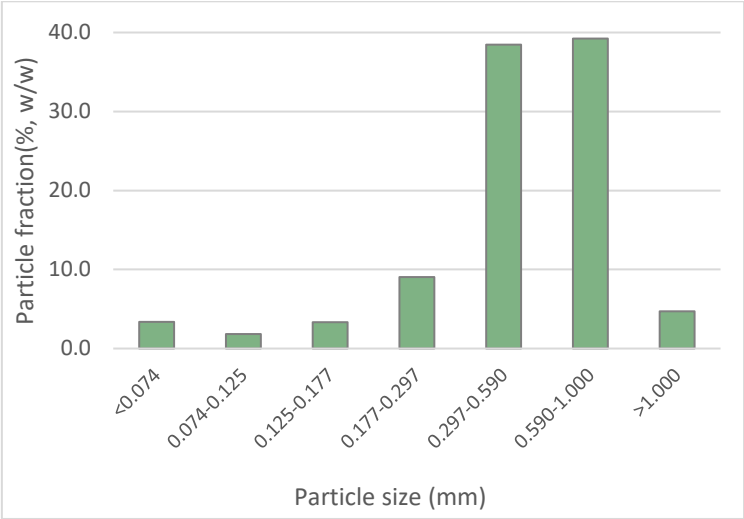


Figure 4.5. Switchgrass particle size distribution.

Switchgrass composition is shown in Table 4.3. This material had a high glucan and low xylan content compared with other studies for switchgrass, which reported values from 32 to 39% for glucan and 20 to 27% for xylan content (Cybulska et al., 2013; Esteghlalian et al., 1997; Garlock et al., 2011; Li et al., 2013a, 2013b; Yan et al., 2010). Differences in the feedstock could be due to differences in variety, location, and weather.

Table 4.3. Switchgrass composition.

Component	Percentage (%) dry basis		
Glucan	43.4	±	0.3
Xylan	16.5	±	1.3
Galactan	ND		
Arabinan	2.7	±	0.1
Mannan	ND		
Acetyl groups	2.5	±	0.3
Soluble lignin	1.2	±	0.1
Insoluble lignin	22.2	±	0.6
Ash	4.5	±	0.1
Extractives	8.5	±	0.9
Total	101.5	±	1.8

ND: Not detected. Detection limit $1x10^{-9}$ g/L

4.3.2. Liquid hot water pretreatment

Table 4.4 shows the recovery results of the different components of the biomass in the solid and liquid fraction, and its removal from the solids, after the LHW treatment. Values were similar to those calculated from data reported by Garlock et al. (2011) for switchgrass treated under LHW conditions at 200°C during 5 min (recovery: 89% glucan, 96.5% xylan, 79% lignin; removal: 14% glucan, 83% xylan, 2% lignin). Recovery percentages for LHW were higher than those found for other pretreatments of switchgrass, such as sulfuric acid-catalyzed clean fractionation (Cybulska et al., 2013), a desirable outcome in a biorefinery approach. The apparent increase in the amount of (103 % recovery) lignin could be due to the formation of "pseudo-lignin". Higher xylan removals while maintaining recoveries over 90% could be reached using a continuous process (Carvalheiro et al., 2016).

Table 4.4. Recovery and removal percentages for different components after LHW pretreatment.

Component	Recovery (%)	Removal (%)
Glucan	86 ± 3	18 ± 3
Xylan	96 ± 9	71 ± 13
Arabinan	90 ± 4	100 ± 4
Acetyl groups	54 ± 12	100 ± 12
Total lignin	103 ± 2	1 ± 2

The composition of the solid and liquid fractions are shown in Table 4.5 Concentrations of acetic acid, formic acid, HMF and furfural (components that could inhibit the following processes) were low, as expected from a low severity pretreatment. An increase in severity could lead to higher xylan removal and higher inhibitors formation. Liu et al. (2015) found that the same pretreatment (LHW, 200ºC) for 10 minutes instead of 5 for switchgrass, had a xylan removal of 93 %, and higher inhibitor concentrations of 8.4 g/L furfural, 0.4 g/L HMF and 5 g/L acetic acid.

Table 4.5. Composition of pretreated solids and hydrolysate obtained after LHW pretreatment.

	Pretreated solids		**Hydrolysate**	
	(g/100g $_{\text{pretreated solid}}$)	(g/100g $_{\text{dry switchgrass}}$)	(g/L$_{\text{hydrolysate}}$)	(g/100g $_{\text{dry switchgrass}}$)
Glucan	42.9 ± 1.2	35.6 ± 1.0	2.7 ± 0.1	1.6 ± 0.1
Glucose	ND	ND	0.1 ± 0.1	0.1 ± 0.1
Xylan	5.8 ± 0.6	4.8 ± 0.5	16.9 ± 0.9	9.9 ± 0.5
Xylose	ND	ND	1.8 ± 0.1	1.0 ± 0.1
Arabinan	ND	ND	2.0 ± 0.1	1.2 ± 0.1
Arabinose	ND	ND	1.7 ± 0.3	1.0 ± 0.2
Acetyl groups	ND	ND	3.3 ± 1.1	1.9 ± 0.6
Soluble lignin	1.1 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	0.9 ± 0.1
Insoluble lignin	27.6 ± 0.3	22.9 ± 0.2	-	-
Formic acid	ND	ND	0.8 ± 0.3	0.5 ± 0.2
HMF	ND	ND	<0.01	<0.006
Furfural	ND	ND	1.1 ± 0.6	0.6 ± 0.3
Ash	2.8 ± 0.1	2.3 ± 0.1	-	-

ND: Not detected. Detection limit $1x10^{-9}$ g/L

4.3.3. Study of solids washing effect

The solids obtained from LHW pretreatment were washed to remove soluble components that could inhibit the enzymatic hydrolysis (xylo-oligomers, phenols, tannic acids) and the glucose fermentation (acetic acid, furfural, and phenols) (Kim et al., 2011; Larsson et al., 1999). The washing step was repeated up to four times. Table 4.6 shows the content of acetic, acid, furfural and glucose at the end of hydrolysis. After two washes, furfural was completely removed, and acetic acid concentration was significantly reduced. Acetic acid and furfural concentrations obtained for the non-washed hydrolysates were lower than those found by Liu et al. (2015) after 4 washes with 500 mL. Acetic acid concentration reached in all cases was below the inhibitory value reported for microorganism fermentation of 100 mmol/L (equivalent to 6 g/L)(Larsson et al., 1999).

Table 4.6. Acetic acid, furfural and glucose after 72 h of hydrolysis for solids with different number of washing steps.

Washing steps	pH of water from the last washing step	Acetic acid (g/L)	Furfural (g/L)	Glucose (g/L)
0	-------	3.48 ± 0.10	1.26 ± 0.03	52 ± 4
1	3.9	1.70 ± 0.02	0.22 ± 0.01	72 ± 4
2	4.0	1.36 ± 0.02	ND	78 ± 1
3	4.3	1.11 ± 0.02	ND	79 ± 1
4	4.4	0.99 ± 0.02	ND	81 ± 1

Figure 4.6 shows the glucan hydrolysis efficiency results for different solids washing conditions. Hydrolysis efficiency after 72 hours increased with the number of washings, from 56 ± 5 % without washing up to 78 ± 4 % and 85 ± 1 % for one and two washes, respectively. This could be due to the removal of xylo-oligomers present in the pretreatment hydrolysate (Kim et al., 2011). Additional washes (three or four) did not have a significant effect on hydrolysis efficiency.

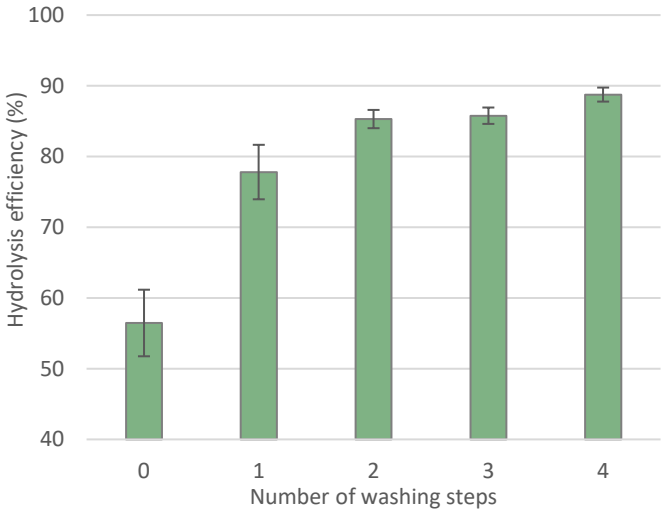


Figure 4.6. Hydrolysis efficiency for solids with different number of washing steps.

4.3.4. Study of the effect of initial pH for different solids content

As expected, initial pH had a significant effect on the cellulose hydrolysis efficiency obtained after 72 hours of hydrolysis for all solids content. Figure 4.7 shows the hydrolysis efficiency values for 15, 20 and 25% w/w solids content. The optimal initial pH value was different for the low and high solids enzymatic hydrolysis. For the lowest solids content studied (15%), the highest efficiency (82 ± 2 %) was obtained for an initial pH of 4.8, this is the pH value recommended by the manufacturer of the enzyme complex. For higher solids content (20 and 25%), the highest efficiencies (82 ± 3, and 63 ± 1 % respectively) were obtained for an initial pH of 6.0.

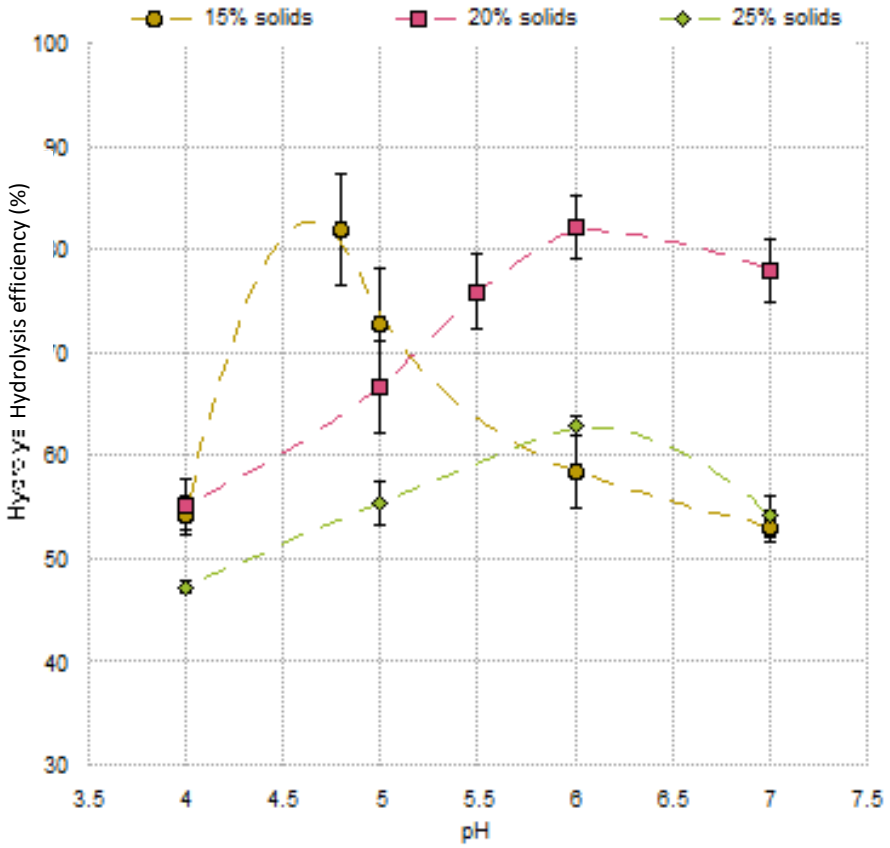


Figure 4.7. Hydrolysis efficiency as a function of pH in the added buffer for 15(●), 20 (▪) and 25 % (◊) solids content.

As shown in Table 4.7, the final pH (after 72 h) was lower than the initial pH. This decrease was progressive as hydrolysis advanced (verified by posterior assays were pH was measured at different times) and could be due to the release of acetyl groups (Horn & Eijsink, 2010). The final pH value for the optimal conditions was similar to the recommended value by the manufacturer of the enzyme complex, for all solids contents. Considering this pH change

during hydrolysis, it is difficult to determine if the difference between optimal initial pH and the pH recommended by the manufacturer could be attributed to the fact that the pH in these cases decreases to a value similar to that recommended by the manufacturer or to the Donnan effect reported by other authors (Lan et al, 2013; Romero et al., 2011). Lan et al. (2013) found that the optimal range of pH for maximum enzymatic cellulose hydrolysis of six different lignocellulosic substrates with different pretreatments was 5.5–6.2 using Cellic® CTec2 working at 1% solids loading. The difference in optimal pH was correlated to the degree of substrate lignin sulfonation, since charged groups in the lignin surface could create a pH gradient within the slurry with a local pH lower than the liquid pH (measured pH did not change considerably through the hydrolysis). Romero et al. (2011) reported that the optimal buffer pH for the enzymatic hydrolysis was higher for high solids loadings for lignocellulosic substrates, measured pH values were not reported.

Table 4.7. Initial conditions and results (after 72 h) of the enzymatic hydrolysis assays.

Solids loading (%, w/w)	Buffer pH	Initial pH	Final pH	Glucose (g/L)	Xylose (g/L)	Cellulose hydrolysis efficiency (%)
15	4.0	4.1 ± 0.1	3.9 ± 0.1	46 ± 2	11 ± 1	54 ± 1
15	4.8	4.8 ± 0.1	4.5 ± 0.1	65 ± 2	16 ± 1	81 ± 2
15	5.0	5.0 ± 0.1	4.6 ± 0.1	55 ± 1	13 ± 1	65 ± 2
15	6.0	5.9 ± 0.1	5.1 ± 0.1	48 ± 3	12 ± 1	57 ± 4
15	7.0	6.8 ± 0.1	5.4 ± 0.1	44 ± 1	12 ± 1	54 ± 2
20	4.0	4.1 ± 0.1	3.9 ± 0.1	81 ± 4	21 ± 1	55 ± 3
20	5.0	4.9 ± 0.1	4.3 ± 0.1	84 ± 6	20 ± 3	67 ± 5
20	5.5	5.5 ± 0.1	4.5 ± 0.1	92 ± 5	22 ± 1	76 ± 4
20	6.0	6.0 ± 0.1	4.7 ± 0.1	100 ± 7	24 ± 1	82 ± 3
20	7.0	6.9 ± 0.1	4.8 ± 0.1	86 ± 6	21 ± 1	78 ± 2
25	4.0	4.1 ± 0.1	3.7 ± 0.1	66 ± 1	19 ± 1	47 ± 1
25	5.0	5.0 ± 0.1	4.0 ± 0.1	77 ± 3	20 ± 1	55 ± 2
25	6.0	6.0 ± 0.1	4.4± 0.1	88 ± 1	26 ± 2	63 ± 1
25	7.0	6.8 ± 0.1	4.4± 0.1	76 ± 3	25 ± 1	54 ± 1

More studies are needed in order to explain the causes of the differences in optimal pH for different solids content. Nevertheless, it is critical to assess optimal pH when working with the enzymatic cellulose hydrolysis of new substrates or under new conditions.

4.3.5. Study of the effect of solids content enzyme dosage and substitution of cellulase by xylanase

Table 4.8 shows the experimental results of the Box-Behnken assays for solids loading (S), enzyme dosage (E) and xylanase substitution (X). Very high sugar concentrations (up to 186 g glucose/L) were obtained. High hydrolysis efficiencies were found for high solids content (>90% for 25% solids content) but only when working with high enzyme dosages (40-70 $mg_{protein}/g_{glucan}$).

Table 4.8. Hydrolysis efficiency, glucose concentration and glucose productivity for the Box-Behnken assays.

Assay number	S (%, w/w)	E ($mg_{protein}/g_{glucan}$)	X (%, w/w)	Glucose (g/L)	Hydrolysis efficiency (%)	time (h)	Productivity (g/Lh)	MESP [1] ($/L)	MESP [2] ($/L)
1	15	10	10	70	75	168	0.42 ± 0.06	0.95	0.93
2	15	70	10	100	100	48	2.08 ± 0.06	1.04	0.91
3	25	10	10	111	60	168	0.66 ± 0.06	0.96	0.94
4	25	70	10	186	100	168	1.10 ± 0.06	0.99	0.90
5	15	40	0	96	99	48	1.99 ± 0.06	0.88	0.81
6	15	40	20	95	100	48	1.98 ± 0.06	0.88	0.81
7	25	40	0	170	94	168	1.02 ± 0.06	0.86	0.79
8	25	40	20	171	94	168	1.02 ± 0.06	0.86	0.79
9	20	10	0	93	68	168	0.55 ± 0.06	0.93	0.91
10	20	10	20	89	66	168	0.53 ± 0.06	0.96	0.93
11	20	70	0	143	99	48	2.98 ± 0.06	1.00	0.88
12	20	70	20	143	100	48	2.98 ± 0.06	0.99	0.87
13	20	40	10	133	98	48	2.77 ± 0.06	0.83	0.76
14	20	40	10	138	100	48	2.87 ± 0.06	0.82	0.75
15	20	40	10	133	96	48	2.77 ± 0.06	0.84	0.77

[1] considering an enzyme cost of 4.24 $/kg protein
[2] considering an enzyme cost of 3 $/kg protein

Figure 4.8 shows the kinetics for the enzymatic hydrolysis of cellulose at 20% solids content and 40 $mg_{protein}/g_{glucan}$.

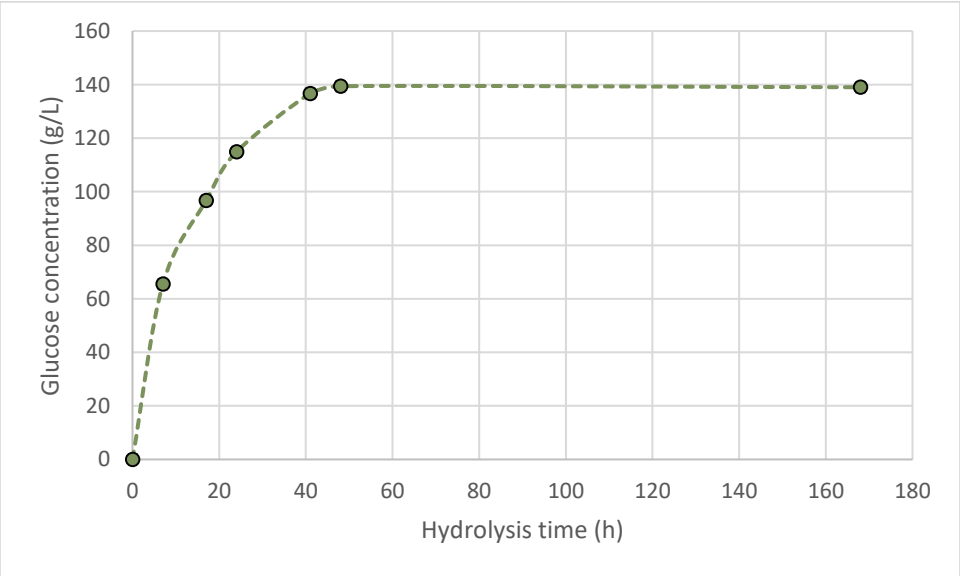


Figure 4.8. Glucose concentration as a function of time, for hydrolysis at 20% solids content and 40 $mg_{protein}/g_{glucan}$

Quadratic models found for glucose concentration (G), and hydrolysis efficiency (H), as a function of coded variables for solids loading (x_1), enzyme dosage (x_2) and xylanase substitution (x_3), are:

$$G\left(\frac{g}{L}\right) = 133.86 + 34.75\,x_1 + 26.13\,x_2 - 16.98\,{x_2}^2 + 11.25\,x_1 * x_2 \quad (r^2 = 0.99) \qquad (4.8)$$

$$H\,(\%) = 97.29 - 3.25\,x_1 + 16.38\,x_2 - 13.66\,{x_2}^2 + 3.75\,x_1 * x_2 \quad (r^2 = 0{,}99) \qquad (4.9)$$

The proposed models were considered a good fit at they show significant regression and non-significant lack of fit (see details in Appendix D). Therefore, they constitute a good representation of experimental data. The response surfaces obtained are shown in Figure 4.9 and Figure 4.10.

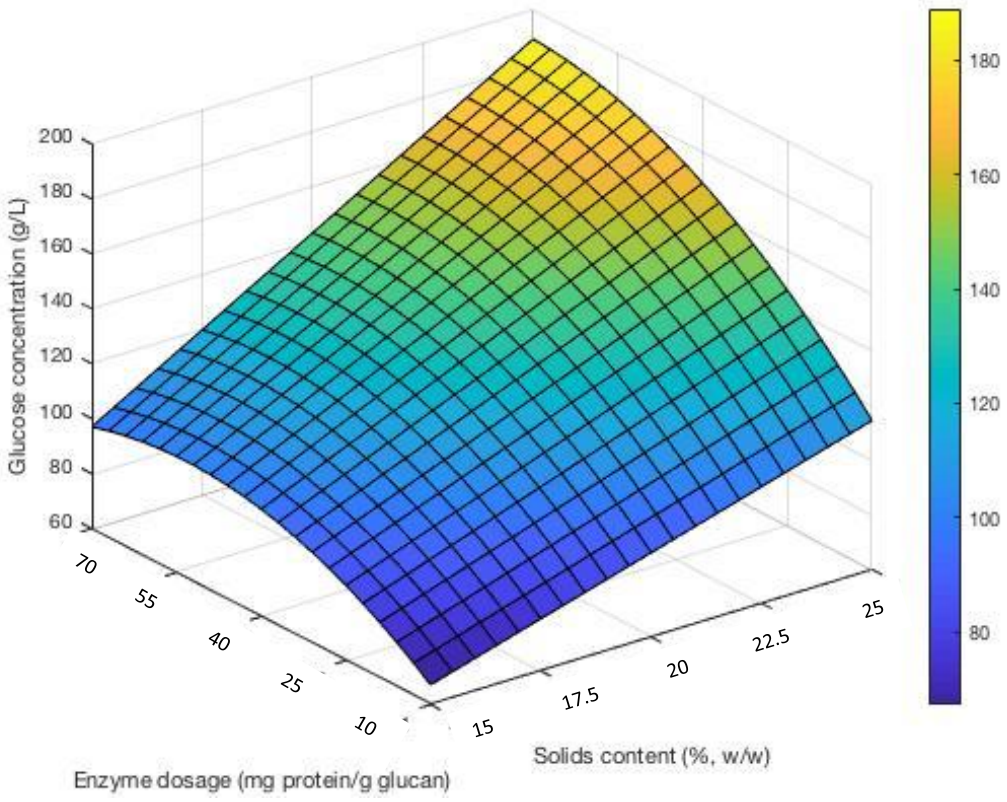


Figure 4.9. Glucose concentration as a function of enzyme dosage (E) and solids content (S) according to the proposed quadratic model.

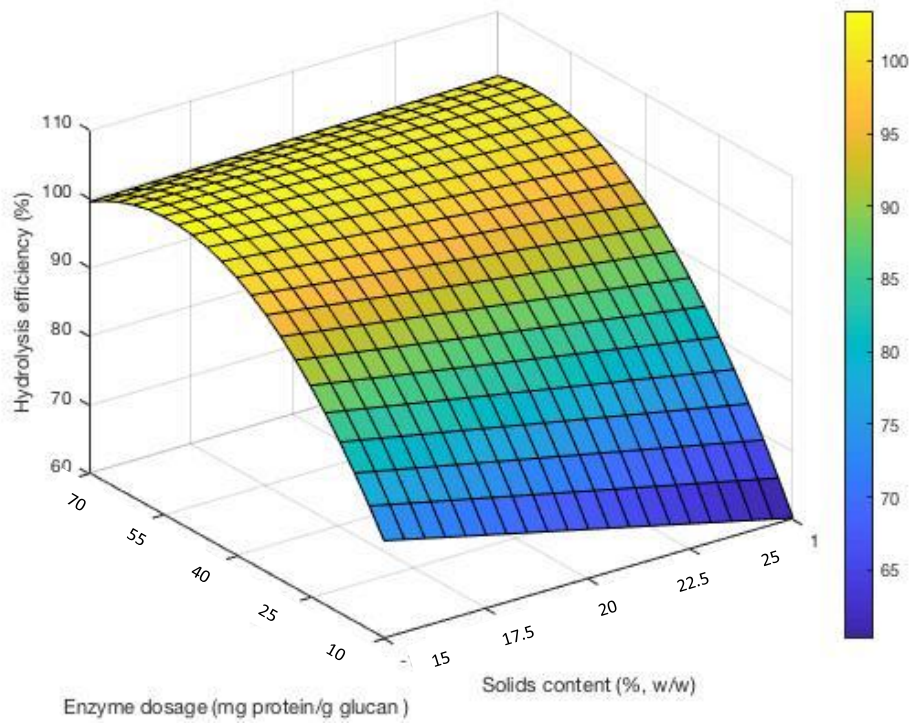


Figure 4.10. Hydrolysis efficiency as a function of enzyme dosage and solids content according to the proposed quadratic model.

Solids content and enzyme dosage both have a significant effect in glucose concentration and hydrolysis efficiency. For hydrolysis efficiency, enzyme dosage was considerably more significant than solids content. Xylanase substitution had no significant effect on any of these variables. Inhibition of the hydrolysis reaction related to the increase of solids content was considerable for low enzyme dosages (10 $mg_{protein}/g_{glucan}$), however, this inhibition seemed to be avoided when enzyme was used in high dosages (70 $mg_{protein}/g_{glucan}$). Cellulose hydrolysis inhibition due to high solids content could be attributed to mass transfer issues and to the reduction of available water (Hsieh et al., 2014). High enzyme dosages studied appeared to be enough to compensate these inhibition effects. Solids content at high enzyme dosages did affect the kinetics of the reaction (longer hydrolysis times).

The assay conditions (from the experimental design in Table 4.8) that minimized selling price for both enzyme costs were: 20 % solids content with 40 $mg_{protein}/g_{glucan}$ (0.83 and 0.76 $/L, for 4.24 and 3 $/$kg_{protein}$ respectively). It can also be observed that there was not a direct correlation between hydrolysis efficiency and minimum selling price for all conditions. For high enzyme cost, it was better to work at a low hydrolysis efficiency of 60% with low enzyme dosage than with a high efficiency of 96 % and a high enzyme dosage. The opposite occurred for a low

enzyme cost (see assays 3 and 4). Therefore, enzyme cost should be considered when setting the goals of an experimental research (e.g. prioritizing hydrolysis efficiency or enzyme dosage).

The differences in minimum selling price observed in Table 4.8 were similar in magnitude to the differences observed between different pretreatment methods (except soaking in aqueous ammonia) for switchgrass (Tao et al., 2011). This shows that improvement of the enzymatic hydrolysis stage is as important as improving pretreatment for the biorefinery sustainability.

For the minimum selling price fitting, xylanase substitution was not considered as a parameter, since it had no effect on the values entered in the simulation model (solids content, total enzyme dosage (mg protein), hydrolysis efficiency, and hydrolysis time, see section 4.2.3). The quadratic models shown below give a good representation of variations of minimum ethanol selling price ($MESP^1$ and $MESP^2$) for an enzyme cost of 4.24 and 3 $/kg protein respectively.

$$MESP^1(\$/L) = 0.84 - 0.01\,x_1 + 0.03x_2 + 0.03{x_1}^2 + 0.13{x_2}^2 - 0.02\,x_1 * x_2 \quad (r^2 = 0.97) \quad (4.10)$$

$$MESP^2(\$/L) = 0.77 - 0.01x_1 - 0.02x_2 + 0.03{x_1}^2 + 0.12{x_2}^2 - 0.01\,x_1 * x_2 \quad (r^2 = 0.91) \quad (4.11)$$

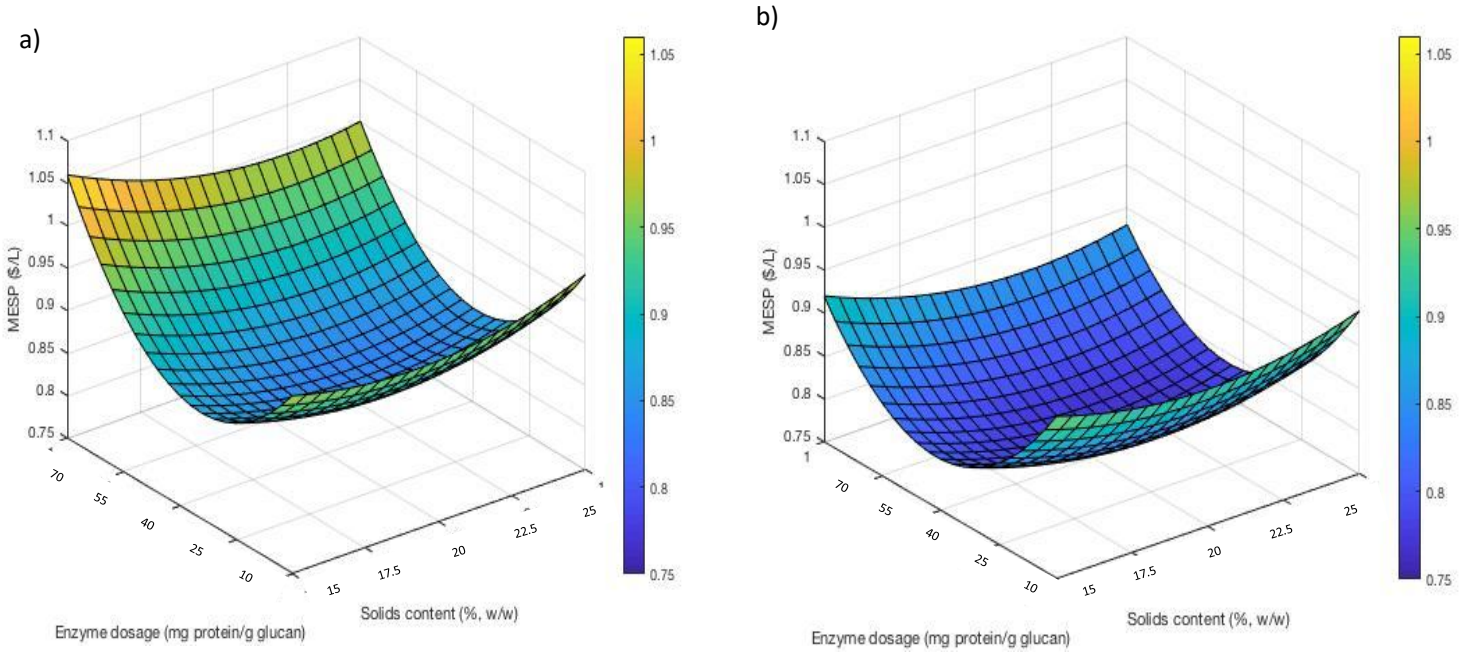


Figure 4.11. Minimum ethanol selling price (MESP) as a function of enzyme dosage (E) and solids content (S) for a) an enzyme cost of $4.24/kg protein and b) an enzyme cost of $3/kg protein according to the proposed quadratic model.

As shown in Figure 4.11, MESP showed a similar tendency for high and low enzyme cost. MESPs variations due to enzyme dosage were larger than those due to solids content. The behavior of MESP vs enzyme dosage can be explained by low enzyme dosages leading to low hydrolysis efficiencies (reducing ethanol yields and increasing cost) and high enzyme dosages adding a higher cost than the benefits from the increase in hydrolysis efficiency.

The conditions that minimize $MESP^1$ and $MESP^2$ found by nonlinear constrained optimization of the quadratic models (Equations 4.10 and 4.11) are shown in Table 4.9.

Table 4.9. Results of nonlinear optimization of models for glucose concentration, hydrolysis efficiency and minimum ethanol selling price.

Optimized variable	Solids content (%)	Enzyme dosage ($mg_{protein}/g_{glucan}$)	Glucose concentration (g/L)*	Hydrolysis efficiency (%)*	MESP ($/L)
MESP for 4.24 $/ kg protein	20.7	36.9	135.7	95	0.838
MESP for 3 $/ kg protein	20.9	42.7	142	99	0.768

*Calculated from the models (equations 4.8 and 4.9).

The enzyme dosage conditions that minimize MESP, were not always associated with the optimal hydrolysis responses (e.g. 95% hydrolysis efficiency). Therefore "technically optimal" conditions (higher efficiency, higher glucose concentration) do not necessarily lead to an economical optimal (lower cost) when other factors such as use of enzymes are involved.

The solids content that minimized MESP was similar for both enzyme costs, but the optimal enzyme dosage showed a significant change. This highlights the importance of accurate estimation of enzyme cost.

A new experimental design in the proximity of the optimal conditions would be desirable to improve the accuracy in the determination of the optimal conditions and values for this process.

4.4. Chapter conclusions

Compared with other compositions reported, switchgrass (Alamo variety) grown experimentally in Uruguay, had a high glucan and low xylan content (43.4 ± 0.3 and 16.5 ± 1.3 % w/w dry base, respectively). From an economic perspective, this is not desirable for the biorefinery scenario as shown in Chapter 3 (Section 3.4).

LHW pretreatment at 200ºC for 5 min proved to be a suitable pretreatment technology for a biorefinery approach, showing high xylan removal (71%) with high components recovery percentages (103 ± 2, 96 ± 9, and 86 ± 3 % of lignin, xylan, and glucan respectively), and low concentrations of inhibitors (acetic acid, formic acid, 5-hydroxymethyl furfural, and furfural).

Washing the solids after pretreatment improved hydrolysis efficiency. Two washing steps with 10 g distilled water per gram of dry matter were enough to reach the maximum hydrolysis efficiency (85 ± 1 %). This could be due to the removal of xylo-oligomers present in the pretreatment hydrolysate. After two washes furfural was completely removed, and acetic acid concentrations were reduced (below the inhibitory value reported for fermentation).

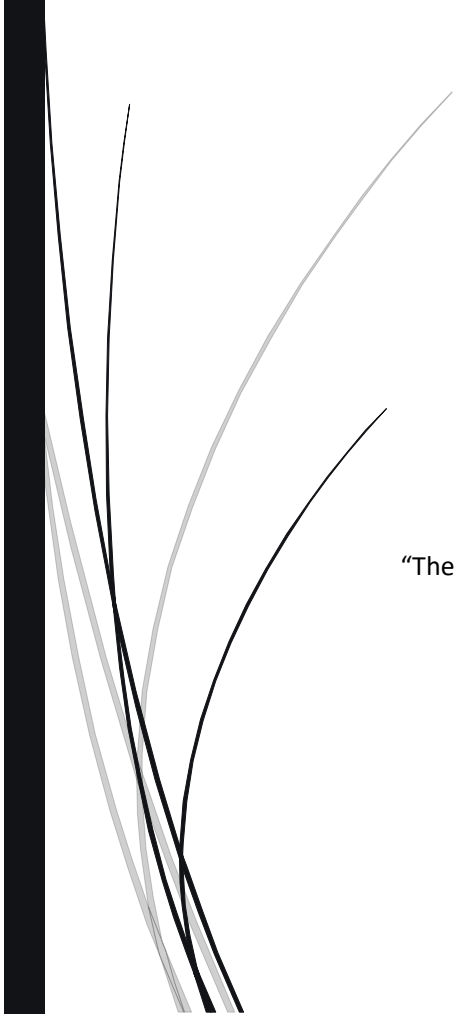
Changes in pH had a significant effect on the efficiency obtained after 72 hours of hydrolysis for all solids content. The optimal initial pH value for hydrolysis varied for the hydrolysis at different solids content. For the lowest solids content studied (15%), the highest efficiency (82 ± 2%) was obtained for an initial pH of 4.8; for higher solids content (20 and 25%), the highest efficiencies (82 ± 3, and 63 ± 1 % respectively) were obtained for an initial pH of 6.0. Further research is needed in order to explain the causes of the observed differences in optimal initial pH. Nevertheless, it is critical to assess optimal pH when working with the enzymatic hydrolysis of new substrates or under new conditions.

When studying the effect of solids content, enzyme dosage, and xylanase substitution, both solids content and enzyme dosage had a significant effect on glucose concentration and hydrolysis efficiency. Enzyme dosage had a most significant effect on hydrolysis efficiency than solids content. Xylanase substitution had no significant effect on any of these variables.

From an economic perspective, the variations in minimum ethanol selling price found for the experimental assays (Box-Behnken) were similar to those reported for different pretreatment methods by Tao et al. (2011). Consequently, optimizing the enzymatic hydrolysis stage is as important as optimizing pretreatment for the economic sustainability of the energy-driven biorefinery.

The solids content that minimized MESP was similar for both enzyme costs (21%), but the optimal enzyme dosage changes from 37 to 43 mg $_{protein}$ /g_{glucan} (for 4.24 and 3 \$/ $kg_{protein}$ respectively), highlighting the importance of accurate estimation of enzyme cost.

Chapter 5: Environmental assessment



"The universe is not required to be in perfect harmony with human ambition. "

Carl Sagan

5.1. Introduction

Evaluation of the environmental performance of biofuels through their life cycle is critical for the sustainable production of biofuels and the development of new technology and environmental policies (MacLean & Spatari, 2009). In the last decades, environmental performance has been assessed through life cycle assessment (LCA). Several works have analyzed fuel bioethanol life cycle (Kemppainen & Shonnard, 2005; Sheehan et al., 2004). Most studies showed an improvement in the environmental performance of biofuels in terms of energy usage and greenhouse gases (GHG) emissions, when compared to gasoline (Farrell et al., 2006).

Some previous works have used life cycle assessment to analyze the performance of bioethanol obtained from switchgrass (Bai et al., 2010; Smeets & Lewandowski, 2009; Spatari et al., 2005). However, their results do not apply to the production of bioethanol from switchgrass in Uruguay, as LCA is highly dependent on location, soil quality, transportation distance, and energy sources.

5.1.1. Life cycle assessment (LCA): definition and procedure

Life cycle assessment is a tool to quantify the environmental effect of the use of natural resources and emissions generated, by gathering information associated with material and energy inputs and outputs of every process involved in a product's life cycle. It is the most accepted tool for the assessment of environmental aspects of biofuel production sustainability (Rathore et al., 2013).

According to the International Organization for Standardization (ISO) life cycle assessment is defined as a technique to evaluate environmental aspects and potential impacts associated to a product by means of: compiling an inventory of inputs and outputs relevant to the system studied; evaluating the potential impact associated to this inputs and outputs and interpreting the results of the inventory analysis and impact evaluation stages in relation to the goals of the study (International Organization for Standardization [ISO1-4040], 2006; International Organization for Standardization [ISO-14044], 2006).

The stages of the LCA procedure as defined by ISO can be observed in Figure 5.1 and will be explained in detail in the following sections.

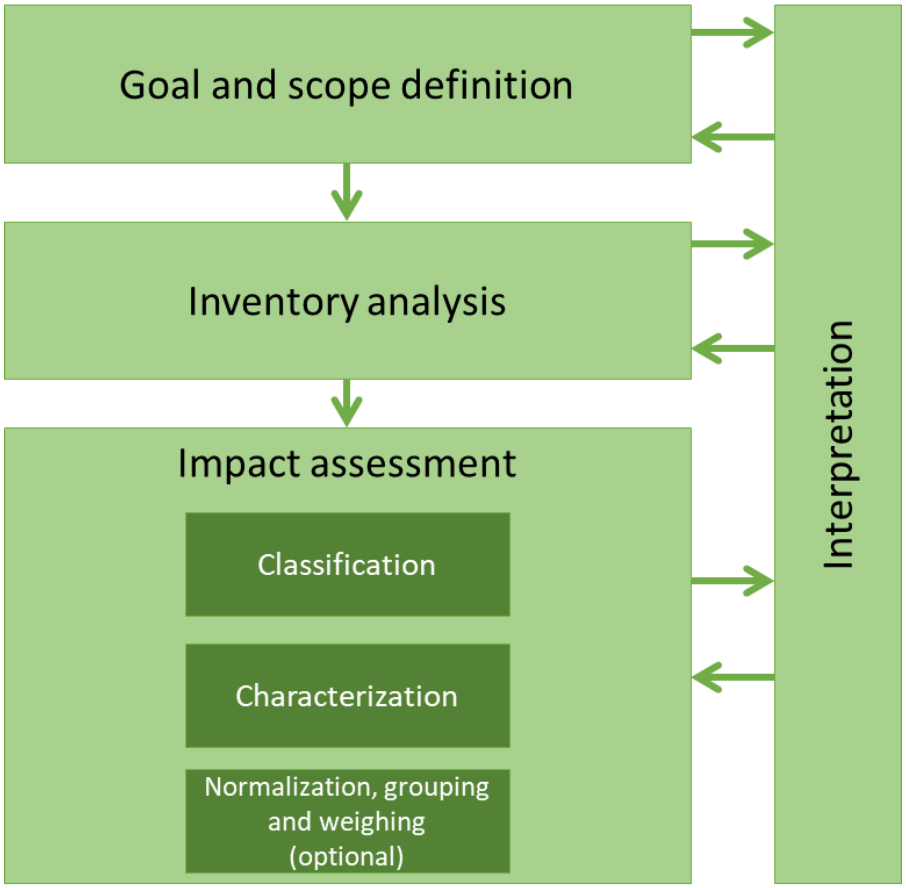


Figure 5.1. LCA methodology according to ISO-14040 and ISO-14044 standards.

5.1.1.1. Goal and scope definition

This definition takes into consideration the context of the study and the intended use of its results (intended application, motivation, and target audience). This definition is of immense importance, as it determines the methodology required for the following stages. For example, studies that aim to provide information for nationwide decisions should be made with data obtained from national averages, while a study that aims to provide information for taking decisions on an existing facility should use data from the facility itself, or local data.

In this stage the following aspects need to be defined:

Functional unit. It is the reference unit for the study and all impacts will be quantified in relation to it (e.g. 1 kg of product). The unit selected must be relevant to the process and must allow comparison with other systems. For bioethanol LCA, the most frequent functional units are area of land used for the feedstock, volume or mass of feedstock, volume or mass of ethanol produced, ethanol heating value, or distance covered in a car (Wiloso et al., 2012).

System boundaries. System boundaries are defined for several dimensions:

- Natural boundaries: They state the beginning and end of the LCA, determining which processes are considered in the LCA and which are not, by drawing the frontier between the technical system modeled in the LCA and the natural system that surrounds it. Depending on the stages comprised in the study the LCA study can be defined as a Cradle to Grave, Cradle to Gate, Gate to Gate, or Gate to Grave, as shown in Figure 5.2. Cradle to Grave analyses the entire process, from resource extraction ("cradle") to end use and disposal ("grave"). Cradle to Gate analysis goes from resource extraction to product production (at the "gate" of the facility). Gate to Gate systems are limited to the product production inside the facility that produces it, while Gate to Grave only studies end use and disposal of the product after it leaves the facility (Fokaides & Christoforou, 2016).

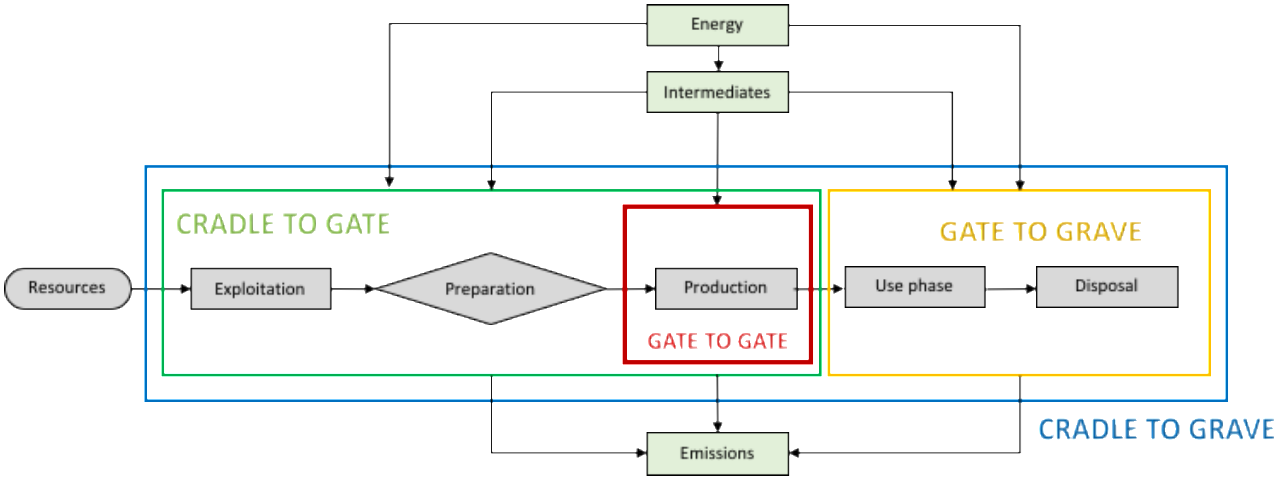


Figure 5.2. System boundaries commonly used for LCAs.

For fuel LCAs, the system is usually defined as Well to Tank (WtT) or Well to Wheel (WtW). As shown in Figure 5.3, WtT includes biomass production, fuel production, and fuel distribution. WtW analyses all the stages of WtT and includes fuel end use.

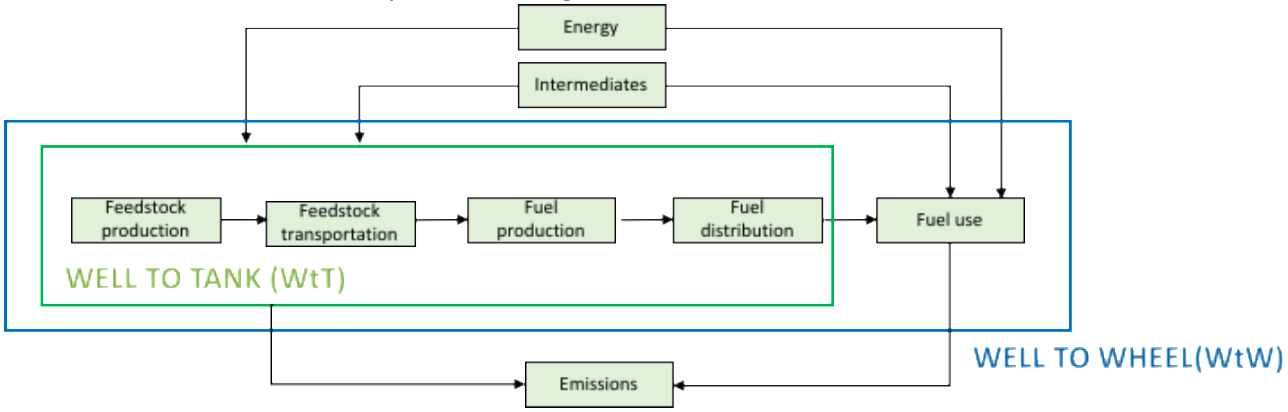


Figure 5.3. System boundaries commonly used for fuel LCAs.

- Geographical boundaries: LCAs must take in consideration the location of every process, as distinct locations can have significant differences in terms of available infrastructure, the source of electricity, waste disposal, transportation distances, and fuels. The environmental reaction to a given contaminant can also depend on the location.
- Time boundaries: It defines the time frame for the study, as it could refer to the present, to the future (next five to ten years) or even analyze impacts in the past.
- Boundaries and cut off criteria for personal and capital: This refers to the decision of including or excluding the impact of manufacturing and maintenance of capital goods (equipment and facilities) and the impacts generated by the staff (food, breathing, and transportation). Other cut-off decisions can relate to exclude product use or waste management from the analysis or exclude some information based on available data, time, or resources.
- Boundaries relating to other life cycle and allocation: In most cases the life cycles of several products have some common processes. This generates an allocation problem, in which it should be decided how much of each process impact belongs to each product. Figure 5.4 shows the most common types of allocation problems. The problem can be solved when a lot of information of the process is available, allowing for an exact calculation of the impact associated with each product. As this information is not always available, other alternatives include: system expansion, in which the other products are included in the system and accounted as a credit for avoiding the production of these products in a different facility; and allocation by partition, in which resource consumption and emissions associated to a process are divided amongst the products. ISO-14044 indicates that if possible, allocation should be avoided either by using system expansion or by dividing the processes. System expansion by burden substitution is the most used method for biofuels (Wiloso et al., 2012).

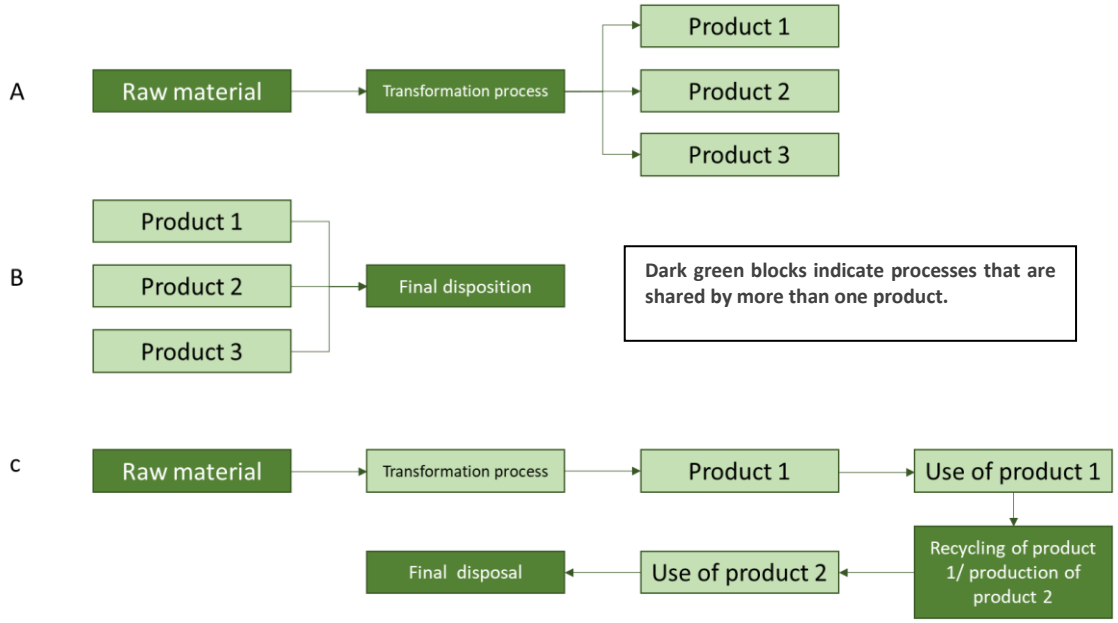


Figure 5.4. Typical configuration of allocation problems.

Types of impacts assessed: The type of impacts to be studied defines the parameters for which data must be collected. According to ISO, impacts can refer to the use of resources, ecological consequences, and human health. These impacts are usually interpreted in more practical categories such as global warming, acidification, and resource deployment. Impact categories for biofuel analysis are usually related to net energy balance and global warming (Wiloso et al., 2012).

System subdivision: It might be useful to separate the technical system studied into a foreground system and a background system. These systems differ on the effect decisions made by the person performing the LCA have on each system**.** The primary system can be affected directly by the methodology chosen for the LCA, while the background system is only affected indirectly. This subdivision is useful for deciding the type of data that should be gathered for each system.

Data quality: The quality of the data compiled defines how representative of reality the model really is. Higher data quality usually requires a bigger workload; therefore, it is necessary to set data quality requirements appropriate for the goal and scope of the study. In order to comply with ISO-14044, quality should be assessed based on: relevance (degree in which the data represents the aspect in question, it includes aspects as geographical, technological and time relevance), reliability (precision, uncertainty and consistency) y accessibility (allows for data to be reviewed and guarantees reproducibility) (Baumann & Tillman, 2008).

5.1.1.2. Life cycle inventory analysis

Inventory analysis is a partial material and energy balance within the limits of the system. It is partial because it focuses only on inputs and outputs that are relevant to the types of impacts studied.

This stage includes the following activities:

Construction of a flow model. Generally, a flow diagram is created, detailing the activities comprised within the system and the material and energy flows associated with these activities.

Data recollection. Data have to be collected for inputs and outputs of every activity modeled, including materials, energy, wastes, and emissions. Other data like transportation distance, technology applied, geographic location, price and/or quantity of products might also be necessary. The source of the data used is an important aspect of data gathering. Data for the background system are usually taken from existing databases, introducing some estimations and assumptions for its use. Data for the primary system are usually gathered from the opinion of experts in the field. If possible, data obtained should be checked by comparing with another database or through material and energy balances (Baumann & Tillman, 2008).

Calculation of resource use and/or contaminant emitted. This quantification is done in relation to the functional unit. All activities are expressed as a function of the product of interest. The flows that link activities and those that go through the system boundaries are calculated. The sum of resource usage and contaminant emissions through the process is determined.

5.1.1.3. Life cycle impact evaluation

This evaluation seeks to analyze the environmental impact associated with the environmental load quantified at the inventory analysis. When the inventory results are interpreted directly (without impact evaluation), the study is a life cycle inventory (LCI) instead of an LCA. Impact evaluation is done after the information is grouped in a smaller number of parameters of higher environmental relevance. This grouping of data is done on three stages:

- Classification: Inventory parameters are arranged according to which type of impact they contribute to. The most common impacts considered are global warming, energy consumption, reduction of the ozone layer, eutrophication, and acidification.
- Characterization: The relative contribution of each emission/resource used to each type of impact is calculated.
- Normalization, grouping or weighting: This stage is done only when a greater level of data conglomeration is needed. In this case, data is normalized, grouped and weighed according to some criteria defined by experts. The result of this stage would be a unique

value representing the environmental impact of the process. According to ISO14040 it is an optional step (ISO, 2006a).

5.1.1.4. Interpretation

In this stage, the findings from the impact evaluation are considered in the context of the goal and scope of the study. The results from the interpretation stage should include information on the limitations of the conclusions of the study, as well as recommendations for decision-making actors. These should offer a complete, comprehensible and coherent lecture of the results of the life cycle assessment in agreement with the goal and scope definition (ISO, 2006a).

5.1.1.5. Computational tools for LCA

Computational tools are useful for the systematical analysis and comparison of the environmental impact of a product or process. These tools include standardized methods for impact analysis as well as useful databases for different sectors and locations, simplifying the inventory analysis. Table 5.1 shows the most used software tools for LCA (Morales et al., 2015).

Table 5.1. List of the main software tools for LCA, adapted from Morales et al. (2015).

Software	Developer
SimaPro	Pré-consultants
GaBi	PE Europe GmbH
Bousted	Bousted Consulting
LCAmanager	SIMPPLE
OpenLCA	GreenDeltaTC
WRATE	UK Enviromental Agency
REGIS	Sinum AG
Euklid	Frauenhofer Institut
WISARD	Pricewaterhouse Coopers
TEAM	Ecobilan-Pricewaterhouse Coopers
Umberto	Ifeu-Institut

For LCAs focusing on fuels impact on global warming, another tool available is the "Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation Model" (GREET), developed by the system evaluation group at Argonne National Laboratory.

SimaPro is a software developed by Pré Consultants that complies with recommendations by the norms ISO 14040 e ISO 14044. It has been used as an LCA tool in studies for products and services including several works on fuel bioethanol production (Hsu et al., 2010; Papong et al., 2017; Pourhashem et al., 2013).

SimaPro offers several databases, including Ecoinvent v3. This is the most used database and includes over 10000 unitary operations and production processes on different locations, based on industrial information gathered by research institutes and consultants with expertise in LCA. This database is the result of updating and integrating different life cycle inventory databases. It includes capital goods and it is updated regularly (Pré Consultants, 2013) (Wernet et al., 2016).

5.1.2. Regulatory framework for the environmental assessment of biofuels

Current regulation for biofuels for Uruguay does not include environmental requirements for biofuels, besides stating that the promotion and regulation aims to reduce greenhouse gas emissions in the terms of the Kyoto protocol (*Ley de Agrocombustibles* N° 18195, https://legislativo.parlamento.gub.uy/temporales/leytemp647719.htm access 12/2018).

The European Union (EU) Renewable Energy Directive and the United States (US) Renewable Fuel Standard set frameworks to determine when a biofuel can be considered useful to meet the renewable fuel targets, as a function of the reduction on GHG emissions, and provide guidelines to calculate them.

The US Renewable Fuel Standard has different reduction thresholds based on the feedstock and technology used for the biofuel, while the European Union Renewable Energy Directive has the same threshold for all biofuels. However, the EU threshold depends on when the facility commenced its operation (Environmental Protection Agency, 2010; European Parliament, 2009; European Parliament, 2015). A summary of these thresholds, as well as the fossil fuel baselines they refer to, can be observed in Table 5.2.

Table 5.2. GHG reduction thresholds and fossil baseline values according to EU renewable energy directive and US renewable fuel standard. Adapted from De Jong et al. (2017).

	GHG reduction threshold		Fossil fuel baseline		Allocation method
	(% of fossil fuel baseline)	Conditions	(gCO_{2eq}/MJ)	Applies to	
EU renewable energy directive	**60**	From 2018 for installations commencing production after 5 October 2015.	83.8	Average fossil fuel at 2010.	Energy allocation except for co-generation of heat and power.
	50	From 2018 for installations that started production before 5 October 2015.			
US renewable fuel standard	**60**	Cellulosic biofuels from lignocellulosic feedstocks.	91.8 93.3	Diesel type fuels. Gasoline type fuels.	Avoided by system expansion.
	50	Advanced biofuels from all feedstocks except cornstarch.			
	20	Conventional renewable fuels (typically corn ethanol).			

The emission requirement for new facilities producing cellulosic biofuels is 60% for both frameworks, but they differ in the baseline value (83.8 and 93.3, for EU and for US respectively)

Considering that in this work allocation was avoided by system expansion, the US standard was taken as reference for the rest of the discussion.

5.2. Materials and methods

5.2.1. Bases for the life cycle assessment

Definition of goal and scope of the study

The goal of this study was to estimate the use of fossil energy and the greenhouse gases emissions during the life cycle of fuel bioethanol produced from switchgrass in Uruguay, aiming to identify environmentally significant factors at the industrial and/or agricultural phase of production. The results of this study should be useful as the basis for decision making by the actors involved in biomass production and industrial ethanol production, and comparable with existing LCAs for local ethanol production from other feedstocks.

Functional unit

The functional unit selected was 1 MJ of ethanol produced, inputs and outputs information will be informed in relation to this unit. The use of an energy unit is consistent with the energetic use of the product and with recommendations from the European Union Renewable Energy Directive for the calculation of GHG emissions in biofuels (European Parliament, 2009; European Parliament, 2015).

System boundaries

Natural boundaries: Figure 5.5 shows the boundaries considered for this product and the processes included within the boundaries of the technical system. The system analyzed considered ethanol distribution but not its use. Therefore, it was categorized as a well to tank analysis (WtT). For some comparisons of GHG emissions, ethanol use was included (Well to Wheel). The limits were chosen to allow comparison with existing LCAs for local biofuel production (Herrera et al., 2015, 2016, 2017).

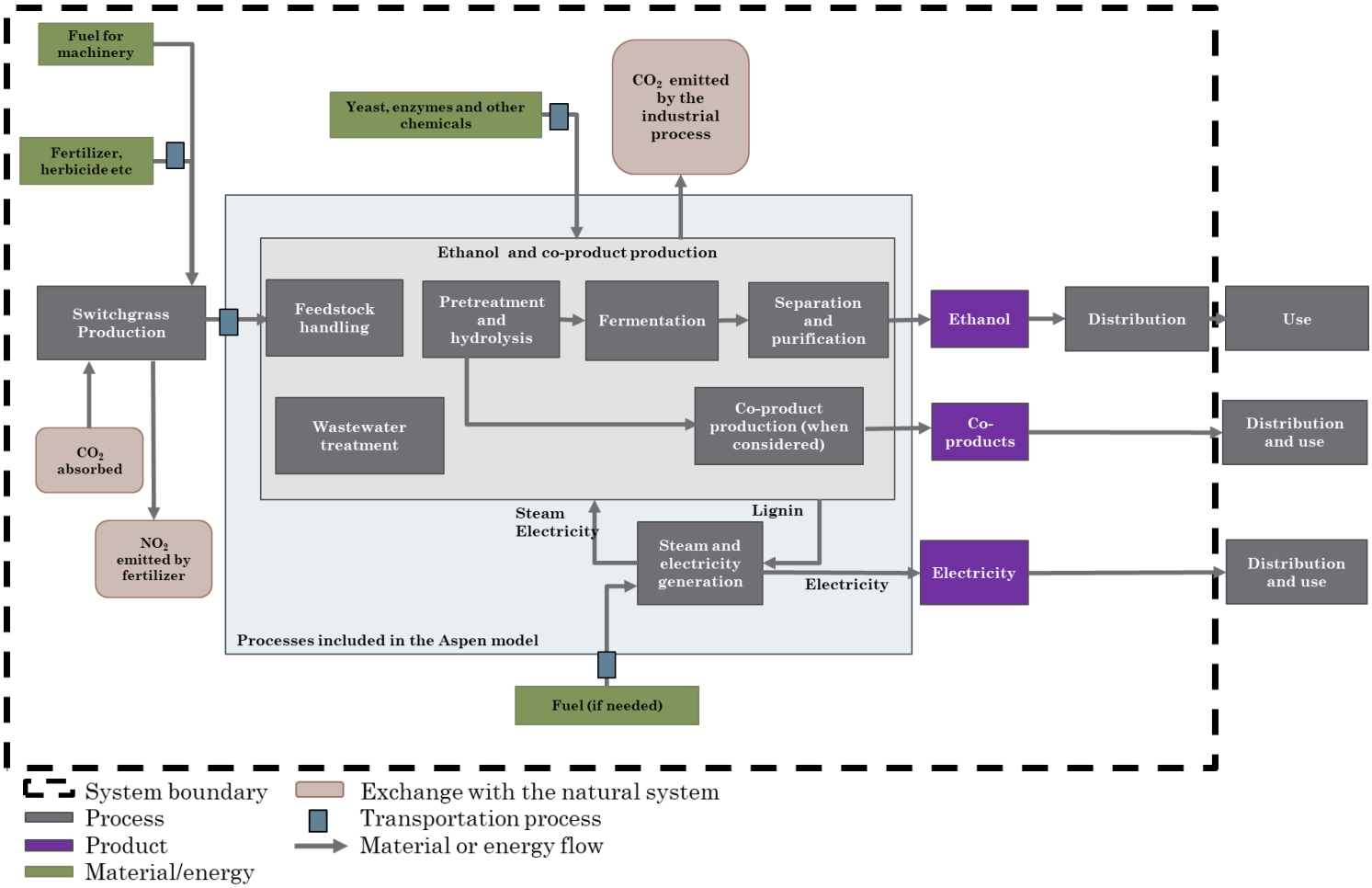


Figure 5.5. System boundaries for the LCA.

- Geographic boundaries: This work considered that both switchgrass and ethanol production would take place in the Paysandú department, Uruguay. The location was selected based on suggestions by the agronomist that supplied the feedstock, and on the fact that bioethanol producing facilities were also located in the area. The exact location would have to be selected analyzing logistics for both feedstock and products. Regarding other materials used in the process, their assumed origins can be seen in Table 5.3. It was considered that these materials were transported by land (by train) for an average of 300 km at the country of origin, ferry transportation to Montevideo and ground transportation to Paysandú (by truck), for 350 km. Transportation of ethanol and chemicals were calculated for the LCI in tkm units, calculated as the weight transported (t) multiplied by the distance (km).

Table 5.3. Origin of raw materials.

Agricultural supplies	
All chemicals	China
Industrial supplies	
Enzymes	Brazil
Corn Steep Liquor	United States
Chemicals and nutrients	China

Switchgrass production was assumed to take place in a 100 km radius of the production facility. This large radius was considered to allow the possibility of switchgrass being grown in the less fertile soils. An average transportation distance of 50-90 km was considered (see Monte Carlo analysis, section 5.2.5).

- Temporary boundaries: This study should give useful information for decision making related to ethanol production in the near future. Therefore, available current data and/or near-term projections with a solid base will be used.
- Cut-off criteria for personal and capital goods: The exclusion of capital goods manufacture and maintenance on LCA is the subject of great debate, mainly due to the varying importance of capital goods in different processes as reported by Frischknecht et al. (2007). Most biofuel LCAs do not supply any information regarding how capital goods are considered. The LCAs performed for other Uruguayan biofuels do not include capital goods (Herrera et al., 2016; Herrera et al., 2015; Herrera et al., 2017). The European Union Renewable Energy Directive indicates that capital goods should be excluded in the LCA focusing on GHG emissions of biofuels (Alberici & Hamelinck, 2010). Considering this information and the goal of this work, capital goods from the foreground system were excluded. For these same reasons, the impact of personnel (feeding, breathing, transportation, etc.) was also excluded.
- Limits regarding other life cycles and allocation: As suggested by ISO standards allocation is avoided by expanding the boundaries of the system to include the co-production of electricity, furfural, acetic and formic acids. The avoided impact of producing these co-products in a separate facility is considered as a credit.

Types of impact analyzed

According to the goals of the study, impact analysis is limited to the non-renewable fossil energy consumption and to the global warming potential (GWP). GWP is defined as the potential to increase the average global temperature of the earth atmosphere and oceans, Greenhouse gas emissions are used as an indicator of the GWP.

System subdivision and data quality

The foreground system was comprised by: feedstock production and transportation, and the industrial production process. Data for this system were collected from information from experts in the agricultural and industrial areas and by simulation results. The background system included the raw materials produced in other countries. Data for this system were

obtained from the Ecoinvent v3, database available at SimaPro, and the U.S. Life Cycle Inventory Database created by NREL (National Renewable Energy Laboratory, 2012).

The U.S. Life Cycle Inventory Database covers materials, products, and processes commonly used in the United States with up-to-date, critically reviewed accessible data that is compatible with international databases.

Methods for impact evaluation

Global warming potential was assessed through the "IPCC 2013" method, developed by the International Panel on Climate Change (IPCC). A time frame of 100 years was chosen.

IPCC characterizes factors for the direct GWP of emissions to the air (except CH_4). This characterization does not include:

- indirect formation of nitrous oxide (N_2O) from nitrogen emissions
- radiative forcing due to emissions of NO_x, water, sulfate, etc. in the lower stratosphere and upper troposphere
- the range of indirect effects given by IPCC
- CO_2 formation from CO emissions (Pre' Consultants, 2014)

To evaluate the use of nonrenewable fossil energy, the cumulative energy demand (CED) method was applied. CED is based on the method published by Ecoinvent version 1.01 and expanded by SimaPro developers for energy resources available in the SimaPro database. Characterization factors for the primary energy resources are divided into five impact categories: Nonrenewable-fossil, Nonrenewable-nuclear, Renewable-biomass, Renewable-wind-solar-geothermal, Renewable–water (Frischknecht, et al., 2007). Nonrenewable fossil energy was the only category analyzed.

The Energy Return on Investment (EROI) was calculated for the WtT system (not including ethanol use) with the data for nonrenewable fossil energy usage, according to the following equation:

$$EROI = \frac{Energy\ from\ ethanol\ (LHV)}{Fossil\ energy\ consumption\ (\ considering\ credits\ from\ co{-}products)} \qquad (5.1)$$

5.2.2. Switchgrass production inventory

Switchgrass production includes seed production, land preparation, crop establishment and growth, harvesting, drying, milling, storage, and transportation. The flow diagram in Figure 5.6 shows the inputs and outputs considered in this fraction of the inventory.

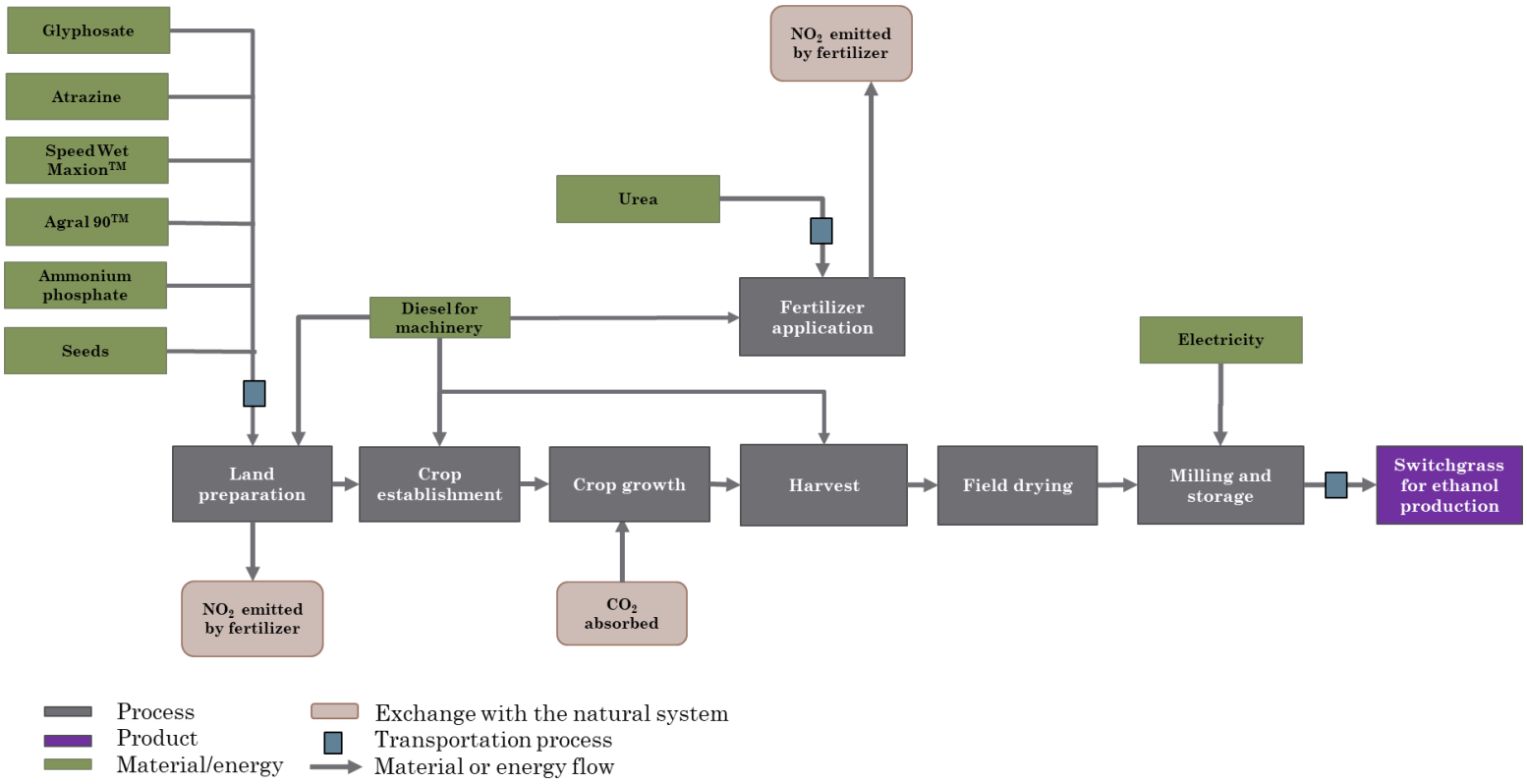


Figure 5.6. Flow diagram of switchgrass production process as considered for the life cycle inventory.

Seeds can be generated from a previous crop production. Switchgrass produced in 0.06 hectare (ha) would give enough seeds to plant one new hectare. Therefore, all the inputs for the switchgrass production phase were multiplied by 1.06 (Olave, 2015; Siri-Prieto personal communication, 2016).

Switchgrass is planted on previously prepared land, and is usually harvested annually for 20 years, after which land preparation needs to be repeated.

In Uruguay, the most common procedure for land preparation is the chemical fallow, which consists of the addition of herbicides, insecticides and adjuvants. Disease and pest control data for switchgrass are very limited (Sanderson et al., 2012). Application of chemicals for disease and pest control was considered unnecessary for this crop at this location (Guillermo Siri-Prieto personal communication 2016). Table 5.4 shows the amount of chemicals needed for switchgrass production.

Table 5.4. Chemicals added at the land preparation stage. Source: Guillermo Siri-Prieto personal communication, 2016.

Type	Name/Brand	L/ha
Herbicide	Glyphosate	8
Herbicide	Atrazine	3
Adjuvant	SpeedWet Maxion™	0.1
Adjuvant	Agral 90™	1
Fertilizer	Ammonium phosphate	100

An annual harvest would be done in August and urea would be added at a rate of 200 kg/ha (Guillermo Siri-Prieto personal communication, 2016). Average crop yield of 15.5 Mg/ha for Uruguay were reported by Siri-Prieto et al. (2017).

Land preparation (ploughing and harrowing), crop establishment, fertilizer application and harvesting stages also involve the use of machinery with an associated fuel consumption. Land preparation and establishment were assumed to take place every 20 years, while urea application and harvesting were considered annual processes.

Information on diesel consumptions at Uruguayan farms was obtained by Herrera et al. (2017) for grain sorghum production. The applicability of these data to switchgrass was confirmed by an agronomy expert (Siri-Prieto personal communication, 2016), for all stages except harvesting. For harvesting, data from literature were used (See Table 5.5).

Table 5.5. Direct diesel consumption during switchgrass production.

Activity	**Diesel consumption (L/ha/year)**	**Source**
Land preparation	1.4	Herrera et al. (2017)
Establishment	0.5	Herrera et al. (2017)
Urea application	1	Herrera et al. (2017)
Harvesting	10.4	Smeets & Lewandowski (2009)

Diesel production and distribution data were obtained from the Ecoinvent database, while emissions associated with diesel combustion were calculated using the emission factors reported by Nemecek & Kagi (2007). A density of 0.849 kg/L was used, calculated as the average of values reported in Uruguay from 2013 to 2017 (http://www.ben.miem.gub.uy/icomplementaria.html).

It was considered that harvested switchgrass was field dried with solar energy and transported to a regional storage facility where it was milled, stored and later transported to the ethanol production facility. Milling energy consumption was considered as 267 MJ/ t, using electricity from the grid (Sokhansanj et al., 2009).

Biogenic carbon

Biogenic carbon can be defined as carbon from biological sources. It includes carbon absorbed by the crop through photosynthesis and released by degradation, soil respiration, or combustion (Biobased Products Working Group, 2010; Harris et al., 2017). Biogenic carbon has an important effect on the agricultural production step and on the overall ethanol production. Therefore, it is fundamental to consider the biogenic carbon flows on the environmental assessment (Wiloso et al., 2012).

Many studies consider biogenic carbon for biofuel and short-lived materials by assuming that the atmospheric carbon fixated in the biomass is the same as the biogenic carbon emissions associated with the product and therefore use the concept of "carbon neutrality".

The assumption on biogenic carbon neutrality has been questioned as its values differ from those obtained by complete inventory (Wiloso et al., 2016). Consequently, in this work biogenic carbon was considered as carbon flows in the inventory instead of assuming biogenic carbon neutrality.

Given the system boundaries previously defined, the biogenic carbon absorbed by the crop through photosynthesis and emitted during fermentation, wastewater treatment, lignin combustion, and ethanol combustion were considered in the balance. Carbon sequestered by the crop was calculated considering the base biomass composition used for modeling as 1.63 g CO_2/g dry switchgrass (carbon content in the extractives was not considered) (Toochi, 2018).

Biogenic carbon flows due to land use change are discussed in the land use section.

Nitrous oxide (N_2O) emissions

Nitrous oxide is a greenhouse gas with a global warming potential 300 times larger than carbon dioxide and a residence time in the atmosphere of over 100 years. Nitrous oxide is produced as a result of microbial activity in the soil, and its production is enhanced by the use of fertilizers (Forster et al., 2007). Factors as soil type, hydrogeology and vegetation also affect N_2O emissions (Wang, 1996).

Quantification studies of N_2O emissions from switchgrass are rare and most analyses estimate it using emission factors (Skinner et al., 2012). There are no experimental data available for N_2O emissions in Uruguay of switchgrass cultivars. The GREET model considers that due to the fact that the production of woody and herbaceous biomass requires little soil disturbance and no irrigation, N_2O and NO emissions from nitrification are reduced, and N_2O emissions can be calculated as a 1.3% expressed as the percentage of N-fertilizer converted to N_2O-N (Wang, 1996). This percentage is similar to what is usually obtained by IPCC methodology, but other studies consider that this percentage should be higher (Crutzen et al., 2008). The Ecoinvent database uses a factor of 2.5% for its calculations (Nemecek & Kagi, 2007). Considering this

uncertainty and data previously reported for switchgrass (Spatari & MacLean, 2010), a range of 0.2 to 5%,of N-fertilizer converted to N_2O-N with a mean of 1.3 % was considered.

Land use change

Distinct uses of soil have different environmental impacts associated with the above- and below-ground carbon stocks. Therefore, the impact of establishing a new crop depends on the previous use of the land. The effect of this change is considered by quantifying direct land use change emissions. Indirect land use change (ILUC) emissions can also occur, as production of older crops could be moved to a new location.

GHG emissions related to land use change (LUC) may be positive (net emissions) or negative (net sequestration). Perennial grasses like switchgrass present high agronomical yields with low fertilizer and sequester more carbon (both below and above ground) than the reference vegetation (especially on marginal lands). Consequently, land use change emissions for these grasses are usually negative increasing soil carbon sequestration, which is favorable from an environmental perspective. This could have an important impact on the LCA results. There is a limited number of studies that have looked at the effect of switchgrass cultivations on soil carbon sequestration. These studies found that it would increase carbon sequestration (Skinner et al., 2012). Nevertheless, these emissions are highly uncertain and hard to quantify, as they depend not only on soil characteristics and former use but also on methods to amortize emissions, agricultural zoning, improved management, intensification measures, etc. (De Jong et al., 2017). Land use change was proven to be one of the most important determinants of uncertainty for life cycle carbon intensity analysis of ethanol produced from switchgrass, and depends heavily on the type of land being converted (Spatari & MacLean, 2010).

Indirect and direct land use change effects were not included in the inventory due to:

- difficulties for an accurate and precise determination,
- likely beneficial impact of land use change,
- this work focuses on the ethanol conversion process,
- local studies do not include these effects.

This is consistent with many LCA studies as reviewed by Wiloso et al. (2012).

5.2.3. Ethanol production system

The ethanol production systems described in Chapter 3 (ethanol and electricity and biorefinery systems) were both considered for the LCA. Results from the material balances and energy balances obtained for the TEA (and described in more detail in Appendix A) were used to quantify material and energy inputs to the process in the different cases studied for the sensitivity analysis. Figure 5.7 shows the diagram flow with the inputs and outputs considered in the ethanol production section of the inventory analysis for the biorefinery scenario (see Chapter 3 for further case description).

Data for acetic acid and formic acid production emissions were obtained from the Ecoinvent database, while data for furfural production was obtained from results found by Raman & Gnansounou (2017) for the conventional production of furfural from vetiver (case PF-1 R). They reported GHG emissions of 0.1 kg CO_{2eq}/kg furfural and 0.5 kg oil/kg furfural, lower than the values reported by Slater et al. (2016) from 0.37 to 0.64 kg CO_{2eq}/kg furfural from corn waste. Values for furfural production from the biomass currently used to produce furfural would be more accurate but were not available.

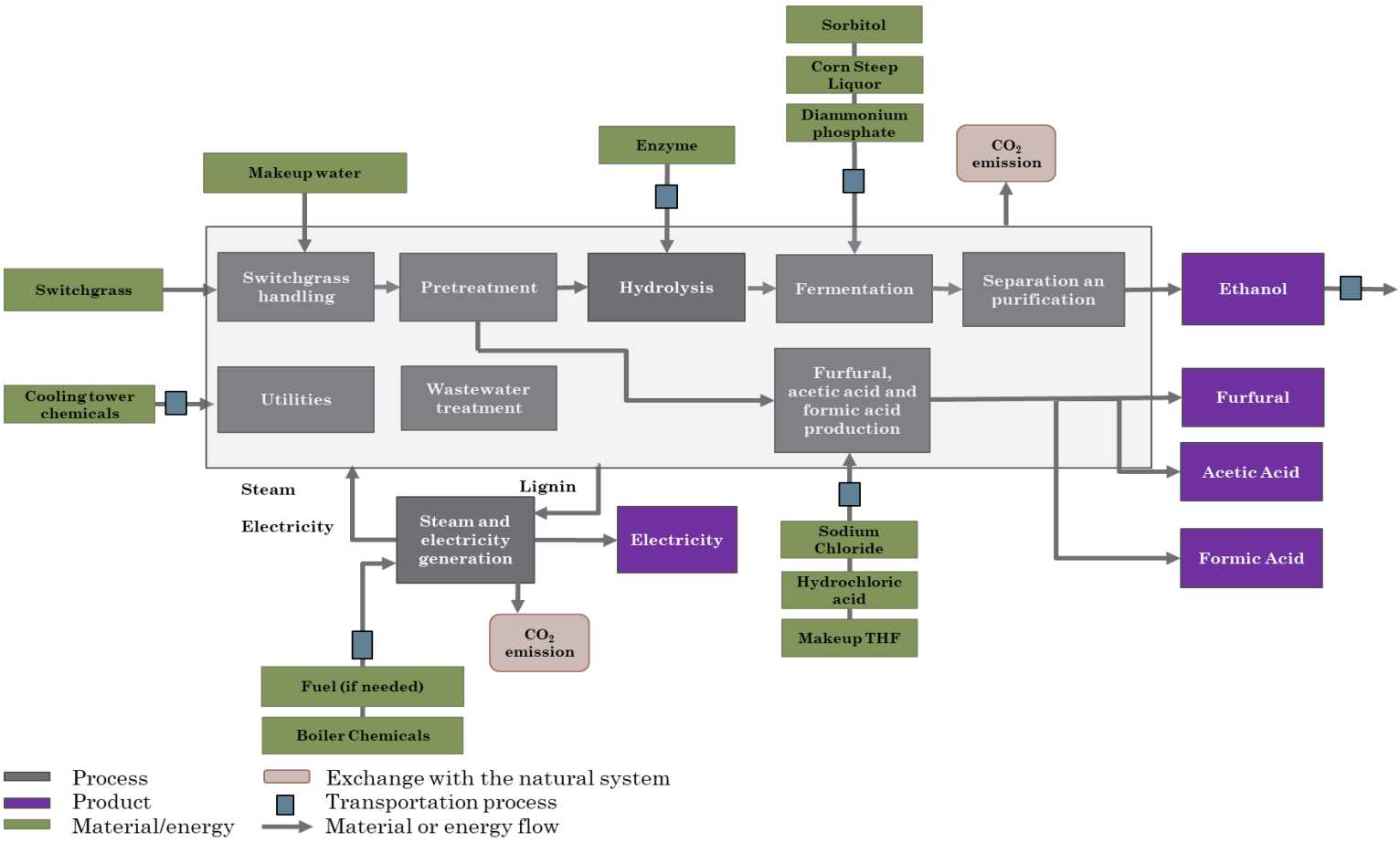


Figure 5.7. Flow diagram for ethanol and co-product production as considered for LCI.

Ethanol distribution

This facility would be located in Paysandú, close to the existing ethanol from grain sorghum plant from ALUR. Ethanol distribution would be similar to the one for this plant, reported as 327 km by Herrera et al. (2017). Transportation of ethanol and chemicals is calculated for the LCI in tkm units, calculated as the weight transported multiplied by the distance. For ethanol, it amounts to 327 tkm /$t_{ethanol}$, equivalent to 0.012 tkm/$MJ_{ethanol}$.

Ethanol use

Emissions from ethanol use were considered outside the boundaries of the study (WtT). Ethanol use was estimated to compare GHG results with other studies that assumed carbon neutrality (biogenic carbon sequestered = CO_2 released on the industrial combustion and fermentation + CO_2 released during ethanol combustion), with studies that included ethanol use, and with other reference values. Ethanol use GHG emissions of 70 gCO_2/ $MJ_{ethanol}$ were considered (calculated with the lower heating value and the equation for complete ethanol combustion).

5.2.4. Sensitivity analyses

Sensitivity analyses were done to assess the effect of some process variables previously identified as affecting emissions and energy use in the industrial phase (see Chapter 3), on the environmental performance of fuel bioethanol. Table 5.6 shows the material and energy balance results that are more relevant to the LCA for each case studied on the sensitivity analysis, for a facility processing 10.4 dry $t_{switchgrass}$/h. Results for other chemicals can be found in Appendix C.

Table 5.6. Cases and main parameters modified for LCA sensitivity analysis

Case	Parameter	Value	Ethanol yield ($MJ_{ethanol}$/$kg_{dry\ switchgrass}$)	Industrial emissions ($kgCO_{2eq}$/$MJ_{ethanol}$)	Electricity as co-product ($MJ_{electricity}$/$MJ_{ethanol}$)	Furfural (kg/ $MJ_{ethanol}$)	Acetic acid (kg/ $MJ_{ethanol}$)	Formic acid (kg/ $MJ_{ethanol}$)	CO_2 sequestration (gCO_2/$g_{dry\ switchgrass}$)
1	**Use of hemicellulose**	Xylose to ethanol	6.50	0.19	0.11		Not generated		1.63
4		Xylose not fermented	4.91	0.27	0.23		Not generated		1.63
5		Biorefinery	5.08	0.20	0.07	0.0075	0.0042	0.0012	1.63
65	**Glucan content (%)**	37	4.53	0.22	0.09	0.0084	0.0047	0.0013	1.55
66		47	5.64	0.19	0.05	0.0067	0.0037	0.0011	1.72
67	**Xylan content (%)**	11.5	4.95	0.20	0.06	0.0054	0.0030	0.0008	1.55
68		21.5	5.22	0.20	0.09	0.0095	0.0053	0.0014	1.72
69	**Lignin content (%)**	19	5.09	0.18	0.03	0.0075	0.0042	0.0012	1.51
70		29	5.08	0.22	0.12	0.0075	0.0042	0.0012	1.76
15	**Enzyme dosage**	6.75	5.08	0.20	0.07	0.0075	0.0042	0.0012	1.63
19	**($mg_{protein}$/g_{glucan})**	40.5	5.08	0.20	0.07	0.0075	0.0042	0.0012	1.63
26	**Hydrolysis efficiency (%)**	95	5.52	0.18	0.04	0.0069	0.0039	0.0011	1.63
29		65	3.91	0.28	0.18	0.0097	0.0056	0.0016	1.63
35	**Fermentation efficiency**	85	4.64	0.23	0.11	0.0082	0.0046	0.0013	1.63
37	**(%)**	65	3.74	0.30	0.20	0.010	0.0057	0.0016	1.63
38	**Solids content (%,w/w)**	25	5.09	0.20	0.09	0.0075	0.0042	0.0012	1.63
42		12.5	5.06	0.20	0.02	0.0075	0.0042	0.0012	1.63

The experimental results of hydrolysis efficiencies obtained from the Box-Behnken design (see Table 4.8) for different enzyme dosage and solids contents, were used on the Aspen® Plus model to generate material and energy balances as previously mentioned in Chapter 4. Results from these material and energy balances (Table 5.7) were used to define the life cycle inventory. The goal of this analysis was to determine the correlation between GHG emissions solids content and enzyme dosage and the correlation between fossil energy use, solids content and enzyme dosage. Results for other chemicals used in the inventories can be found in Appendix C.

Table 5.7. Main parameters modified in the LCI for analysis of experimental results.

Case	Ethanol yield ($MJ_{ethanol}$/kg $_{switchgrass}$)	Industrial emissions ($kgCO_{2eq}$/MJ $_{ethanol}$)	Electricity as co-product ($MJ_{electricity}$/MJ $_{ethanol}$)	Furfural (kg/MJ $_{ethanol}$)	Acetic acid (kg/MJ $_{ethanol}$)	Formic acid (kg/MJ $_{ethanol}$)	Sequestration (gCO_2/g $_{dry\ biomass}$)
BB1	4.43	0.24	0.07	0.0086	0.0048	0.0014	1.63
BB2	5.77	0.17	0.01	0.0066	0.0037	0.0013	1.63
BB3	3.65	0.31	0.23	0.0104	0.0065	0.0017	1.63
BB4	5.79	0.17	0.04	0.0066	0.0036	0.0011	1.63
BB5	5.73	0.17	0.01	0.0066	0.0037	0.0011	1.63
BB6	5.73	0.17	0.01	0.0066	0.0037	0.0011	1.63
BB7	5.48	0.18	0.06	0.0070	0.0039	0.0011	1.63
BB8	5.48	0.18	0.06	0.0070	0.0039	0.0011	1.63
BB9	4.08	0.27	0.15	0.0093	0.0052	0.0015	1.63
BB10	3.97	0.28	0.16	0.0096	0.0054	0.0015	1.63
BB11	5.74	0.18	0.03	0.0066	0.0037	0.0011	1.63
BB12	5.80	0.17	0.03	0.0066	0.0037	0.0011	1.63
BB13	5.69	0.17	0.04	0.0067	0.0037	0.0011	1.63
BB14	5.78	0.17	0.03	0.0066	0.0036	0.0011	1.63
BB15	5.58	0.18	0.05	0.0068	0.0038	0.0011	1.63

5.2.5. Monte Carlo Analysis

Switchgrass was produced as an experimental crop in Uruguay. Therefore, several important agricultural parameters are still uncertain. The uncertainties considered for the foreground system and for the electricity grid mix are shown on Table 5.8 and Table 5.9. The effect of these uncertainties and of the uncertainties associated with the data from the database, was studied through a Monte Carlo Analysis.

Monte Carlo is a method that determines the probability of a given result, through multiple simulations that randomly select values of the parameters from the uncertainty distribution assigned. The simulation results (in this case GHG emissions and fossil energy use) are obtained as the means and standard deviations that characterize them in terms of uncertainty distributions.

Table 5.8. Data distribution used on the Monte Carlo Analysis.

Parameter	Low value	Mean value	High value	Distribution function	Sources
Switchgrass yield (dry t/ha/year)	8.5	15.5	22.5	Normal	Siri Prieto et al. (2017)
Feedstock transport (km)	30	70	110	Normal	Herrera et al. (2015, 2017); Olave, (2015)
Urea application (kg/ha/year)	100	200	300	Normal	Guillermo Siri- Personal communication, 2016 (see section 5.2.2.)
N_2O-N emissions (% of N-fertilizer)	0.2	1.3	5.0	Triangular	

Note: For normal distribution low and high values correspond to the 2.5 and 97.5 percentile respectively and are calculated as the mean value ± 2σ (standard deviation).

Electricity grid mix

One of the co-products of the ethanol production process is the remaining electricity generated from burning lignin and biogas, after thermal and electrical energy needs for the industrial process have been satisfied. This electricity is assumed to replace the electricity from the Uruguayan grid mix.

The variability of the electricity grid composition was also considered in the Monte Carlo Analysis. Average percentages and their variations were determined based on the national energy balance from 2014 to 2017 (available online at: http://www.ben.miem.gub.uy/matrices.html access 05/2018). This time period was selected because from 2013 to 2014 there was a significant change in the energy matrix towards more renewable sources, changing high percentages of fossil energy for wind energy. Percentages considered for the study and their standard deviation (σ) are shown on Table 5.9.

Table 5.9. Electricity grid mix by energy source under Uruguayan conditions.

Energy source	Mean value (%)	σ (%)	Distribution function
Thermal (Fossil) gas oil+fuel oil	8.5	4.8	Normal
Thermal (Fossil) - Natural gas	0.2	0.3	Normal
Thermal (Biomass)-	18.4	1.9	Normal
Wind	14.2	7.9	Normal
Solar	0.7	0.7	Normal
Hydroelectric	56.9	7.2	Normal

For the Monte Carlo analysis hydroelectric energy percentage was calculated as 100% minus the rest of the percentages for the LCI, to avoid combinations that do not add to 100%.

5.2.6. Statistical analyses and optimization

Analysis of variance

In order to compare the results obtained from the stochastic Monte Carlo WtT LCA, one-way analysis of variance was performed using the website: http://statpages.info/anova1sm.html Counts (1000), means, standard deviations obtained from the Monte Carlo results (see section 5.3) for each case analyzed, were used.

A Tukey Honestly Significant Difference (HSD) post-hoc test was performed on the same website to indicate which groups were significantly different (p<0.05). Results are shown in Appendix D.

Multiple objective optimization

Multiple objective optimization was performed to optimize environmental and economic factors (GHG and MESP). The Multiobjective Genetic Algorithm Solver from MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States) was used. The result of this optimization is given by an evenly distributed set of points on the Pareto front, and are presented in Appendix D. From these results, one set of values was selected, prioritizing MESP over GHG, as all GHG values complied with the emission reduction requirements.

5.3. Results and discussion

5.3.1. Inventory analysis

Formulas and parameters were used to relate the different materials and processes to the functional unit. This allows to accurately evaluate the changes in the life cycle inventory for the different cases analyzed. Parameters defined for the LCI are shown on Table 5.10. The inventories generated for switchgrass and ethanol production (1 kg and 1MJ respectively), as a function of these parameters, are shown in Table 5.11 and Table 5.12. Inventories for other materials and energy that were defined for this work (such as electricity grid, enzymes, CSL and furfural) can be found in Appendix C (Tables 1 to 6).

Table 5.10. Parameters defined for the LCI.

Parameter			**Value**
Symbol	**Definition**	**Units**	
Y	Switchgrass agricultural yield	$kg_{switchgrass}/ha$	15500 (normal distribution $2\sigma = 7000$)
N	Urea addition	kg_{urea}/ha	200 (normal distribution $2\sigma = 100$)
sequ	Sequestered carbon	$kgCO_2/kg_{switchgrass}$	Case dependent (See Table 5.6 and Table 5.7)
Furfu	Furfural production	$kg_{furfural}/MJ_{ethanol}$	Case dependent (See Table 5.6 and Table 5.7)
Acet	Acetic acid production	$kg_{acetic\ acid}/MJ_{ethanol}$	Case dependent (See Table 5.6 and Table 5.7)
Form	Formic acid production	$kg_{formic\ acid}/MJ_{ethanol}$	Case dependent (See Table 5.6 and Table 5.7)
Electr	Surplus electricity sold to the grid	$MJ_{electricity}/MJ_{ethanol}$	Case dependent (See Table 5.6 and Table 5.7)
YE	Ethanol yield	$MJ_{ethanol}/kg_{switchgrass}$	Case dependent (See Table 5.6 and Table 5.7)
CO2	GHG emissions (industrial process)	$kgCO_{2eq}/MJ_{ethanol}$	Case dependent (See Table 5.6 and Table 5.7)
CSL	Corn steep liquor consumption	$kg_{CSL}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
DAP	Diammonium phosphate consumption	$kg_{DAP}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
Sorb	Sorbitol consumption	$Kg_{sorbitol}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
Enzyme	Enzyme consumption	$kg_{enzyme}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
Water	Water consumption	$kg_{water}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
NaCl	Sodium chloride consumption	$kg_{NaCl}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
HCl	Hydrochloric acid consumption	$kg_{HCl}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
THF	Tetrahydrofuran consumption	$kg_{THF}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)
Ammonia	Ammonia consumption	$kg_{ammonia}/kg_{switchgrass}$	Case dependent (See Appendix C, Table C.7, C.8 and C.9)

Note: Switchgrass kg in this table is considered in dry basis.

Table 5.11. Life cycle inventory for the production of 1 dry kg of switchgrass.

Resources as named in database	Database	Amount	Unit
Materials/fuels			
Glyphosate {RoW}| production | Alloc Def,S	Ecoinvent 3	0.7208 (kg/ha) **/Y**	kg
Atrazine {RoW}| production | Alloc Def, S	Ecoinvent 3	0.189 (kg/ha) **/Y**	kg
Adjuvant simulated as: Soap stock (coconut oil refining), at plant ID Energy	Agri-footprint	0.0583 (kg/ha) **/Y**	kg
Nitrogen fertiliser, as N {RER}| diammonium phosphate production Alloc Def, S	Ecoinvent 3	0.562 (kg/ha) **/Y**	kg
Nitrogen fertiliser, as N {RoW}| urea ammonium nitrate production Alloc Def, S	Ecoinvent 3	0.23 (kg/ha) ***N/Y**	kg
N2O emissions/N fertilizer ($kgCO_{2eq}$/N)	Defined for this work	(0.23***N** (kg/ha) +0.562(kg/ha)) **/Y**	kg N
Diesel, at regional storage/RER S	Ecoinvent System process	11.78 (kg/ha) **/Y**	kg
Diesel combustion in farm machinery	Defined for this work	11.78 (kg/ha) **/Y**	kg
Chemical transport at origin: Transport, freight, rail/RER S	Ecoinvent System process	$300\ km\left(\frac{(0.7208+0.189+0.0583+\frac{106}{20}+N)(\frac{kg}{ha})}{Y\times 1000}\right)t$	tkm
Chemical transport from China: Transport, transoceanic freight ship/OCE S	Ecoinvent System process	$21011\ km\left(\frac{(0.7208+0.189+0.0583+\frac{106}{20}+N)(\frac{kg}{ha})}{Y\times 1000}\right)t$	tkm
Chemical transport Uruguay: Transport, lorry >32t, EURO5/RER S	Ecoinvent System process	$350\ km\left(\frac{(0.7208+0.189+0.0583+106/20+N)(\frac{kg}{ha})}{Y\times 1000}\right)t$	tkm
Switchgrass transport: Transport, lorry >32t, EURO5/RER S	Ecoinvent System process	0.0769* (Normal distribution 2σ = 0.0308)	tkm
Electricity/heat			
Switchgrass grinding: Electricity grid mix uy	Defined for this work	0.293*	MJ
Emissions to air			
Carbon dioxide		**-sequ**	

*Values consider that switchgrass is milled and transported with a 9 % water content.

Table 5.12. Life cycle inventory for the production of 1 MJ of ethanol.

	Database	Amount	Unit
Avoided products			
Furfural	Defined for this work.	**Furfu**	kg
Acetic acid, 98% in H2O, at plant/RER S	Ecoinvent System process.	**Acet**	kg
Formic acid from methyl formate, at plant/RER S kg Undefined	Ecoinvent System process.	**Form**	kg
Electricity grid mix uy	Defined for this work.	**Electr**	MJ
Resources			
Materials/fuels			
Switchgrass at plant	Defined for this work (See table).	**1/YE**	kg
Corn Steep Liquor	Defined for this work based on NREL LCI inventory.	**CSL/YE**	kg
Diammonium phosphate, as N, at regional storehouse/RER S	Ecoinvent System process.	**DAP/YE***(14/132)	kg
Sorbitol, modeled as: Chemicals organic, at plant/GLO S	Ecoinvent System process.	**Sorb/YE**	kg
Enzyme cellulase Novozymes	Defined for this work based on NREL LCI inventory.	**Enzyme/YE**	kg
Process water, ion exchange, production mix at plant, from groundwater RER S	ELCD	**Water/YE**	kg
Sodium chloride, powder {RoW}| production|Alloc Def, S	Ecoinvent 3.	**NaCl/YE**	kg
Hydrochloric acid, 36% in H2O, from reacting propylene and chlorine at plant/RER S	Ecoinvent System process.	**HCl/YE**	kg
Tetrahydrofuran {RoW}| production | Alloc Def S k	Ecoinvent 3.	**THF/YE**	kg
Ammonia, liquid {RoW} ammonia production, partial oxidation, liquid | Alloc S	Ecoinvent 3.	**Ammonia/YE**	kg
Chemicals transport at origin: Transport, freight, rail/RER S	Ecoinvent System process.	$300\ km\left(\frac{CSL + DAP + Sorb + Enzyme + NaCl + HCl + TH}{YE \times 1000}\right.$	tkm
From China: Transport, transoceanic freight ship/OCE	Ecoinvent System process.	$21011\ km\left(\frac{DAP + Sorb + NaCl + HCl + THF + Ammonia}{YE \times 1000}\right)t$	tkm
From US: Transport, transoceanic freight ship	Ecoinvent System process	$10788\ km\left(\frac{CSL}{YE \times 1000}\right)t$	tkm
From Brazil: Transport, transoceanic freight ship/OCE	Ecoinvent System process	$3195\ km\left(\frac{Enzyme}{YE \times 1000}\right)t$	tkm
Chemicals transport at Uruguay: Transport, lorry >32t, EURO5/RER S	Ecoinvent System process	$350\ km\left(\frac{CSL + DAP + Sorb + Enzyme + NaCl + HCl + TH}{YE \times 1000}\right.$	tkm
Ethanol distribution: Transport, lorry >32t, EURO5/RER S	Ecoinvent System process	0.012	tkm
Chemicals for boiler and chiller. Chemicals inorganic, at plant/GLO S	Ecoinvent System process	0.0003**/YE**	kg
Emissions to air			
Carbon dioxide		**CO2**	kg

5.3.2. Impact evaluation and life cycle interpretation

Analysis of different production processes and the use of hemicellulose

Well to tank (WtT) greenhouse gas emissions and fossil energy analyses were performed for different scenarios of the fuel bioethanol production from switchgrass. Figure 5.8 shows the results with the standard deviation calculated with the stochastic Monte Carlo analysis.

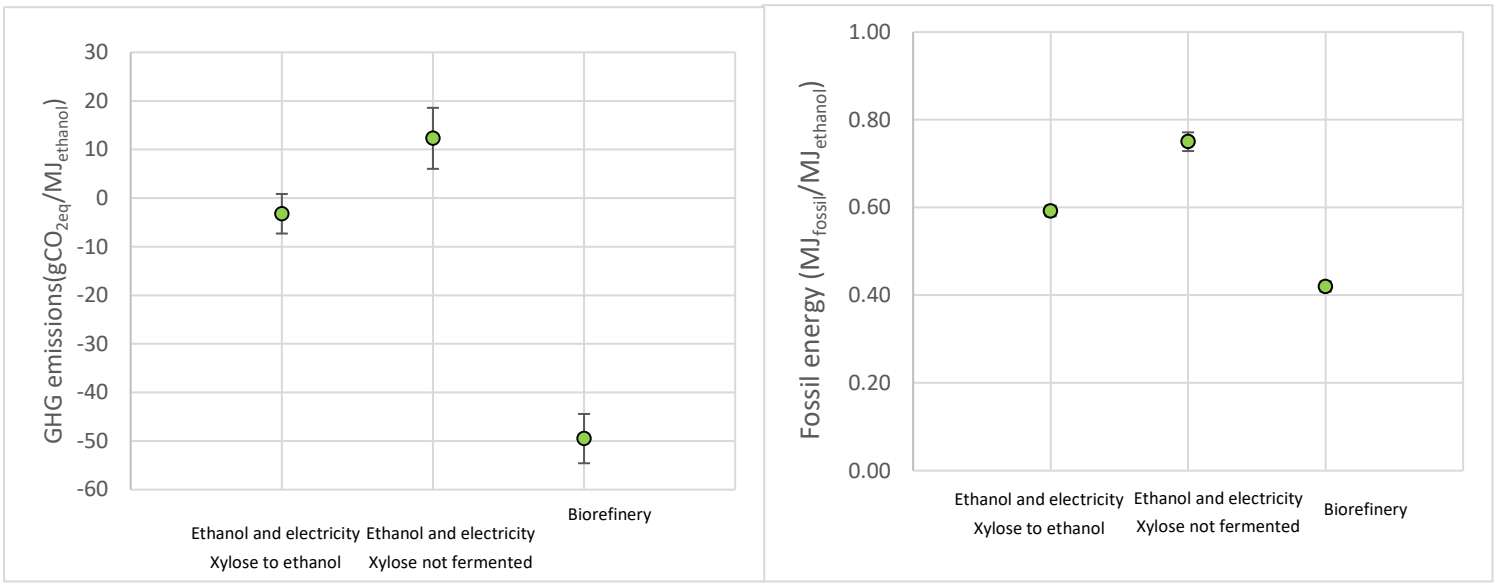


Figure 5.8. Variations in a) GHG emissions and b) fossil energy as a function of xylose use scenarios. Cases 1, 4 and 5. Results correspond to stochastic WtT LCA (not including ethanol combustion) with error bars showing standard deviation (σ).

Table 5.13 and Table 5.14 show the contribution of different processes and materials to the global warming potential (measured as GHG emissions) and to the nonrenewable fossil energy use respectively, per MJ of ethanol produced in the facility. Ethanol use is included in the GHG emission tables for comparison with references. The value obtained by adding the values for different contributions may not coincide with the total mean value "Total WtT", as inventory analysis results correspond to one run of the LCA model on SimaPro (out of one thousand used to the determine the total mean and standard deviation).

Table 5.13. GHG emissions results ($gCO_{2eq}/MJ_{ethanol}$) for the different uses of xylose.

	Ethanol and electricity	Xylose not fermented	Biorefinery
Sequestered carbon	-251	-332	-321
Switchgrass production and transport	9	12	12
CO_2 emissions on the industrial process	190	270	200
Chemicals production and transport	48	63	69
Ethanol distribution	1	1	1
Co-products (furfural, acetic acid, formic acid)	0	0	-12
Electricity	-3	-6	-2
Total (WtT)	-3 ± 4	12 ± 6	-49 ± 5
Total (including) ethanol use	67 ± 4	82 ± 6	21 ± 5

Table 5.14. Fossil energy results ($kJ_{fossil}/MJ_{ethanol}$) and EROI ($MJ_{ethanol}/MJ_{fossil}$) for different xylose uses.

	Ethanol and electricity	Xylose not fermented	Biorefinery
Switchgrass production and transport	35	46	44
Chemicals production and transport	567	753	825
Ethanol distribution	21	21	21
Co-products (furfural, acetic acid, formic acid)	0	0	-449
Electricity	-34	-71	-22
Total (WtT)	592 ± 12	750 ± 21	420 ± 12
EROI (WtT)	1.69	1.33	2.38

GHG emissions considering ethanol use for all scenarios were lower (21-82 $gCO_{2eq}/MJ_{ethanol}$) than the reference emissions for fossil fuel of 93.3 $gCO_{2eq}/MJ_{ethanol}$ used as reference by the US regulatory framework (see Table 5.2). Nevertheless, only the bioethanol produced in the biorefinery scenario could meet the reduction requirement set in this document, that corresponds to emissions lower than 37.3 $gCO_{2eq}/MJ_{ethanol}$ (Environmental Protection Agency, 2010).

Energy return on investment (EROI) for this three scenarios was lower than 3 $MJ_{ethanol}/MJ_{fossil}$, which corresponds to the EROI associated with fossil fuels at the point of use as calculated by Hall et al. (2009). Nevertheless, it was higher than the value of 0.72 reported for switchgrass (Hall et al., 2011).

The scenario in which xylose fermentation was not considered for ethanol production in a facility producing only electricity as co-product (xylose not fermented, case 2, see Chapter 3 for further detail), had the worst environmental performance both in terms of GHG emissions and fossil energy consumption. This shows the environmental importance of the use of xylose either for the production of more ethanol through fermentation or for the production of other chemicals (e.g. furfural).

Results for the bioethanol produced in the ethanol and electricity process (case 1, see Chapter 3 for further detail) can be compared in terms of GHG emissions with the results obtained in a similar LCA by Spatari et al. (2005) for bioethanol from switchgrass through dilute acid pretreatment in Canada. GHG emissions obtained in this work were lower for the switchgrass production stage (9.25 *vs* 22.4 $CO_{2eq}/MJ_{ethanol}$), and higher for the industrial process (233.36 vs 118 $gCO_{2eq}/MJ_{ethanol}$). Nevertheless, total GHG emissions considering ethanol use (67 $gCO_{2eq}/MJ_{ethanol}$) were within the range reported by Spatari & MacLean (2010) in an analysis focused on uncertainties for the production of ethanol from switchgrass using dilute acid pretreatment (-10 to 100 $gCO_{2eq}/MJ_{ethanol}$ WtW emissions, confidence interval 95%).

Lower GHG emissions for switchgrass production under Uruguayan conditions can be explained by the agricultural yields of 15.5 t/ha considered for this work, reported by Siri-Prieto et al. (2017), compared with the 8 t/ha considered by Spatari et al. (2005), and low nutrient addition considered for the Uruguayan soil (3 kg N/dry t *vs* 7.5 kg N/dry t). Figure 5.9 and Figure 5.10 show the percentile distribution of GHG emissions and fossil energy usage of different processes and materials for the switchgrass production stage.

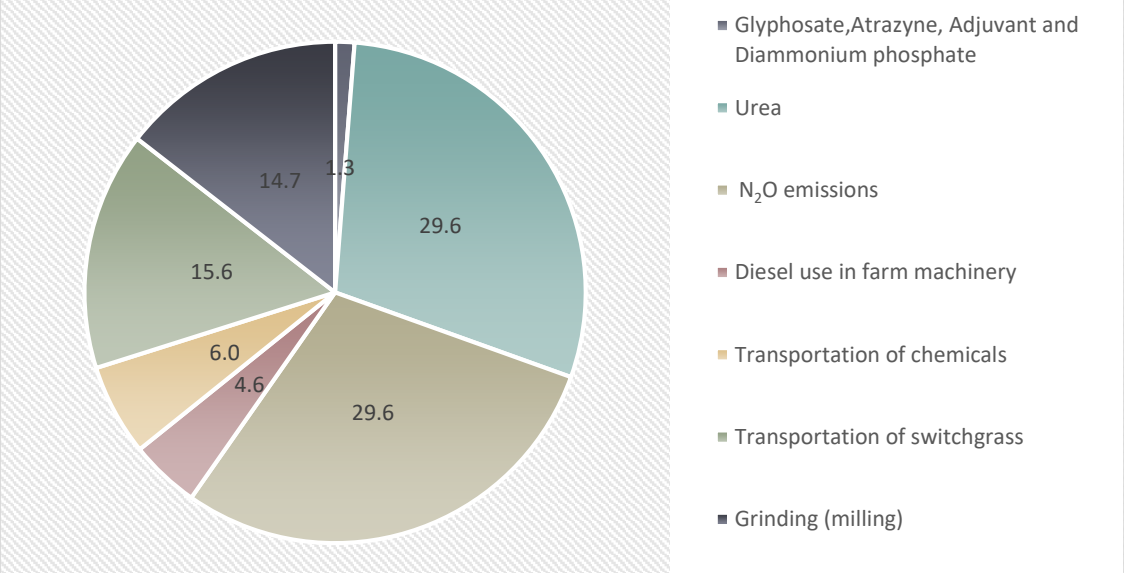


Figure 5.9. Distribution of GHG emissions on the switchgrass production phase for all scenarios.

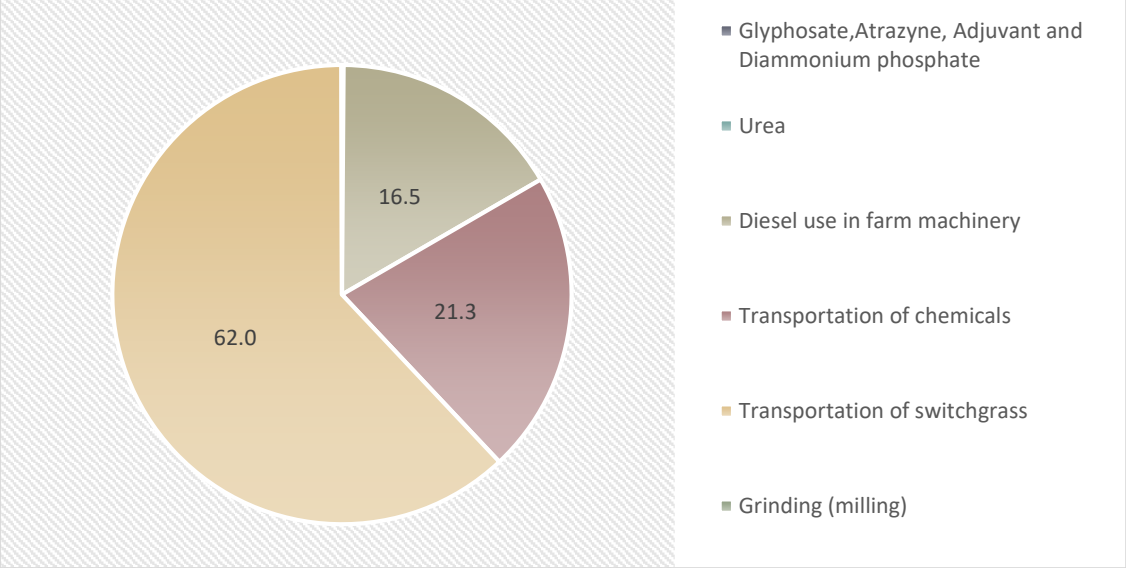


Figure 5.10. Distribution of fossil energy on the switchgrass production phase for all scenarios.

As Figure 5.12 and Figure 5.11 show, the highest percentages of GHG emissions and of fossil energy consumption from chemicals were due to the environmental impact associated with the enzymes. Enzyme related GHG emissions account for a 60-70% of the total emissions (not considering biogenic carbon or credits, see Table 5.13). This is consistent with other works that reported the elevated relative weight of enzymes and chemicals on the environmental impact of biofuels (Hong et al., 2012; Janssen et al., 2016; MacLean & Spatari, 2009).

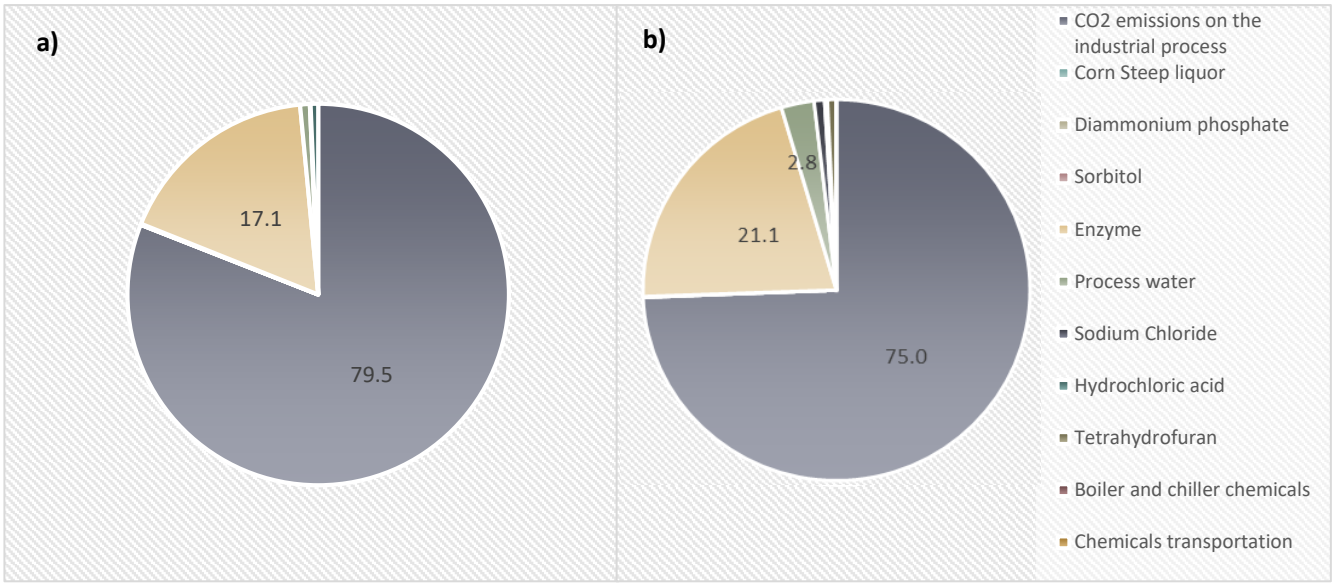


Figure 5.12. GHG emissions on the industrial phase (ethanol and co-products production) for a) ethanol and electricity (case 1) and b) biorefinery (case 5).

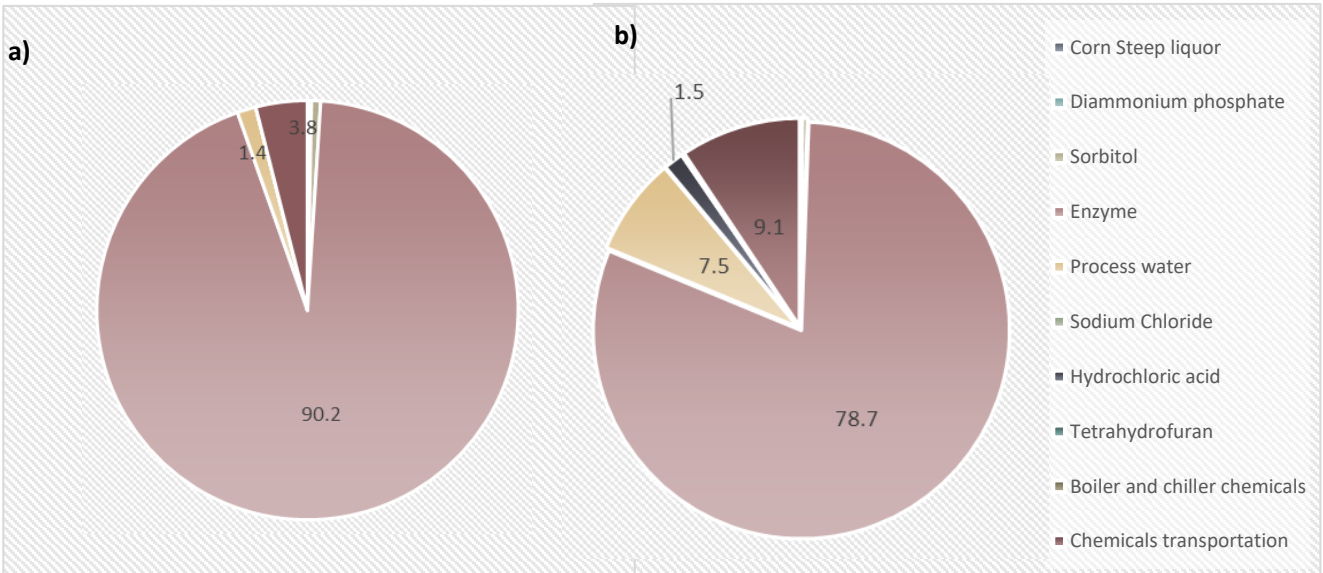


Figure 5.11. Distribution of fossil energy on the industrial phase (ethanol and co-products production) for a) ethanol and electricity (case 1) and b) biorefinery (case 5).

Higher GHG emissions (compared with Spatari et al. (2005)) were found for the ethanol production stage. This could be associated with the limited information available on the use of enzymes when that work was published (MacLean & Spatari, 2009). The specific activity of the enzyme, dosage and associated emissions reported were considerably different than those used in this work. The lower enzyme dosage explains the differences on the industrial GHG emissions. The impact of enzymes will be further discussed when the effect of enzyme dosage is analyzed.

Credits from co-products contribute to the final value of both GHG emissions and fossil energy consumption. Credits associated with electricity were lower than those found by Spatari et al. (2010) reported as 2 to 3 times higher than the negative fossil energy and emissions of the process. This is due to the high percentage of renewable energy in the Uruguayan electricity grid mix, resulting in less GHG emissions and fossil energy displaced. In this scenario it would be convenient to explore the possibility of using the lignin to make value-added chemicals.

Credits from the co-products on the biorefinery process (case 5, see Chapter 3 section 3.2.2 for further detail), could have an important contribution to the environmental performance, by displacing products produced by traditional processes. Acetic and formic acid substituted the same chemicals produced by the most frequent production method, with GHG saving of 6.49 and 3.53 $gCO_{2eq}/MJ_{ethanol}$ and energy savings of 195 and 73 $kJ_{fossil}/MJ_{ethanol}$ respectively. Even though it is produced in higher quantities, savings due to furfural credits were lower (1.62 $gCO_{2eq}/MJ_{ethanol}$ and 184 $kJ_{fossil}/MJ_{ethanol}$). This was expected, as furfural is currently produced from biomass.

An important contribution to GHG emissions comes from the carbon dioxide emitted from the fermentation, wastewater treatment and boiler. Capturing part of the carbon dioxide generated during fermentation could be beneficial. Carbon dioxide capture reduces GHG emissions but requires energy. Despite the increase on energy usage, carbon dioxide capture could still lead to GHG savings, as reported by Herrera et al. (2017).

Another difference between the biorefinery (case 5) and the ethanol and electricity (case 1) processes is the use of process water due to the higher quantity needed for washing the pretreated solids in the biorefinery. Consequently, the environmental impact of process water was higher for that scenario in terms of both, GHG emissions and fossil energy (see Figure 5.11). Impact of process water is probably over estimated as the database inventory used does not take into the account the renewable nature of the energy grid mix in Uruguay. Consequently, results shown are conservative and a better understanding of local impacts of water use would be desirable to improve the accuracy of the analysis. Nevertheless, it is important to minimize water use.

Effect of switchgrass composition

Well to tank (WtT) greenhouse gas emissions and fossil energy analysis for ethanol produced from switchgrass with different contents of key components, calculated with the stochastic Monte Carlo analysis, are shown on Figure 5.13.

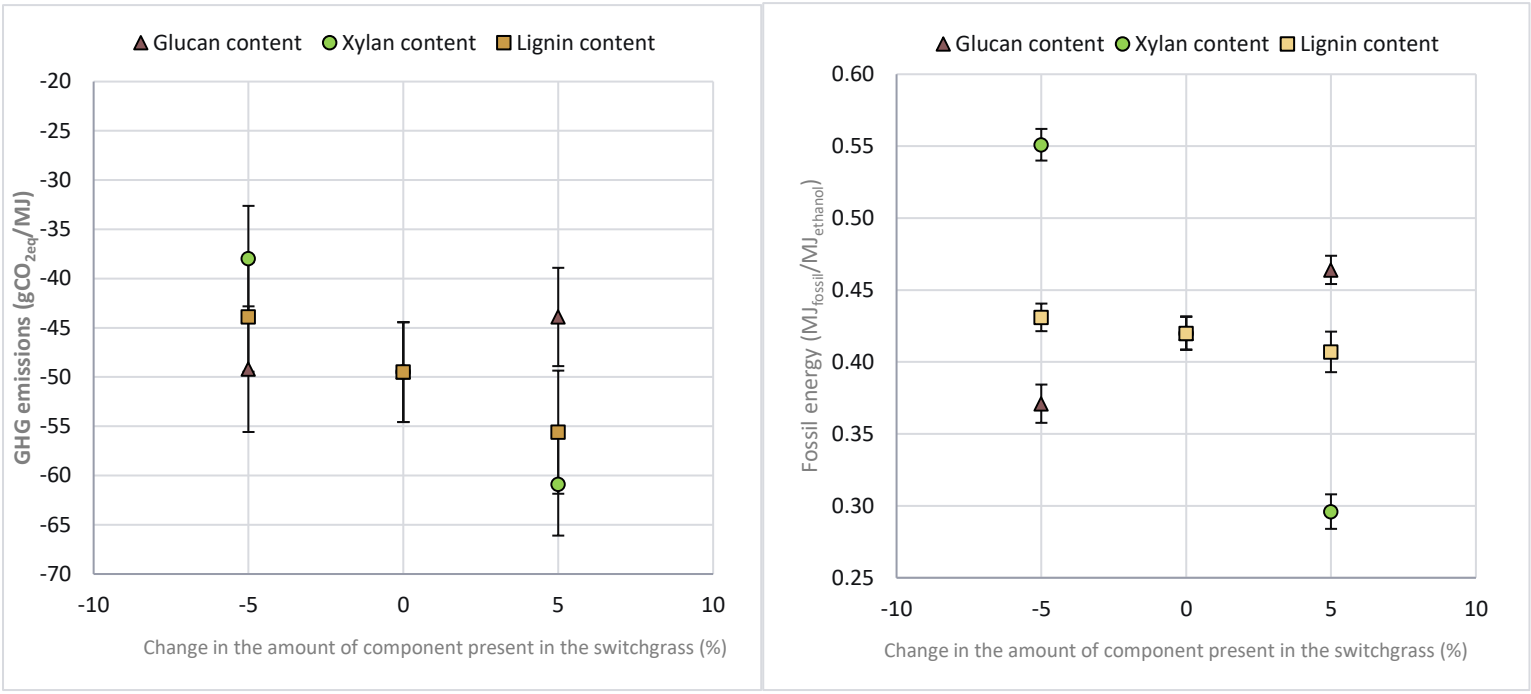


Figure 5.13. Variations in a) GHG emissions and b) fossil energy as a function of switchgrass composition. Cases 5 and 65-70. Results correspond to stochastic WtT LCA (not including ethanol combustion) with error bars showing standard deviation (σ).

Changes in xylan composition had the greatest effect on both GHG emissions and fossil energy usage, due to variations in emission savings due to credits, and to variations in the ratio of sequestered carbon per ethanol generated. This can be observed on Table 5.15 and Table 5.16.

In the proposed biorefinery design, high xylan content was found desirable from both an environmental an economical perspective (see Chapter 3, Section 3.4). Low xylan content in this scenario could lead to a biofuel that does not meet the US requirement for GHG emissions.

Table 5.15. GHG emissions results ($gCO_{2eq}/MJ_{ethanol}$) for different switchgrass compositions. Cases 5 and 65-70

	Base composition	Glucan 37%	Glucan 47%	Xylan 11.5%	Xylan 21.5%	Lignin 19%	Lignin 29%
Sequestered carbon	-321	-342	-305	-313	-330	-297	-346
Switchgrass production and transport	12	13	11	12	11	12	12
CO_2 emissions on the industrial process	200	220	190	200	200	180	220
Chemicals production and transport	69	70	68	68	70	69	69
Ethanol distribution	1	1	1	1	1	1	1
Co-products (furfural, acetic acid, formic acid)	-12	-13	-10	-8	-15	-12	-12
Electricity	-2	-2	-1	-2	-2	-1	-3
Total (WtT)	-49 ± 5	-49 ± 6	-44 ± 5	-38 ± 5	-61 ± 5	-44 ± 6	-56 ± 5
Total (including ethanol use)	21 ± 5	21 ± 6	26 ± 5	32 ± 6	9 ± 5	26 ± 6	14 ± 5

Table 5.16. Fossil energy results ($kJ_{fossil}/MJ_{ethanol}$) and EROI ($MJ_{ethanol}/MJ_{fossil}$) for different switchgrass compositions. Cases 5 and 65-70

	Base composition	Glucan 37%	Glucan 47%	Xylan 11.5%	Xylan 21.5%	Lignin 19%	Liginin 29%
Switchgrass production and transport	44	50	40	46	43	44	44
Chemicals production and transport	825	832	820	824	824	824	827
Ethanol distribution	21	21	21	21	21	21	21
Co-products (furfural, acetic acid, formic acid)	-449	-504	-405	-321	-565	-449	-449
Electricity	-22	-28	-16	-19	-28	-9	-37
Total (WtT)	420 ± 12	371 ± 13	464 ± 10	551 ± 11	296 ± 12	431 ± 10	407 ± 14
EROI (WtT)	2.38	2.69	2.16	1.81	3.38	2.32	2.45

Effect of enzyme dosage

Contribution of enzymes on the environmental impacts studied (GWP through GHG emissions and fossil energy use) for bioethanol is very high (60% of non-biogenic emissions for the biorefinery scenario, not considering credits), consistent with previous reports for biofuels published in the last decade. Prior to work by MacLean and Spatari (2009), studies and widely used models for estimating greenhouse gas emissions did not consider the effects of enzymes and chemicals, but since then studies that accurately consider enzyme use show the importance of enzymes in terms of greenhouse gas emissions(Hong et al., 2012; Janssen et al., 2016; MacLean & Spatari, 2009).

The elevated impacts associated with the enzymes were due to the fossil energy used during its production (Janssen et al., 2016). The LCI data from the NREL database for Celluclast from 2013 was used here for Cellic® CTec2 (see Appendix C). This database reported an associated GHG emission of 4.09 $kgCO_{2eq}/kg_{enzyme}$ and a fossil energy use of 52 MJ_{fossil}/kg_{enzyme}. Reports for Cellic® CTec3 from 2015 inform GHG emissions of 5.5 $kgCO_{2eq}/kg_{enzyme}$ and a fossil energy use of 69 MJ_{fossil}/kg_{enzyme} (Olofsson et al., 2017). The improvement in enzyme formulation and costs does not seem to be associated with a decrease on the environmental impacts associated to the production of enzyme, although improvements in formulation can lead to lower dosages which would decrease their contribution to the environmental impact of bioethanol.

Alternatives to mitigate the effect of enzymes include: on site enzyme production, enzyme recycling, integrated enzyme and ethanol generation (Janssen et al., 2016; Olofsson et al., 2017). Considering the current facilities operating in Uruguay, these are not probable scenarios. Therefore, the best alternative is a reduction on the amount of enzyme consumed. As shown on Figure 5.14, GHG emissions and fossil energy were very sensitive to enzyme dosage, as previously reported by MacLean & Spatari (2009). This tendency also applies to the bioethanol produced in the ethanol and electricity scenario, consequently a considerable reduction of the enzyme dosage could be enough for this fuel to meet the US requirements of GHG emissions savings.

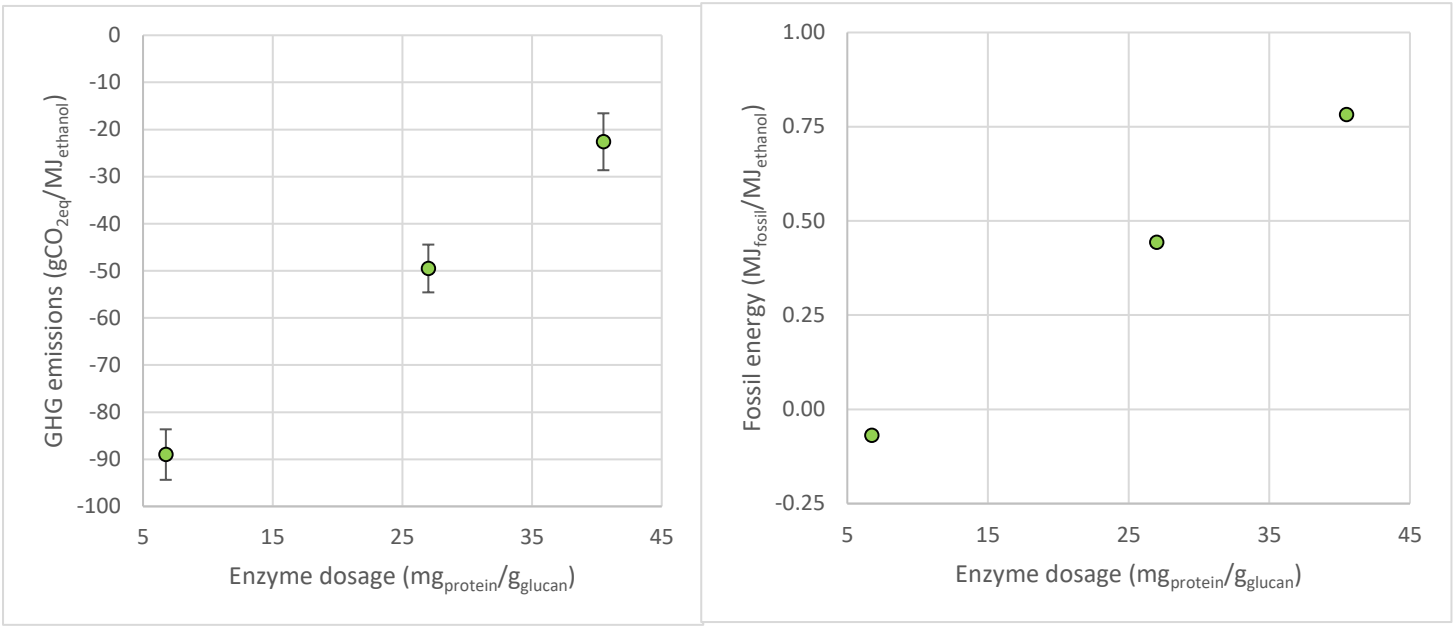


Figure 5.14. Variations in a) GHG emissions and b) fossil energy as a function of enzyme dosage. Cases 5, 15 and 19. Results correspond to stochastic WtT LCA (not including ethanol combustion) with error bars showing standard deviation (σ).

Table 5.17 and Table 5.18 show the great differences in the GHG emissions and fossil energy use in the chemicals production and transport category, due to changes in enzyme dosage.

Table 5.17. GHG emissions results ($gCO_{2eq}/MJ_{ethanol}$) for different enzyme dosages. Cases 5, 15 and 19.

	Enzyme dosage (6.75 $mg_{protein}/g_{glucan}$)	Enzyme dosage (27 $mg_{protein}/g_{glucan}$)	Enzyme dosage (40.5 $mg_{protein}/g_{glucan}$)
Sequestered carbon	-321	-321	-321
Switchgrass production and transport	12	12	12
CO_2 emissions on the industrial process	200	200	200
Chemicals production and transport	29	69	95
Ethanol distribution	1	1	1
Co-products (furfural, acetic acid, formic acid)	-12	-12	-12
Electricity	-2	-2	-2
Total (WtT)	-89 ± 5	-49 ± 5	-23± 5
Total (including ethanol use)	-19 ± 5	21 ± 5	47 ± 5

Table 5.18. Fossil energy results ($kJ_{fossil}/MJ_{ethanol}$) and EROI ($MJ_{ethanol}/MJ_{fossil}$) for different enzyme dosages. Cases 5, 15 and 19.

	Enzyme dosage (6.75 $mg_{protein}/g_{glucan}$)	Enzyme dosage (27 $mg_{protein}/g_{glucan}$)	Enzyme dosage (40.5 $mg_{protein}/g_{glucan}$)
Switchgrass production and transport	44	44	44
Chemicals production and transport	314	825	1163
Ethanol distribution	21	21	21
Co-products (furfural, acetic acid, formic acid)	-449	-449	-449
Electricity	-22	-22	-22
Total (WtT)	-91± 16	420 ± 12	759 ± 11
EROI (WtT)	-	2.38	1.32

Effect of hydrolysis and fermentation efficiencies

Variations in hydrolysis efficiencies had the same effect as variations in fermentation efficiencies on the environmental impacts studied, as shown in Figure 5.15, Table 5.19 and Table 5.20. Both variables affect the amount of ethanol generated in the same way. An increase in ethanol yield is correlated with a decrease in environmental impacts, when these improvements are achieved maintaining enzyme dosage.

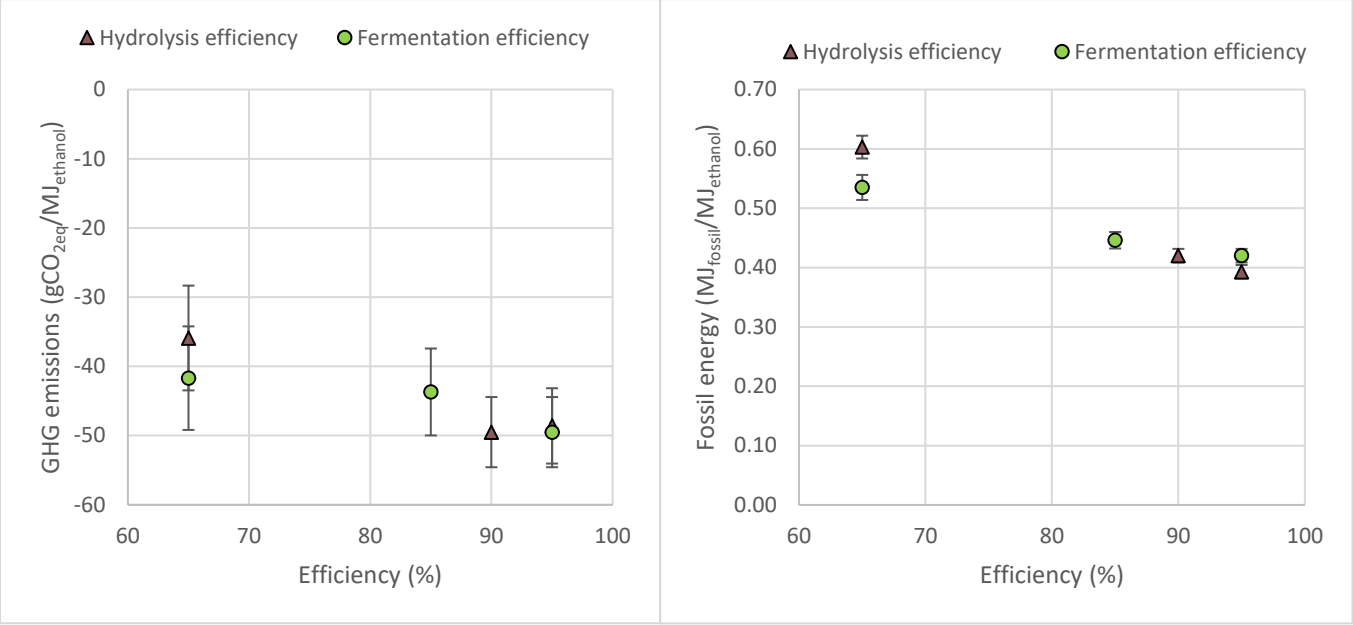


Figure 5.15. Variations in a) GHG emissions and b) fossil energy as a function of hydrolysis efficiency for a fixed fermentation efficiency of 95%, fermentation efficiency for a fixed hydrolysis efficiency of 90%. Cases 5, 26, 29, 35 and 37. Results correspond to stochastic WtT LCA (not including ethanol combustion) with error bars showing standard deviation (σ).

Ethanol yield having a significant effect on environmental factors was an expected result, based on the findings in Chapter 3, and on other works on ethanol production (Janssen et al., 2014). Nevertheless, the net positive effect of a higher yield was not an obvious conclusion. Lower yields increase the amount of material that can be burned, generating more electricity credit. In a country with a higher portion of fossil energy on the electricity grid mix, the GHG and energy savings could offset the effect of a lower ethanol production (Spatari & MacLean, 2010). Table 5.19 and Table 5.20 show the GHG emission and fossil energy use results respectively for different hydrolysis and fermentation efficiencies.

Table 5.19. GHG emissions results ($gCO_{2eq}/MJ_{ethanol}$) for different hydrolysis (H) and fermentation efficiencies(F). Cases 5, 26, 29, 35 and 37

	H (95%), F (92 %)	**H (65%),** F (92 %)	**H (90%), F (92 %)**	H (90%), **F (82.5 %)**	H (90%), **F (63 %)**
Sequestered carbon	-275	-371	-321	-317	-368
Switchgrass production and transport	10	13	12	12	13
CO_2 emissions on the industrial process	168	249	200	208	254
Chemicals production and transport	64	90	69	76	94
Ethanol distribution	1	1	1	1	1
Co-products (furfural, acetic acid, formic acid)	-11	-15	-12	-13	-16
Electricity	-1	-4	-2	-3	-5
Total (WtT)	-49 ± 5	-36 ± 5	-49 ± 5	-44 ± 6	-42 ± 8
Total (including ethanol use)	21 ± 5	34 ± 8	21 ± 5	26 ± 6	28 ± 8

Table 5.20 Fossil energy results ($kJ_{fossil}/MJ_{ethanol}$) and EROI ($MJ_{ethanol}/MJ_{fossil}$) for different hydrolysis (H) and fermentation efficiencies(F). Cases 5, 26, 29, 35 and 37

	H (95%), F (92 %)	**H (65%),** F (92 %)	**H (90%), F (92 %)**	H (90%), **F (82.5 %)**	H (90%), **F (63 %)**
Switchgrass production and transport	41	58	44	49	60
Chemicals production and transport	760	1073	825	905	1122
Ethanol distribution	21	21	21	21	21
Co-products (furfural, acetic acid, formic acid)	-414	-584	-449	-492	-610
Electricity	-12	-56	-22	-34	-62
Total (WtT)	393 ± 11	603 ± 19	420 ± 12	446 ± 14	535 ± 21
EROI	2.54	1.66	2.38	2.24	1.87

Effect of solids content

An increase in solids contents led to energy savings on the industrial stage as discussed in Chapter 3. Due to the renewable nature of the electricity that would be replaced by the surplus generated, this process improvement did not translate into a considerable reduction of the environmental impact, as can be observed on Figure 5.16.

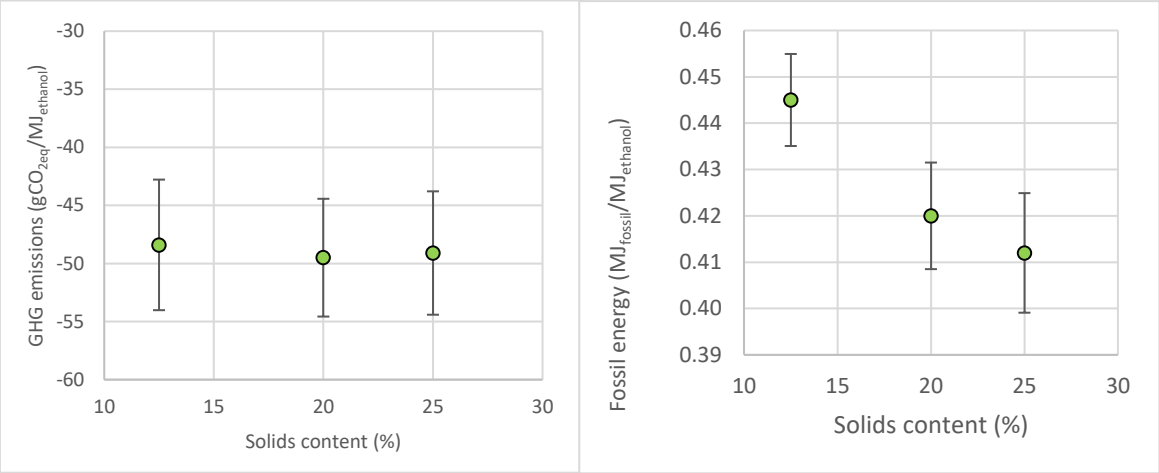


Figure 5.16. Variations in a) GHG emissions and b) fossil energy as a function of solids content. Cases 5, 38 and 42. Results correspond to stochastic WtT LCA (not including ethanol combustion) with error bars showing standard deviation (σ).

Effect of solids content and enzyme dosage using experimental results

Well to tank (WtT) greenhouse gas emissions and fossil energy analysis were performed for the bioethanol production with the experimental hydrolysis yields and conditions previously studied in the Box-Behnken design assays. Stochastic Monte Carlo analysis results are shown on Table 5.21.

Table 5.21. GHG emissions and fossil energy for the experimental design assays, obtained through stochastic WtT LCA.

Assay number	S (%, w/w)	E ($mg_{protein}/g_{glucan}$)	WtT GHG ($gCO_{2eq}/MJ_{ethanol}$)	Fossil energy use ($kJ_{fossil}/MJ_{ethanol}$)
1	15	10	-96 ± 6	-169 ± 13
2	15	70	-40 ± 5	486 ± 8
3	25	10	-104 ± 7	-295 ± 24
4	25	70	-40 ± 4	482 ± 9
5	15	40	-64 ± 5	200 ± 9
6	15	40	-64 ± 5	200 ± 9
7	25	40	-66 ± 5	189 ± 11
8	25	40	-66 ± 5	189 ± 11
9	20	10	-97 ± 7	-213 ± 18
10	20	10	-98 ± 7	-221 ± 18
11	20	70	-31 ± 5	491 ± 9
12	20	70	-39 ± 5	481 ± 9
13	20	40	-67 ± 5	188 ± 9
14	20	40	-63 ± 5	190 ± 9
15	20	40	-62 ± 5	189 ± 10

A model to represent the variation of greenhouse gas emission (GHG) as a function of dimensionless normalized variables for solids loading (x_1) and enzyme dosage (x_2) was found (see Equation 5.2).

$$GHG\left(\frac{gCO_{2eq}}{MJ}\right) = -64.81 + 30.65x_2 + 1.88\, x_1 * x_2 - 3.66\, x_2{}^2 \ (r^2 = 0{,}98) \quad (5.2)$$

The proposed quadratic model is a good representation of variations on the mean of GHG emissions as shown by its significant regression and non-significant lack of fit. To take into account the effect of uncertainty on the data, a standard deviation of 5 $gCO_{2eq}/MJ_{ethanol}$ was assigned to values calculated using the model.

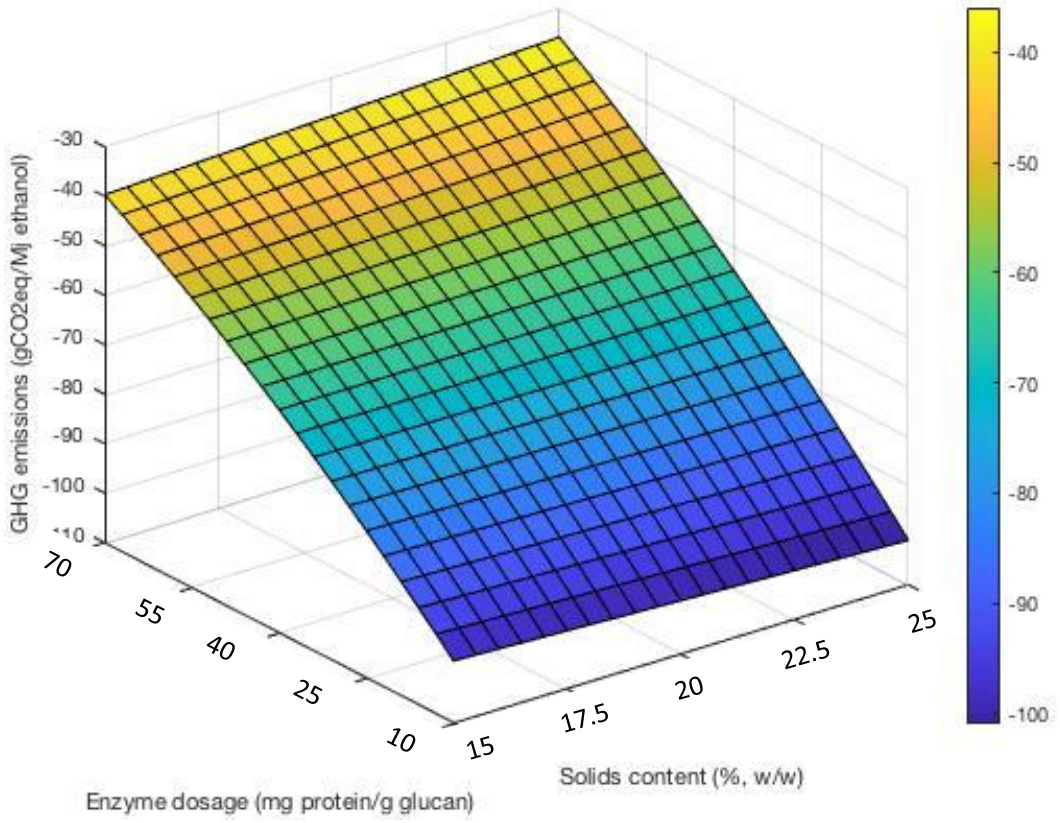


Figure 5.17 Variations of GHG emissions as a function of enzyme dosage and solids content, according to the proposed quadratic model.

As shown both by Equations 5.2 and Figure 5.17, enzyme dosage was the only significant factor for GHG emissions (solids content was not significant but was kept on the model to ensure lack of fit). The minimum enzyme dosage (10 $mg_{protein}/g_{glucan}$) minimized GHG emissions and would lead to negative fossil energy use (as shown by assays 1,3, 9 and 10).

A multi objective optimization was used to find an economical-environmental optimal focusing on reducing GHG emissions and minimizing MESP for the base enzyme cost (4.24 $/kg protein). An enzyme dosage of 36.8 $mg_{protein}/g_{glucan}$ and solids content of 20.7 % were the optimal conditions, producing bioethanol with an associated GHG value of -68 $\pm$ 5 g $CO_{2eq}/MJ_{ethanol}$ and a MESP of 0.838 $/L. The fossil energy use for this conditions would be close to 190 $\pm$ 9 $kJ_{fossil}/MJ_{ethanol}$, which correspond with an EROI of 5, higher than the EROI associated to fossil fuels at the point of use as calculated by Hall et al. (2009). This fuel bioethanol would have an adequate MESP for advanced biofuels (competitive at oil prices over 100$/barrel) and would comply with GHG emissions reduction requirements. These values were obtained from models with good fit, that used experimental data on hydrolysis efficiency for different solids content and enzyme dosage, therefore, an environmentally sustainable production (in terms of GHG end fossil energy) of fuel bioethanol from switchgrass could be possible with the technology and yields currently available on the laboratory.

Comparison with other studies

Comparison with other studies from switchgrass (apart from the already mentioned in the discussion) are difficult due to the differences in systems analyzed and assumptions for the LCA methodology (see Table 5.22).

Table 5.22. Comparison of GHG and EROI for different scenarios of ethanol production from biomass through fermentation.

Case	GHG ($gCO_{2eq}/MJ_{ethanol}$)	EROI ($MJ_{ethanol}/MJ_{fossil}$)	Comments	Source
Sugarcane in Paysandú, Uruguay	33.45	7.0	Does not consider ethanol use. Does not account for sequestration. Considers CO_2 from fermentation.	Herrera et al. (2015)
Sweet sorghum in Paysandú, Uruguay	19.7-58.5	-	Attributional life cycle assessment. IPCC tier 1 and 3. Different crop rotations	Adler et al. (2018)
Grain sorghum in Paysandú, Uruguay	19.9-33.9	-	Attributional life cycle assessment. IPCC tier 1 and 3. Different crop rotations	Adler et al. (2018)
Grain sorghum in Paysandú, Uruguay	28.6	2.4	Does not consider ethanol use. Does not account for sequestration. Consider CO_2 from fermentation.	Herrera et al. (2017)
Grain sorghum in Paysandú, Uruguay	35.7	2.5	Considers carbon neutrality and is therefore comparable with the GHG including use.	Olave (2015)
Switchgrass to ethanol (dilute acid)	23.1	-	Low enzyme dosage.	Spatari et al. (2005)
Switchgrass to ethanol (dilute acid)	-10-100 approx.	-	Includes LUC. Uncertainty analysis	Spatari & MacLean (2010)
Switchgrass to ethanol (AFEX)	20-100 approx.	-	Includes LUC. Uncertainty analysis	Spatari & MacLean (2010)
Switchgrass to ethanol	-23.1	-	Includes LUC. Not clear on how industrial phase chemicals are considered.	Adler et al. (2007)
Switchgrass biorefinery (phenols)	20-43	3.6	No allocation, GHG emissions assigned to ethanol for comparison. Does not specify enzyme usage. Considers LUC.	Cherubini & Jungmeier (2010)
Switchgrass to ethanol (AFEX)	51		Allocation based on energy content. Expressed per MJ of E85 fuel. Enzyme production simulated.	Bai et al. (2010)
Switchgrass to ethanol (dilute acid)	30-60		Onsite enzyme production. Variation on several conditions is studied.	Hsu et al. (2010)
Switchgrass to ethanol and electricity (considering ethanol use)	67	1.6		This work
Switchgrass biorefinery (considering ethanol use)	21	2.38		This work
Switchgrass biorefinery with low enzyme dosage (considering ethanol use)	-19	-		This work
Switchgrass biorefinery with high xylan content (considering ethanol use)	9	3.38		This work
Switchgrass biorefinery experimental optimal (considering ethanol use)	2	5		This work

In comparison to the LCAs for ethanol production in Uruguay from other feedstocks, the ethanol produced from switchgrass with the ethanol and electricity process was worse in terms of environmental performance. The bioethanol produced in the biorefinery fared better in terms of GHG emissions but not in terms of fossil energy usage. Even though the processes and yields are diverse, a great part of the difference can be explained by the impact of enzymes. The ethanol production from sugarcane does not require enzymes and grain sorghum required very low

quantities of different enzymes (information on the impact considered for their production was not shown) (Herrera et al., 2015, 2017). Bioethanol produced from switchgrass in a biorefinery using lower enzyme dosage (case 15) or with higher xylan content in switchgrass (case 68) had very good environmental performances, but they are not currently easy to achieve.

The bioethanol produced from switchgrass in a biorefinery working at the optimal conditions (for both economics and GHG emissions) found from the models for experimental data had a better environmental performance than the ethanol from the other feedstocks analyzed in Uruguay.

Considerations about the electricity displaced

The environmental analyses previously presented assumed that the electricity displaced had the same source distribution as the average electricity grid mix calculated over the past four years (See Table 5.9).

Different assumptions could be made about the nature of the energy displaced. It could be considered that the surplus electricity displaced the marginal unit of electricity at peak demand (usually mostly fossil), or that it displaced energy from biomass (renewable). To assess how these considerations affect the grid electricity credit and environmental results, extreme scenarios of 0% grid displacement and 100% grid displacement of gas/fuel oil were analyzed.

As shown in Table 5.23 and Table 5.24 the 100 % grid displacement of gas/fuel oil would considerably improve the environmental results due to higher credits. In this scenario changes in lignin would have a similar effect to the effect of changes in xylan in the environmental performance of the process, with high lignin content and high xylan content being the conditions with lower GHG emissions and fossil energy consumption. This would also affect the conclusions regarding the effect of hydrolysis and fermentation efficiencies, with higher efficiencies leading to worst environmental performance, consistent with works with high electricity credits as Spatari & MacLean (2010). In this scenario solids contents do affect environmental performance, with higher solids contents having less GHG emissions and fossil energy consumption. Nevertheless, the replacement of high amounts of fossil energy is not very likely in the current situation for Uruguay where only 4% of the energy used in 2017 was from fossil sources.

The scenario in which all the grid displacement is attributed to renewable energy (0 % displacement of gas/fuel) shows very similar results to those found for the replacement of energy with the same distribution as the whole grid, as the fossil fraction was already quite low (9 ± 5 %)

Table 5.23. WtT GHG emissions ($gCO_{2eq}/MJ_{ethanol}$) results for different assumptions about electricity displaced.

Case	Description	Percentage of the energy displaced that comes from fossil fuels.		
		0	9 ± 5 (base case)	100
1	Ethanol and electricity	-1 ± 4	-2 ± 4	-27 ± 4
4	Xylose not fermented	15 ± 6	13 ± 6	-38 ± 6
5	Biorefinery	-48 ± 6	-50 ± 5	-65 ± 5
65	Low glucan content	-48 ± 6	-49 ± 6	-69 ± 17
66	High glucan content	-43 ± 5	-44 ± 5	-55 ± 5
67	Low xylan content	-37 ± 6	-38 ± 5	-51 ± 7
68	High xylan content	-60 ± 5	-61 ± 5	-80 ± 6
69	Low lignin content	-44 ± 5	-44 ± 6	-51 ± 5
70	High lignin content	-54 ± 6	-56 ± 6	-82 ± 6
15	Low enzyme dosage	-88 ± 5	-89 ± 5	-104 ± 5
19	High enzyme dosage	-22 ± 5	-23 ± 6	-38 ± 5
26	High hydrolysis efficiency	-48 ± 5	-49 ± 5	-57 ± 5
29	Low hydrolysis efficiency	-33 ± 7	-36 ± 8	-75 ± 7
35	High fermentation efficiency	-42 ± 7	-44 ± 6	-68 ± 6
37	Low fermentation efficiency	-39 ± 8	-42 ± 7	-85 ± 8
38	High solids content	-48 ± 5	-49 ± 5	-69 ± 7
42	Low solids content	-48 ± 5	-48 ± 6	-53 ± 7

Table 5.24. WtT Fossil energy consumption ($kJ_{fossil}/MJ_{ethanol}$) results for different assumptions about electricity displaced.

Case	Description	Percentage of the energy displaced that comes from fossil fuels.		
		0	9 ± 5 (base case)	100
1	Ethanol and electricity	626 ± 8	592 ± 12	253 ± 8
4	Xylose not fermented	821 ± 10	750 ± 21	40 ± 10
5	Biorefinery	442 ± 10	420 ± 12	206 ± 11
65	Low glucan content	398 ± 11	371 ± 13	93 ± 10
66	High glucan content	478 ± 9	464 ± 10	309 ± 9
67	Low xylan content	570 ± 10	551 ± 11	366 ± 11
68	High xylan content	323 ± 9	296 ± 12	18 ± 9
69	Low lignin content	441 ± 10	431 ± 10	339 ± 10
70	High lignin content	443 ± 10	407 ± 14	36 ± 10
15	Low enzyme dosage	-70 ± 10	-91 ± 16	-307 ± 10
19	High enzyme dosage	779 ± 10	759 ± 11	542 ± 10
26	High hydrolysis efficiency	404 ± 9	393 ± 11	269 ± 9
29	Low hydrolysis efficiency	658 ± 13	603 ± 19	48 ± 13
35	High fermentation efficiency	479 ± 10	446 ± 14	106 ± 11
37	Low fermentation efficiency	595 ± 13	535 ± 21	-83 ± 15
38	High solids content	439 ± 10	412 ± 13	134 ± 10
42	Low solids content	451 ± 10	445 ± 10	384 ± 10

Uncertainty analysis and limitations of the study

The uncertainties in the data were considered for both the foreground and background systems. Uncertainties used in the foreground system were presented in Table 5.8 and Table 5.9. For the background system, uncertainties consisted of those already defined in the corresponding database (Ecoinvent database includes uncertainty in most of its flows). The percentage of values including uncertainty data amounted to 63.3%, and they were analyzed using the Monte Carlo stochastic method.

The results shown in the previous sections included the uncertainty informed as the standard deviation (σ) for total WtT GHG emissions and fossil energy in the Tables and Figures. The effect of different parameters previously discussed was analyzed taking into account these uncertainties. Significant differences (p<0.05) were found for every parameter. Therefore, conclusions from the sensitivity analysis for environmental sustainability aspects reported are reliable since they considered uncertainties.

Uncertainty on the data does affect the comparison of GHG results found with the GHG of fossil fuel baselines, and GHG reduction requirements set by the US regulatory framework. Figure 5.18 shows GHG emissions in gCO_{2eq}/MJ_{fossil} with error bars of two times the standard deviation of that value (2σ). This means that 95% of the data with a normal distribution would be included in the error.

It can be concluded with confidence, that bioethanol produced in all scenarios would lead to reduction in GHG emissions in comparison with the fossil fuel reference, except for case 4 (xylose not fermented to ethanol nor used for co-products). When comparing with the reduction requirements set by the regulatory frameworks, a confident conclusion (95% confidence) is not possible for some production scenarios. It can be said with confidence that bioethanol from switchgrass would meet the GHG emission requirements when produced in a biorefinery operating in the following conditions:

- base conditions (case 5)
- using switchgrass with low glucan content (case 65)
- using switchgrass with high xylan content (case 68)
- using switchgrass with high lignin content (case 70)
- using low enzyme dosage (case 15)
- high hydrolysis and fermentation efficiencies (case 26)
- high and low solids content (cases 38 and 42)
- working at the experimental conditions from some of the Box-Behnken assays (BB1, BB3, BB5-BB10 and BB13-BB15)

- working at the conditions found to optimize GHG and MESP (OPT).

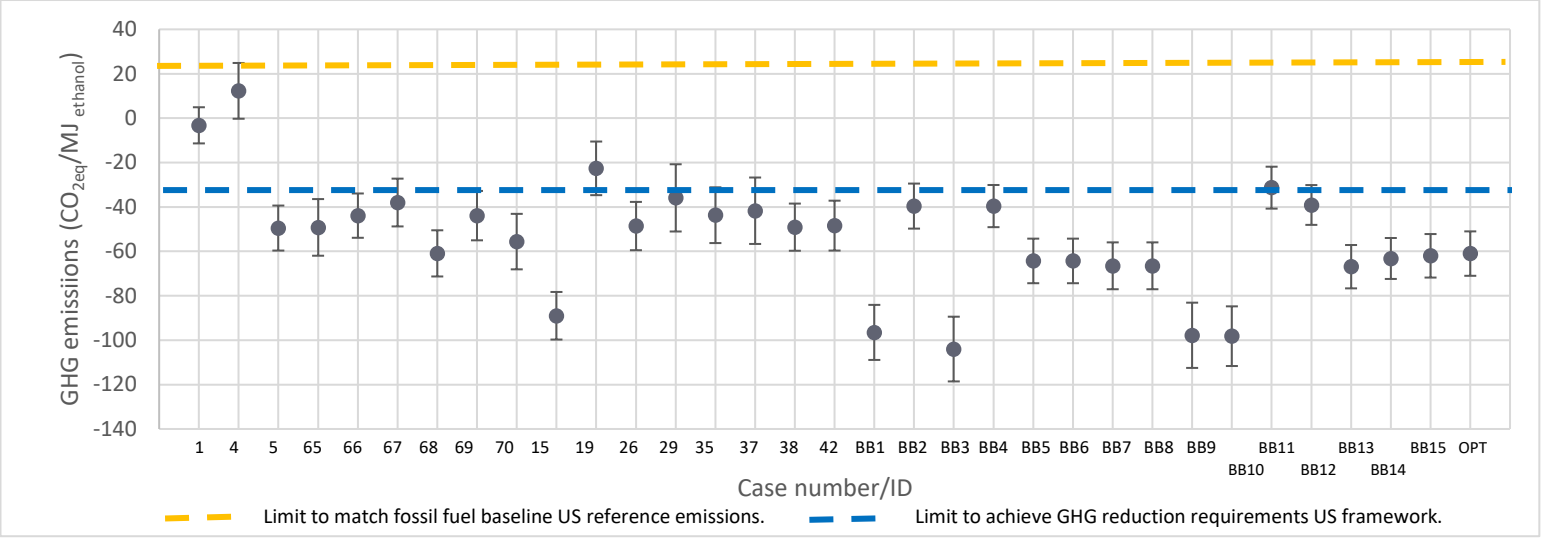


Figure 5.18. GHG emissions data for all cases analyzed, including error corresponding to two times the standard deviation (2σ) obtain through stochastic Monte Carlo analysis of the WtT system.

Uncertainty in the foreground data could be reduced with more information regarding switchgrass production in Uruguay. More or larger studies on agricultural yields and fertilizer use for switchgrass in Uruguay like the one from Sir-Prieto et al. (2017), would be desirable. Data on N_20 emissions from fertilizer, land use change and logistics for switchgrass in Uruguayan soil are not available. Nevertheless, these could be more accurately estimated using a DayCent modeling approach to estimate soil organic carbon change and N_2O as shown by Adler et al. (2018) or using Tier 3 models to approximate N_2O emissions (Del Grosso et al., 2010; Adler et al., 2012), with more knowledge about the land in which switchgrass could be stablished. These studies are necessary to decrease uncertainty and improve precision on the results.

As mentioned through this work, enzyme, and co-products have a great impact on LCAs. More or better literature data for these stages would be desirable to improve the precision of GHG and fossil energy values obtained.

A more accurate estimation of ethanol use emissions could be done including estimates on ethanol gasoline mixes and average engine efficiency with this fuel in the near term for Uruguay, but if this information is not accurate and precise it could lead to a higher error in the determinations than the assumptions made here.

5.4. Chapter conclusions

Bioethanol produced from switchgrass in all scenarios studied would reduce GHG emissions when compared with fossil fuel baseline (93.3 g CO_2/MJ $_{fossil\ fuel}$) (except when xylose is not fermented nor used for co-products).

Switchgrass production under Uruguayan conditions had lower GHG emissions than reported for other locations due to the agricultural yield (15.5 t/ha) and low nutrient requirement (3 kg N/t).

High GHG emissions on the ethanol production stage were associated with enzyme usage. It accounts for a 60-70% of the GHG emissions (not considering biogenic carbon or credits). This is consistent with other works that informed a high impact of enzymes and chemicals on the environmental performance of biofuels (Hong et al., 2012; Janssen et al., 2016; MacLean & Spatari, 2009).

A reduction in the amount of enzyme consumed, due to improvements in enzyme formulation or to optimization of hydrolysis conditions had the most significant effect on GHG emissions and fossil energy of all the variables analyzed. A reduction of the enzyme dosage used would be enough for bioethanol produced in the ethanol and electricity process to meet GHG emissions savings US (Environmental Protection Agency, 2010).

Bioethanol produced in the biorefinery was better than the ethanol and electricity facility in terms of the environmental performance for the impacts analyzed and meets the reduction requirement set by the US regulatory framework.

Credits associated with electricity were low due to the high contribution of renewable energy to the Uruguayan electricity grid mix, resulting in less GHG emissions and fossil energy displaced. In this scenario an alternative use for the lignin such as the production of high value co-products should be explored.

Even though it was produced in higher quantities, savings due to furfural credits were low in comparison to those from formic and acetic acid. This was expected, as furfural is already produced from biomass. Different co-products (produced from oil) could be selected depending if the main objective relates to higher reduction of GHG emissions, minor fossil energy usage, or market value.

For the biorefinery scenario, xylan was the most significant switchgrass component in terms of its effect on the environmental impacts studied. High xylan content was desirable in the proposed biorefinery design.

Hydrolysis efficiency and fermentation efficiency increased ethanol production yields and led to lower environmental impacts.

An increase in solids contents led to energy savings on the industrial stage but this process improvement did not translate into a considerable reduction of the environmental impact of the produced biofuel. This is due to the renewable share of the electricity that would be replaced by the surplus electricity generated.

The bioethanol produced from switchgrass in the ethanol and electricity process had a worst environmental performance than other ethanol production alternatives for Uruguay (sugarcane, sorghum grain). However, the bioethanol produced from switchgrass in the biorefinery was better in terms of GHG emissions than the alternatives. Some of the biorefinery scenarios analyzed, such as working with low enzyme dosage or with a switchgrass with high xylan, produced ethanol with a great environmental performance, but they are not currently achievable.

Bioethanol produced in a biorefinery working at the optimal conditions for both economics (MESP) and environmental aspects (GHG emissions) found from the models based on experimental data had a good environmental performance (better than the Uruguayan alternatives), complied with US emission reduction requirement, and had good process economics (within the expected range for advanced biofuels and competitive with fossil fuel for oil prices above 100 $/ barrel).

Therefore, environmentally sustainable production (in terms of GHG end fossil energy) could be possible with the technology and yields currently available on the laboratory. Scale-up of these processes is a critical aspect of its technical feasibility.

Chapter 6: Conclusions

"The feeling is less like an ending than just another starting point."

Chuck Palahniuk

Switchgrass (Alamo variety) grown experimentally in Uruguay, had a high glucan and low xylan content (43.4 ± 0.3 and 16.5 ± 1.3 % w/w dry base, respectively). High xylan content was desirable from both an economic and environmental perspective, in a biorefinery scenario. Therefore, crop improvement should be aimed to higher xylan contents if hemicellulose is used for the production of furfural, acetic and formic acid.

LHW pretreatment at 200ºC for 5 min proved to be a suitable pretreatment technology for a biorefinery approach, showing high xylan removal (71%) with high recovery percentages (103± 2, 96 ± 9, and 86 ± 3 % of lignin, xylan, and glucan respectively), and low concentrations of inhibitors (acetic acid, formic acid, 5-hydroxymethyl furfural, and furfural).

Techno-economic analysis results based on literature data and experimental switchgrass composition showed that:

- The production cost obtained for ethanol in a facility producing only ethanol and electricity was within the expected price range for advanced alcohol fuels and could compete with oil prices above 100 $/ barrel.
- An energy-driven biorefinery strategy producing furfural, acetic and formic acid led to a lower MESP, increasing the value obtained from biomass to sustain fuel/energy production, which should be considered in order to minimize cost.
- Operative parameters such as enzyme dosage, hydrolysis and fermentation efficiencies and solids content had a high impact on MESP and should be taken into account to decrease MESP.

Washing the solids after pretreatment improved hydrolysis efficiency. Two washing steps with 10 g distilled water per gram of dry matter were enough to reach the maximum hydrolysis efficiency (85 ± 1 %).

Changes in initial pH had a significant effect on the efficiency obtained after 72 hours of hydrolysis for all solids content. The optimal initial pH value for hydrolysis was different for the low and high solids hydrolysis (4.8, 6 and 6 for 15%, 20%, and 25% solids content respectively).

Models obtained from experimental results for enzymatic hydrolysis of cellulose show that solids content and enzyme dosage had a significant effect on glucose concentration and hydrolysis efficiency. Xylanase substitution had no significant effect on any of these variables.

The variations in MESP found for the experimental assays were similar to the differences reported between different pretreatments for switchgrass. Consequently, optimizing hydrolysis conditions could be as important as pretreatment selection to minimize MESP.

The solids content that minimized MESP were similar for both enzyme costs (21 %), but the optimal enzyme dosage increased from 37 to 43 mg $_{protein}$ /g_{glucan} (for 4.24 and 3 $/ $kg_{protein}$ respectively), highlighting the importance of accurate estimation of enzyme cost.

Bioethanol produced from switchgrass in all scenarios studied would reduce GHG emissions when compared with fossil fuel baseline.

The biorefinery ethanol production scenario was better than the ethanol and electricity process in terms of the environmental impacts analyzed and the bioethanol produced would meet the GHG emissions reduction requirement set by the US regulatory framework.

Switchgrass production under Uruguayan conditions had lower GHG emissions than reported for other locations due to its agricultural yield (15.5 t/h) and to low nutrient requirements (3kg N/t).

High percentage of GHG emissions from the ethanol production stage were associated with enzyme usage (60-70% of emissions). A reduction on the amount of enzyme consumed, had the most significant effect on GHG emissions and fossil energy of all the variables analyzed.

The renewable nature of the Uruguayan electricity grid mix led to low credits associated with electricity co-product. This explains why an increase in solids contents does not reduce the environmental impact even if it leads to energy savings on the industrial stage.

Credits associated with furfural were relatively low compared with acetic and formic acid due to the fact furfural is already produce from biomass.

The bioethanol produced from switchgrass in the ethanol and electricity process had a worst environmental performance than other ethanol production alternatives for Uruguay (sugarcane, sorghum grain). However, the bioethanol produced from switchgrass in the biorefinery was better in terms of GHG emissions than these alternatives. Some of the biorefinery scenarios analyzed, such as working with low enzyme dosage or with a switchgrass with high xylan, produced ethanol with a great environmental performance, but they are not currently achievable.

Xylan content and enzyme dosage are parameters that highly influence economic (MESP) and environmental aspects (GHG emissions and fossil energy use). Agronomic research should focus on improving xylan content in the switchgrass. Research and development aimed at reducing enzyme dosage use while achieving high hydrolysis efficiencies is still of uttermost importance.

Bioethanol produced in a biorefinery working at the optimal conditions for both economics (MESP) and environmental aspects (GHG emissions) found from the models based on experimental data had a good environmental performance (better than the Uruguayan alternatives), complied with US emission reduction requirements, and had good process economics (within the expected range for advanced biofuels and competitive with fossil fuel for oil prices above 100 $/ barrel).

Therefore, an environmentally and economically sustainable production (in terms of GHG end fossil energy, and for oil prices higher than 100 $/barrel or including carbon taxes respectively) could be possible with the technology available and yields achieved on the laboratory. Scale-up of these processes is a critical aspect of its technical feasibility. Other environmental and social aspects should be taken in consideration for a sustainable production, but they are outside the scope of this work

Future work should focus on the following areas:

Further enzymatic hydrolysis studies should aim to understand the causes of differences in optimal initial pH for different solids content. Understanding this interaction could lead to optimization of enzyme activity and therefore to reductions in enzyme dosage use, since as it was previously discussed, enzyme dosage greatly affects economic and environmental aspects. More research focusing on enzyme use reduction is needed.

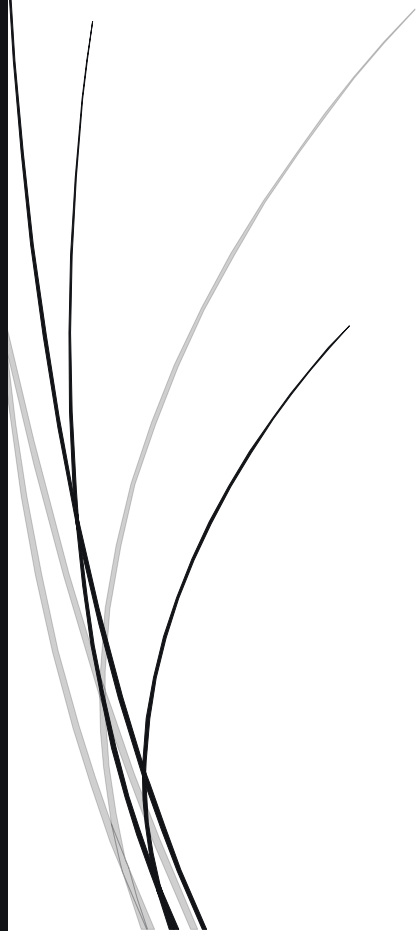
New experimental assays should be performed to study the effect of enzyme dosage and solids content for parameter values closer to the conditions found to optimize MESP and GHG emissions (21% solids content, 37 mg $_{protein}/g_{glucan}$) in order verify or improve the accuracy in the determination of the optimal conditions.

Validation of the enzymatic hydrolysis models for glucose concentration and hydrolysis efficiency at a larger scale.

Study of the fermentation at the optimal hydrolysis conditions (minimizing GHG and MESP), optimizing nutrient addition.

Improving the reliability of the life cycle assessment model, with more information and better estimations for agricultural yields, fertilizer use, data on N_20 emissions from fertilizer, land use change and logistics for switchgrass in Uruguay, are generated.

Chapter 7: References



"An original idea.

That can't be too hard.

The library must be full of them."

Stephen Fry

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