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Effect of solids loading on ethanol production: Experimental, Economic and Environmental analysis.

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Highlights:

- Hydrolysis and fermentation were conducted at 19%, 30%, and 45% solids
- Corn stover at 45% solids loading released 205 ± 25.8 g/L glucose and resulted in 115.9 ± 6.7 g/L ethanol after 60h of fermentation
- Techno-economic analysis revealed an ROI of 8% at 45% solids loading

Abstract

This study explores the effect of high-solids loading for a fed batch enzymatic hydrolysis and fermentation. The solids loading considered was 19%, 30% and 45% using wheat straw and corn stover as a feedstock. Based on the experimental results, techno-economic analysis and life cycle assessments were performed. The experimental results showed that 205 ± 25.8 g/L glucose could be obtained from corn stover at 45% solids loading after 96h which when fermented yielded 115.9 ± 6.37 g/L ethanol after 60h of fermentation. Techno-economic analysis showed that corn stover at 45% loading yielded the highest ROI at 8% with a payback period less than 12 years. Similarly, the global warming potential was lowest for corn stover at 45% loading at -37.8 gCO₂ eq./MJ ethanol produced.

Keywords: solids loading; enzymatic hydrolysis; fermentation; lignocelluloses; techno-economic analysis; environmental impacts

1 Introduction

The need for liquid fuels is increasing the global oil consumption expected to reach 100 million barrels by 2019 (US Energy Information Administration, 2017) and ethanol is one of the promising alternatives to replace fossil fuels. Though the in-field feedstock availability is high between 10-50 billion tons (dry weight) (Rajendran & Taherzadeh, 2014; Zhao et al., 2009) the challenges range from harvesting and transportation logistics, process challenges such as complexity of biomass structure and overall hydrolysis efficiency. Several attempts were made in the past decades to produce ethanol at an economically competitive prices however, there are several hurdles such as increasing the efficiency of the enzymes, efficient release of glucose during hydrolysis, decreasing the energy consumption during pretreatment step, and availability of commercial scale equipment (Mussatto et al., 2010). Considerable efforts were made with respect to the conversion of lignocelluloses to ethanol including developing various pretreatment methods, studying the enzyme interactions with cellulose and lignin, increasing the solids loading during enzymatic hydrolysis and several types of process integration improvements such as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) (Brodeur et al., 2017; da Silva Martins et al., 2015; Hahn-Hägerdal et al., 2006; Jin et al., 2017; Liu et al., 2015; Margeot et al., 2009).

Solids loading during enzymatic hydrolysis is usually in the range of 10- 20% w/w basis (Humbird et al., 2011) however some studies considered a higher solids loading between 25-40% (Jørgensen et al., 2007; Zhang et al., 2010; Zhu et al., 2011). Few concerns with the high-solids loading are that the mixing of the materials becomes very difficult beyond 20% solids due to unavailability of free water, dead zone formation due to improper mixing, available free

water decreases with time as the sugar concentration increases and the product inhibition of the cellulases has a significant effect on the hydrolysis efficiencies (Zhang et al., 2010). To address these concerns, fed-batch methods were proposed where solids were loaded at regular intervals so that the mixing is not impacted and can yield more glucose at high-solids loaded (da Silva Martins et al., 2015; Liu et al., 2015). To the best of our knowledge, no previous work reported solids loading exceeding 40%.

The overall goal of this study was to provide a holistic view of the effect of solids loading for the lignocellulosic ethanol production through experimental, economic and environmental perspectives. The first objective of this study was to evaluate the effect of solids loading (19%, 30%, and 45%) on lignocellulosic biomass (Corn stover and wheat straw) for enzymatic hydrolysis and ethanol fermentation. The second objective was to use the experimental data from this work in conjunction with other reported literature and develop process models to analyze the techno-economic feasibility of a biomass processing facility with a capacity of 60,000 dry MT/year. Sensitivity analysis was performed on the several factors including, capacity, ethanol price, biomass cost, and enzyme cost. The third objective was to use the mass and energy balance data from the techno-economic studies to investigate environmental impacts through a life cycle assessment.

2 Materials and methods

2.1 Biomass

Wheat straw (WS) and corn stover (CS) were considered for the enzymatic hydrolysis and fermentation studies including. The dilute acid pretreated biomass was obtained from a pilot facility at Boardman, Oregon. The composition of the pretreated biomass was measured

according to the NREL procedures (Sluiter et al., 2008; Sluiter et al., 2012). The obtained biomass was washed with three folds' water (W/W) and the pH was adjusted to 5.5 using sodium hydroxide. The pH adjusted and washed biomass was squeezed with hand press to remove additional water and air dried at ambient temperatures until the moisture content was 30% (W/W) (Sluiter et al., 2008; Sluiter et al., 2012). This air-dried biomass was used for all the experiments and further analysis. All experiments were conducted in triplicates.

2.2 Reactor

A stainless reactor with 3 L working volume was used for enzymatic hydrolysis and fermentation. The overall volume was 3.5 L with an internal diameter of 14.5 cm and a height of 21.5 cm. A helical impeller with 10.16 cm diameter was used in the reactor to support complete mixing (Fig. 1). The helical impeller was driven by IKA impeller drive (model: EUROSTAR 60 CS1) which could control impeller speed. The impeller rotational speed was set at 150 rpm for hydrolysis process (He et al., 2014). The reactor was placed in a water bath at 50°C for enzymatic hydrolysis, and 30°C for the fermentation process.

2.3 Enzymatic hydrolysis

Three fed-batch enzymatic hydrolysis experiments were conducted for the two biomasses (WS and CS). The first experiment used WS with a final solid loading of 19% w/w. The experiment was started with 10% solids and remaining biomass was added in two increments of 4.5% w/w at 3h intervals. For the second experiment, WS was initiated with 20% (W/W) solids and then remaining biomass was added in two increments at 3h intervals to reach a final solids content of 30% (W/W). Third fed-batch enzymatic hydrolysis was conducted using corn stover initiated with 30% (W/W) solids. The remaining biomass was added at 4h intervals to make up to 45%

solids. The cellulose enzymes were donated by Novozymes (Ctec2) and were added at the rate of 20 mg protein/g glucan in a single dose at the start of the experiment. This was done to achieve high enzymatic hydrolysis rates initially to liquefy biomass and reduce the insoluble solids content. All the experiments were conducted in triplicates and samples were taken at every 3h for the first 24h; subsequently, the sampling frequency was decreased to one sample every 24h. The samples were centrifuged at 10,000 rpm for 5 min and the supernatant was stored at -20°C until further analysis using HPLC.

2.4 Fermentation experiments

Active dry yeast, *Saccharomyces cerevisiae*, from BIO-FERM® XR was inoculated at a concentration of 1.0 g/L to the enzymatically hydrolyzed slurry. Urea (1.0 g/L) was added to provide nitrogen requirements of the yeast. Tetracycline (stock solution: 5mg in 70% ethanol) was added at a rate of 400 µl/L from the stock solution to limit the bacterial activity. After inoculation with yeast and other chemicals as described above the enzymatically hydrolyzed slurry was fermented at 30°C for a maximum period of 120 h. The rotational speed of the impeller was reduced from the enzymatic hydrolysis experiments to 30 rpm. Samples were taken every 3h for the first 24h and subsequently once in a day. The samples were centrifuged and stored until further analysis as described above.

2.5 Analytical methods

The stored samples from enzymatic hydrolysis and fermentation experiments were analyzed for various sugars and ethanol concentration using HPLC. Biorad Aminex HPX-87H column was used for the analysis which was equipped with an appropriate guard column. Sulfuric acid (5 mM) was used as an eluent, at a flow rate of 0.6 mL/minute and the column temperature was

maintained at 60°C. A statistical evaluation was carried out for the three different experiments using T-test with a 95% confidence interval. The glucose and ethanol concentrations in the various experiments were used to run the statistical evaluation in Minitab 16 software.

2.6 Techno-economic analysis

The experiments conducted in this study were modeled to understand the effect of solids loading on enzymatic hydrolysis and fermentation. Eight scenarios were simulated including the three experimental studies described above and other literature reported values for wheat straw and corn stover. The scenarios were labeled based on their feedstock and solids loading, for example, CS45 refers to corn stover at a solid loading of 45%. Similarly, wheat straw was labeled as 'WS', and Banagrass was labeled as 'BG'. The process simulations were carried out using Intelligen Superpro Designer V8.5.

Model development

The process model was developed based on NREL (Humbird et al., 2011) and Kumar and Murthy (2011). The process model for all scenarios is provided as supplementary files to facilitate transparency and reproduction of data. The composition of biomass used in different scenarios is mentioned in Table 1. Figure 2 shows the schematics of the different processes used in the techno-economic analysis. The composition of Banagrass and Energycane were obtained from a pilot facility in Oregon, whereas the composition of WS and CS was obtained from Jørgensen et al. (2007) and Shekiri III et al. (2014) respectively. The biomass produced from the agricultural fields were transported to the processing facility where it was stored for 10 days before further processing. The stored biomass was washed with recycled water from the process to remove any dirt or debris and comminuted using a knife mill. Dilute sulfuric acid

at 0.75% (W/W) was added to the size-reduced biomass to carry out pretreatment at 158°C and 0.55 Mpa for 10 min. After pretreatment, the slurry was centrifuged to separate solid and liquid fractions. The cellulose-rich solid stream was washed with three times water (Frederick et al., 2014; Rajan & Carrier, 2014) and the pH was adjusted to 4.0-6.0 pH before enzymatic hydrolysis. The liquid fraction rich in dissolved solids and hemicelluloses were overlimed and pH adjusted to precipitate calcium sulfate as gypsum (byproduct) using hydro-cyclone and purified through vacuum filtration.

The liquid stream after gypsum separation was combined with the solids stream and cellulase enzymes were added at the rate of 20 mg protein /g glucan (Humbird et al., 2011). After enzymatic hydrolysis, yeast, diammonium phosphate were added. Depending on the process simultaneous saccharification and fermentation (SSF) or separate hydrolysis and fermentation (SHF) the reactors were added to the process flow. The enzyme loading, solids loading, hydrolysis conversion, ethanol conversion, hydrolysis time, and fermentation time for the experimental studies and other reported literature used in developing process models are mentioned in Table 2. The ethanol produced after fermentation was distilled in three columns. Beer column is the first column in which ethanol was concentrated to 37-40% (W/W), followed by stripper column where ethanol was purified to 41-45% (W/W). The ethanol concentration reached 93% w/w after the rectification column and 99.5% w/w purity was obtained using molecular sieves, to break ethanol-water azeotrope. The pure ethanol was denatured using 1.0% gasoline before it was sold as the primary product.

The solids from the distillation columns containing lignin were sent to the boiler for steam generation and electricity production. The boiler was operated at 10% excess oxygen and the steam was produced at 257°C and 4.5MPa (Mandegari et al., 2017; Singh et al., 2016). The flue gas exit temperature was 200°C and the steam was expanded using gas turbine for electricity production. The waste water from different sections was collected and sent for wastewater treatment including anaerobic digestion, aerobic oxidation, and belt filtration. About 25% of the liquid fraction was split to the multi-effect evaporator and process water was recycled back to the process. A fraction of the high-pressure steam produced was consumed in pretreatment reactor.

2.7 Economic analysis

2.7.1 Assumptions and assessments

The plant was designed based on a feedstock capacity at 60,000 dry MT/year with an annual operation of 7,920 h and a 20-year life. The economic assumptions used in this study are listed in Table 3. The cost of gypsum was based on Statista (2016). The salvage value for the equipment was assumed to be 5.0% of its installed cost and straight-line depreciation method was employed (depreciation period – 10 years) (Kumar & Murthy, 2011). The tax rate was kept at 40%, while the interest rate was set at 9.0% and the utility prices were based on earlier studies (Kazi et al., 2010; Kumar & Murthy, 2011; Kwiatkowski et al., 2006; Laser et al., 2009).

2.7.2 Sensitivity analysis

A sensitivity analysis was carried out for the three experimental treatments to assess the impact of important parameters on plant economics. The factors considered with a $\pm 10\%$, and $\pm 20\%$ variation in the parameter values for sensitivity analysis were the price of ethanol,

biomass cost, enzyme cost and capacity of the plant. Return on investment (ROI) was used as an indicator to check the sensitivity.

2.8 Life cycle assessments

2.8.1 Goal, scope and system boundaries

The main goal for the life cycle assessments was to develop a *cradle-to-gate* life cycle inventory and estimate the different environmental impacts for the three experimental studies carried out. This functional unit for the ethanol production was 1 MJ ethanol and system boundary for this LCA study is shown in Figure 2. Within the system boundary, agricultural inputs including fertilizers, water, electricity, machinery harvest, and transportation were included as a part of biomass preparation. The biomass processing includes the use of sulfuric acid, enzymes, yeast, gasoline, fermentation, and emissions from the processing plant. The byproducts such as excess electricity and gypsum were considered as avoided products and physical allocation was used. Open LCA (Version 1.6.0) was used to perform LCA (Open LCA, 2017), while eco-invent (V 3.1) database was used for integrating the life cycle inventory (LCI), and TRACI 2.1 was used as the impact assessment method (Bare, 2011; Bare et al., 2012; Bare, 2002). The system expansion method was used to avoid allocation and the coproducts such as electricity or gypsum were considered as avoided products. For LCA, this method is preferred as per the ISO 14044 (2006) standards (ISO. Technical Committee ISO/TC 207, 2006).

2.8.2 Life cycle inventory

The data from the techno-economic evaluation including mass and energy details were imported for inventory analysis. The consumption of different raw materials and production of products from the techno-economic analysis scenarios were used for the LCI. For the yeast

production, a separate process was built based on Dunn et al. (2012). The biomass details including crop yields, irrigation, harvest, and emissions were based on Li et al. (2012) (WS) and Murphy and Kendall (2013) (CS). The data imported were analyzed using Ecoinvent database and integrated processes were used to conduct an impact assessment.

2.8.3 Life cycle impact assessment

Tool for the Reduction and Assessment of Chemical and other environmental impact (TRACI 2.1), developed by USEPA (Ryberg et al., 2014), was used as impact assessment method. Ten various categories were included in TRACI of which seven indicators are environmental related and the other three are human health related. The impact categories are as follows: acidification, ecotoxicity, eutrophication, global warming, ozone depletion, photochemical ozone formation (POF), resource depletion – fossil fuels, carcinogenics, noncarcinogenics and respiratory effects. The inputs and outputs from the processes are attached as a supplementary file which can be accessed in OpenLCA with Ecoinvent database.

3 Results and discussion

3.1 Experimental studies

Three experiments were carried out varying the feedstock (wheat straw and corn stover) and solids loading. The wheat straw was loaded at 19% and 30% whereas the corn stover was loaded at 45% in the fed batch hydrolysis reactor. Wheat straw and corn stover were chosen as the substrate as they have high cellulose content and are available across the globe (Barten, 2013; Liu et al., 2005). The cellulose content in the pretreated corn stover was 60.9% (W/W) and that of wheat straw was 54.6% (W/W). The results of the composition were in agreements with the other literature reported by (Kumar et al., 2009; Pakarinen et al., 2014). Figure 3

shows the glucose released during enzymatic hydrolysis and ethanol concentration during fermentation.

During the enzymatic hydrolysis, the highest glucose release was obtained in CS45 and the final concentration after 96h was 205 ± 25.8 g glucose/L. In the first 12h, the productivity of glucose was high at the rate of 12.4 g/L.h and declined to 0.7g/L.h until the end of hydrolysis. Jørgensen et al. (2007) used wheat straw as a substrate at 40% solids loading and reported a 37% hydrolysis efficiency, whereas in this study CS45 had 80% conversion efficiency (Table 2).

Similarly, WS19 and WS30 had a peak glucose concentration of 95.6 ± 3.2 g/L and 166 ± 15.7 g/L after 114 and 144 h hydrolysis. Achieving this high glucose concentration could be mainly attributed to the fed-batch strategy used for loading the biomass. Other researchers (Saha et al., 2011) reported a glucose release of 86 g/L using wheat straw loaded at solids loading of 15%, whereas this study reported 11% higher hydrolysis efficiency at a 4% higher solids loaded to the system.

The enzymatically hydrolyzed samples were fermented using yeast to produce ethanol. WS30 reached a final ethanol concentration of 81.9 ± 1.2 g/L after 60h of fermentation and the productivity was highest at 3.2 g/L.h in the first 24 hours decreasing to 0.14g/L.h, since most of the glucose was consumed in the first 24 h. WS19 had a peak ethanol concentration of 46.8 ± 1.0 g/L after 60h of fermentation and results were comparable with literature reported: He et al. (2014) reported 48.7 g/L after 72h fermentation using corn stover as a raw material for a solids loading of 25%. Pakarinen et al. (2014) used wheat straw at solids loading of 20% and had a final ethanol concentration of 75 g/L which could be due to the addition of polyethylene glycol in that study, which binds to lignin and increases the accessibility of enzymes increasing

hydrolysis efficiency and resulting in higher ethanol production. Jørgensen et al. (2007) reported an ethanol concentration of 47 g/L at 40% solids loading whereas CS45 had a higher ethanol concentration of 115.9 ± 6.7 g/L which could be due to: 1. The lower enzyme loading by the other study (7 FPU/gDM), 2. The fed-batch loading in this study, and 3. The duration of hydrolysis in the other study was lower (48 h) whereas for CS45 the hydrolysis went until 96h. Final ethanol concentrations of 115.9 ± 6.7 g/L needs a minimum of 227 g/L glucose assuming 100% conversion. However, the glucose concentrations after hydrolysis were only 205 ± 25.8 g/L which implies that the cellulose enzymes were active during fermentation process and resulted in additional sugar production.

3.2 Techno-economic analysis

The process schematics and overall mass balance for the eight scenarios are presented in Figure 4. The complete process flow can be obtained from supplementary file Figure 2. For the experimental scenarios, the results showed that higher the solids loaded higher the ethanol produced. The ethanol production was highest for CS45 where 347.9 L/dry MT was produced which was 16.5% higher reported by NREL at 20% solids loading (298.6 L/dry MT)(Humbird et al., 2011). Table 4 shows the list of raw materials used in the process simulations for various scenarios. The water consumption ranged between 230,000 to 300,000 MT/year. Most of the consumption occurred during the washing biomass after pretreatment with three times the volume of the pretreated biomass. Many laboratory studies indicate that this step is critical to achieving high enzymatic hydrolysis efficiency but is unfortunately largely ignored in techno-economic studies reported in the literature. This is a critical step as washing the pretreated

biomass removes toxic substances such as phenolics and other inhibitors formed during pretreatment procedures (Frederick et al., 2014; Rajan & Carrier, 2014).

Compared with WS20, WS19 (this study) produced 29% higher ethanol production which could be attributed to the lower enzymes loading in WS20. The lower enzyme loading decreased the glucose release efficiency by 10% which was reflected in ethanol production and overall conversion. The overall conversion for WS20 was 53%, while it was 74% for WS 19 (this study).

Similarly, WS40 had a lower enzyme loading which produced the lowest ethanol (7,356 MT/year) in contrast to CS45 which had the highest ethanol production at 16,492 MT/year for an identical feedstock processing capacity of 60,000 dry MT/year. Table 5 shows the utility consumption and electricity production from lignin and other solids. As the solids loading increased, the electricity consumption increased, which decreased the net electricity produced from the process (Fig. 4). The highest electricity production was seen in WS40 (13,499 MWh/year) which (the lowest ethanol production scenario) could be attributed to higher amounts of the unfermented solids sent to boiler resulting in higher electricity production. Of the electricity produced, between 25-30% was consumed on the site except for BG20, EC20, and CS45. BG20 and EC20 had high moisture content which increased the power consumption, while CS45 consumed 33% of the power produced which was due to the higher solids loaded. The capital investments for the different scenarios ranged between \$33-48 Million. The CS45 scenario had the lowest capital investment (\$33 Million) which could be mainly attributed to the smaller size of the reactor used at 45% solids loading (Fig 5A). The working volume for WS19 stood at 6,000 m³ whereas for CS45 it was 42.5% lower. This reduction in mainly attributed to the higher solids loading resulting in reduced overall CAPEX of the plant. The

production cost ranged between \$ 0.8-1.1/L except WS40 (Fig 5B). Due to the low enzyme loading, high solids and low ethanol yield the production cost of WS40 was \$1.74/L. The production cost was lowest for CS45 at \$0.8/L ethanol. The OPEX to CAPEX ratio ranged between 0.4-0.5 and was highest for CS45. The most profitable scenario was CS45 which yielded 8.06% ROI after 20 years of operation (Fig. 5B). The production cost for the all the scenarios except WS40 were in the range of \$0.84-1.13/L and the results were comparable with other studies reported in the literature (Gnansounou & Dauriat, 2010; Kazi et al., 2010; Kumar & Murthy, 2011; Wingren et al., 2003; Wingren et al., 2004) which reported between \$0.6-1.2/L. Compared with literature, the production cost reported from the simulations were higher which could be attributed to the two reasons: 1. The capacity of the plant considered at 60,000 dry MT/year while most of the plant capacities reported in literature were 150,000-250,000 MT/year; 2. The cost of biomass used in this study \$80/dry MT, while most literature reported \$60/dry MT as the feedstock acquisition price.

3.3 Sensitivity analysis

The crucial factors which affect the overall economics such as selling price of ethanol, purchase cost of biomass and capacity of the plant considered were varied by $\pm 10\%$ and $\pm 20\%$. The sensitivity analysis was performed for the three experimental studies. The selling price of ethanol was the most crucial factor and increasing the price by +20% for CS45 increased the ROI to 14.2%. The capacity of the plant is the second most important factor, wherein increasing the capacity by 20% from the 60,000 MT/year base case increased the ROI to 7.6% for the WS30 scenario. The biomass cost is the third most important factor wherein a 20% reduction in the biomass cost from the \$80/ dry MT base case value increased the ROI to 4.8% in WS19 scenario

(supplementary file Fig. 3). The ethanol prices follow the crude oil prices and therefore the crude oil prices indirectly affect the overall profitability of the plant. When the crude oil price increase to more than \$80/barrel most of the ethanol processing plants using lignocelluloses are profitable, whereas reducing the crude oil prices adversely affect the profits (data not shown).

3.4 Environmental impacts

3.4.1 Carbon balance

The elemental carbon balance was performed for the experimental studies carried out and can be obtained from the supplementary file (Supplementary Fig. 4). The carbon dioxide sequestration while cultivation of wheat straw and corn stover for every ton of biomass was 378 kg and 345 kg respectively. The sequestered carbon was partially captured in the ethanol product, while remaining carbon was released in different forms during ethanol production. Out of sequestered carbon for WS19, 14.5% was released from the fermentation process, 55.6% from the boiler and 29.5% was retained in the ethanol produced. Similarly, for CS45, 16.1% of sequestered carbon was released from fermentation, while the carbon fractions from boiler and ethanol were 50.9% and 32.5% respectively.

3.4.2 Life cycle assessments

The inputs from the techno-economic analysis were used for life cycle inventory, and LCA was conducted using Open LCA (V1.6). TRACI 2.1 was used as an impact assessment method, and the functional unit was 1MJ ethanol. Ten environmental impacts for the three experiments are shown in Fig. 6. In general, a decreasing trend of all environmental impact metrics was observed with the increase in the solids loading which was the result of increasing ethanol

production with increasing solids loading without a concomitant increase in plant energy requirements.

Global warming indicator is measured as kg CO₂ eq. releases per functional unit. The global warming in WS19, WS30 and CS45 was 17.9, -4.5 and -37.8 g CO₂ eq. The GREET (2016) reported a global warming potential of 30.8 gCO₂ eq./MJ from corn stover ethanol which was higher than the value reported in this study. This could be mainly attributed the higher ethanol production from CS45 (16.5%) compared with GREET and NREL. Similar results by other studies were comparable to the results from this study, where Luo et al. (2009) reported 50 g CO₂ eq./MJ ethanol produced and Cavalett et al. (2013) reported of 24.2 g CO₂ eq./MJ ethanol. Acidification refers to the increase in the hydrogen ion concentration which increases the addition of acids in the environment, and it was measured in kg SO₂ equivalent (Bare et al., 2012). The acidification was highest for WS19 at 5.7×10^{-4} kg SO₂ eq. The other environmental indicator eutrophication refers to the increase of aquatic systems like algae or weeds due to the nutrients release from the process which was measured in kg N Eq. Eutrophication was lowest in CS45 1.9×10^{-4} kg N Eq.

4 Conclusion

Three experiments were conducted using wheat straw and corn stover as feedstocks. Corn stover at 45% solids loading (CS45) released highest glucose concentration at 205 ± 25.8 g/L and a peak ethanol concentration of 115.9 ± 6.7 g/L. The results from the experiments and other reported literature were used in the techno-economic analysis which showed that CS45 could yield the highest profit with a payback period less than 12 years at an ROI of 8.06% after 20

years of operation. Regarding LCA, global warming potential decreasing trend with an increase in solids loading and it lowest for CS45 with $-37.8 \text{ gCO}_2 \text{ eq./MJ ethanol}$.

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Supplementary files information

There are 10 supplementary files attached to this manuscript of which 8 files are the process models developed for the techno-economic analysis and file 9 contains all supplementary information such as statistical evaluation, sensitivity analysis, and elemental carbon balance. File 10 contains the LCA data which could be accessed with the eco-invent database.

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Figure 6. Life cycle assessments for the three experimental studies carried out.

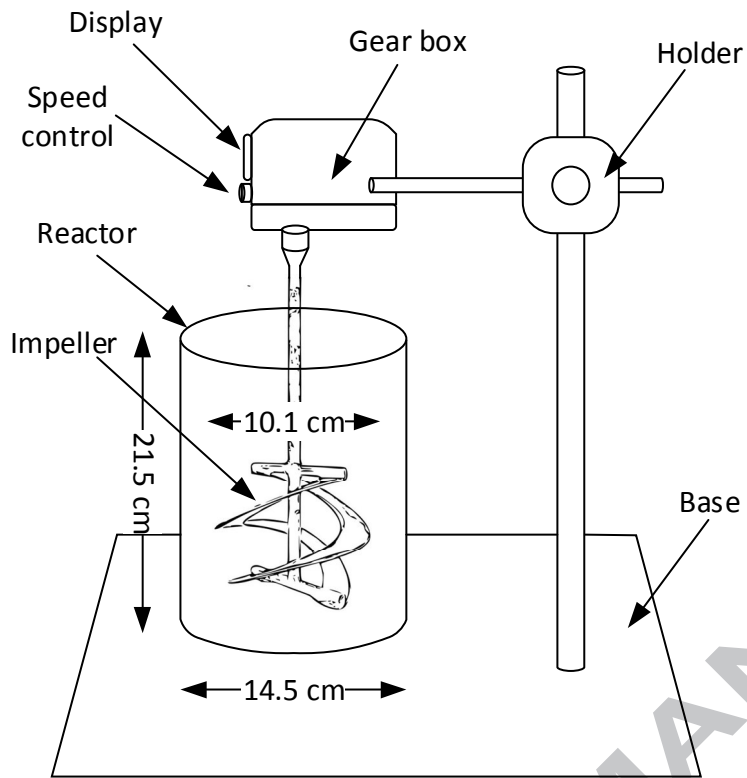


Figure 1.

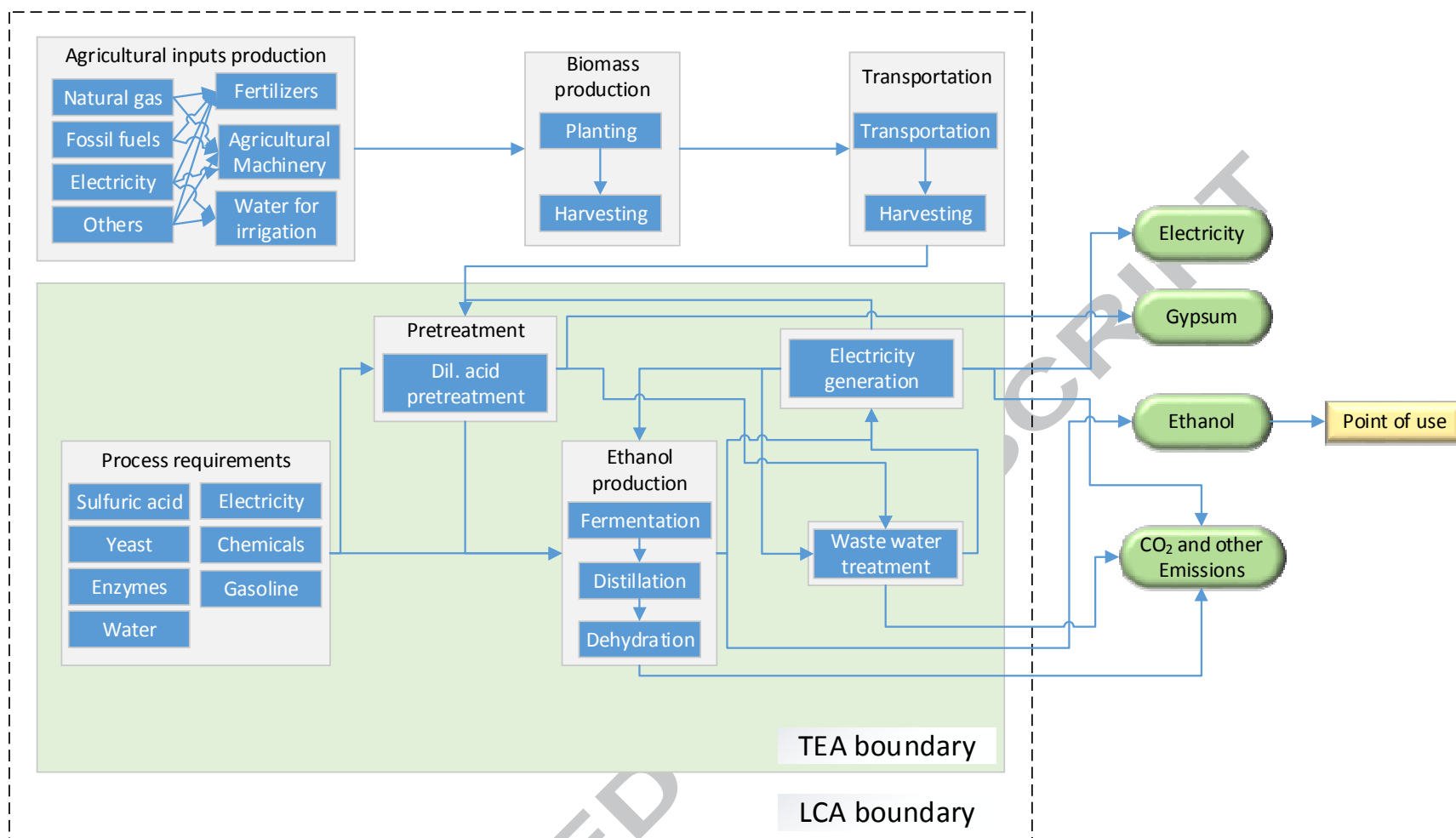


Figure 2.

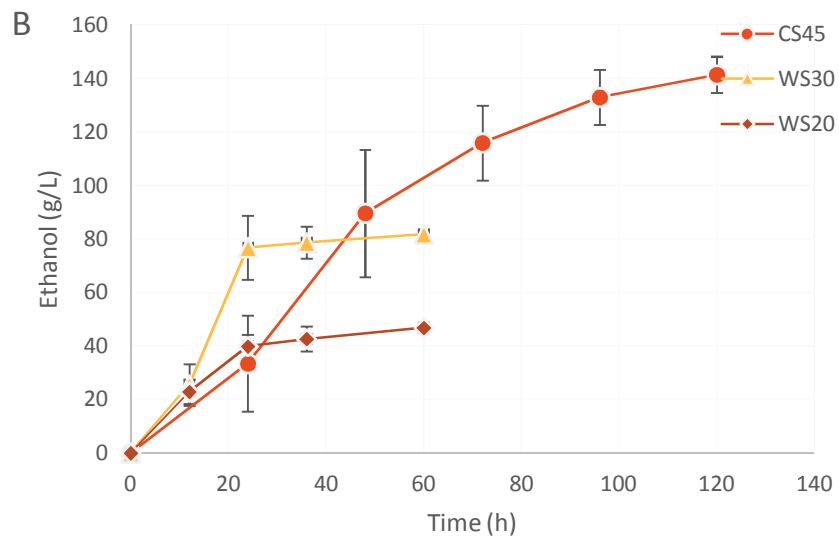
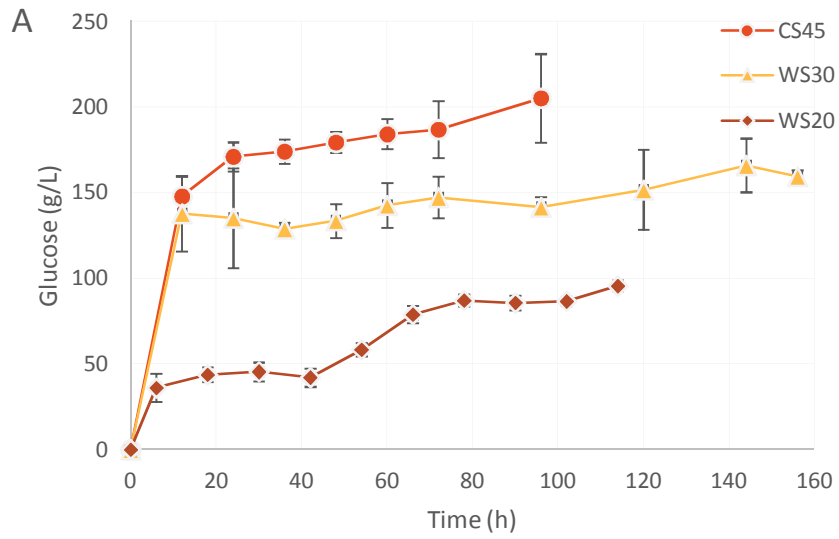
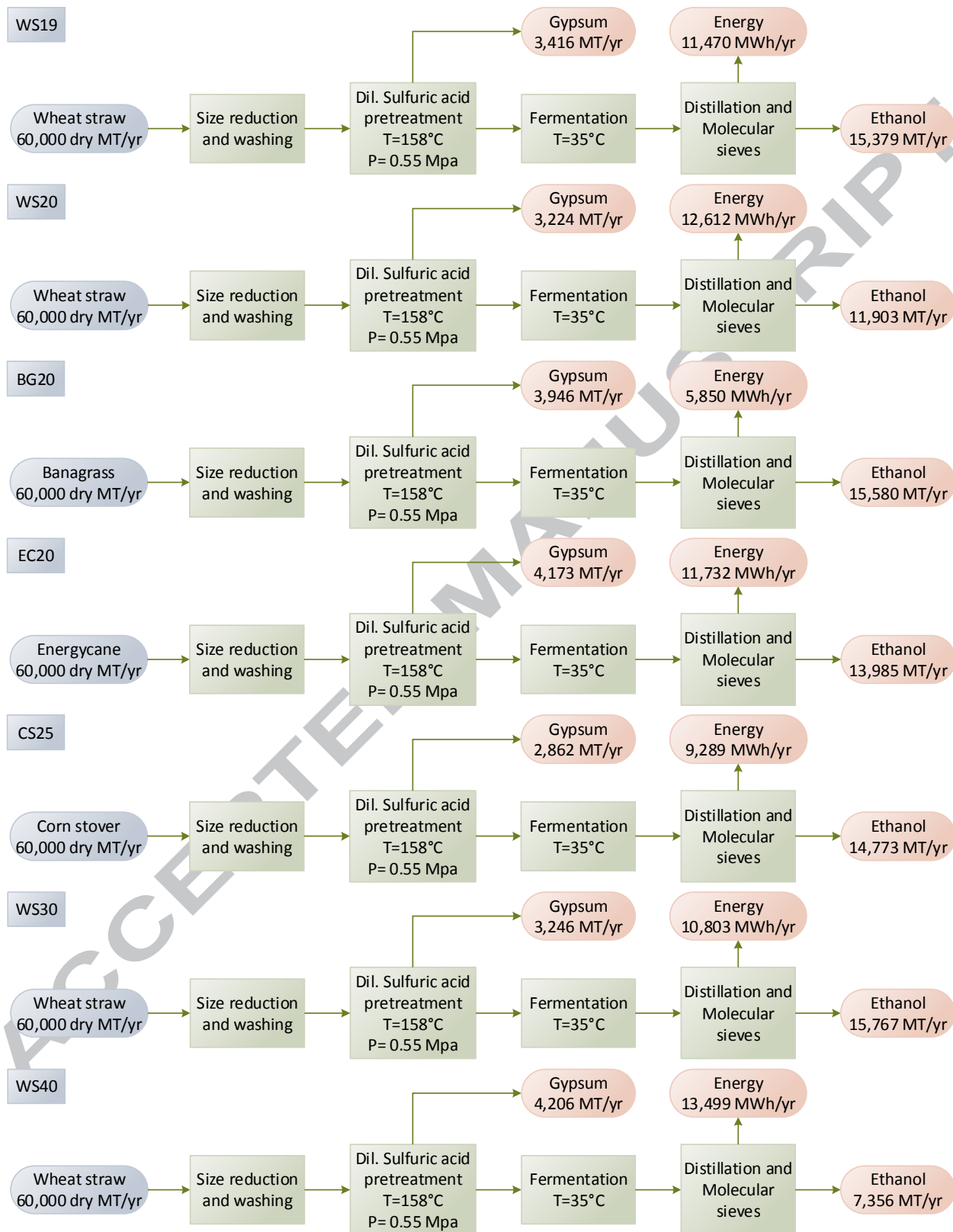


Figure 3.



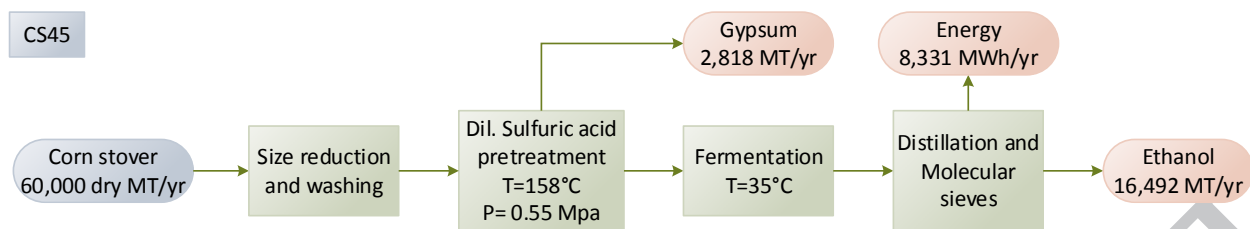


Figure 4.

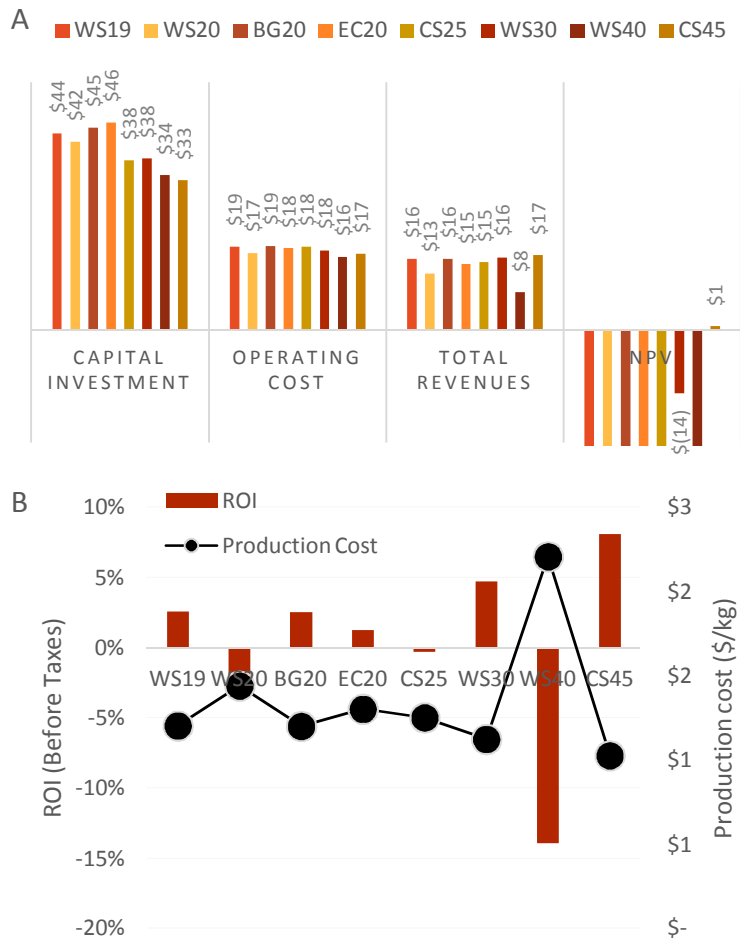


Figure 5.

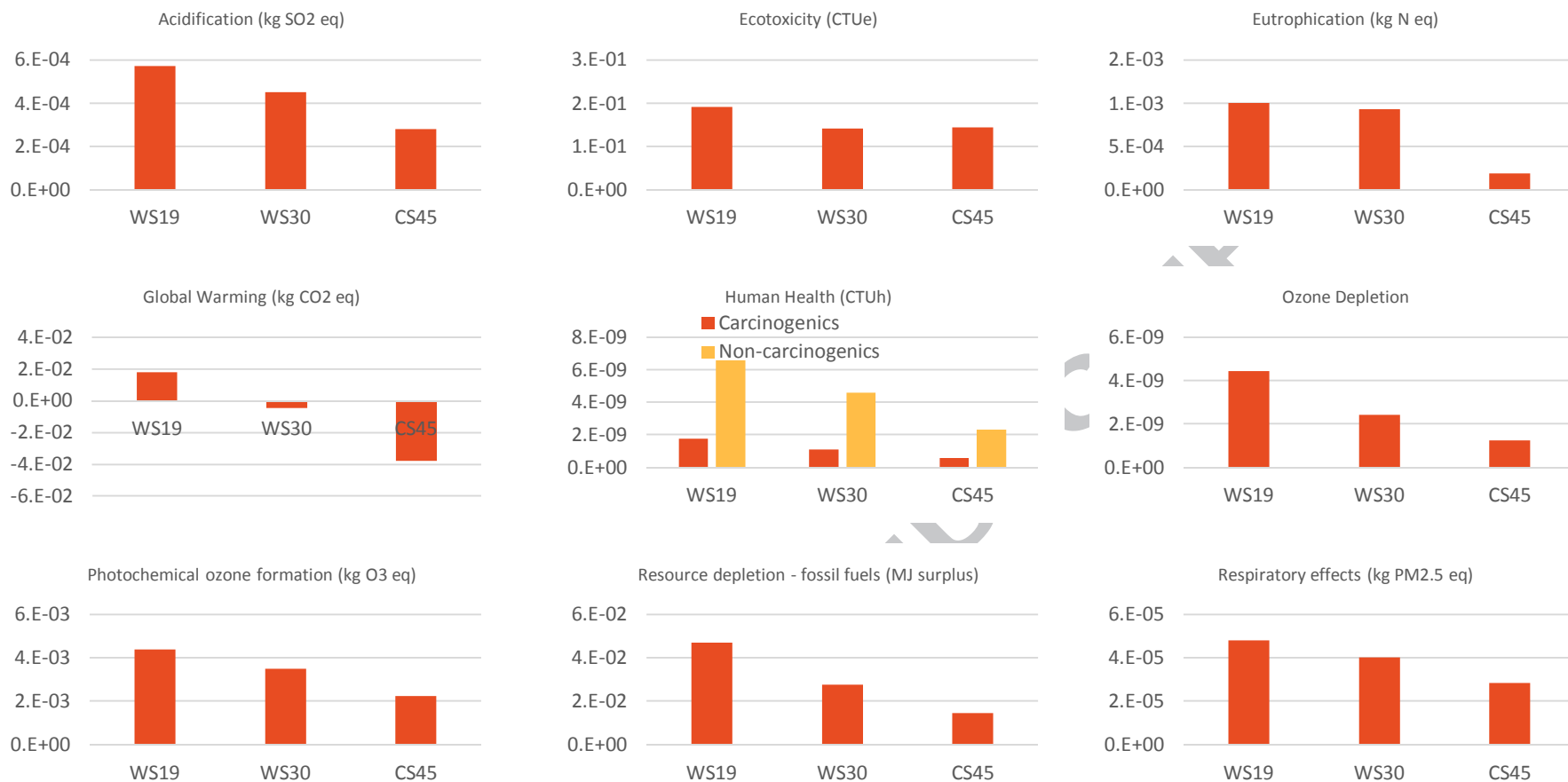


Figure 6.

Table 1. The composition of various biomass used on the wet and dry basis.

Reference	WS		BG		EC		CS	
	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis
	(Jørgensen et al., 2007)				Pilot study		(Shekiro III et al., 2014)	
Ash	6.0%	7.1%	2.6%	9.6%	1.9%	6.5%	6.8%	9%
Cellulose	29.6%	34.8%	10.2%	37.5%	10.1%	33.4%	29.6%	37%
Extractives	13.0%	15.3%	3.6%	13.1%	7.9%	26.4%	10.2%	13%
Hemicellulose	21.4%	25.2%	6.4%	23.4%	6.4%	21.2%	21.4%	27%
Lignin	15.0%	17.6%	4.5%	16.4%	3.8%	12.6%	12.0%	15%
Moisture	15.0%		72.7%		70.0%		20.0%	

Table 2. Various process parameters used in this study to carry out the process simulation.

Scenario	Solids loading	Type of process	Enzyme loading	Hydrolysis Time (h)	Fermentation Time (h)	Ethanol (g/L)	Hydrolysis Conversion	Ethanol conversion	Total conversion	Reference
WS19	19%	SHF	20 mg/g	78	60	46.8	76%	97%	74%	This study
WS20	20%	SSF	7 FPU/gDM	96	48	31.9	66%	80%	53%	(Jørgensen et al., 2007)
BG20	20%	SSF	20 mg/g	72	48	44.6	81%	95%	77%	Pilot plant
EC20	20%	SSF	20 mg/g	72	48	37.9	81%	95%	77%	Pilot plant
CS25	25%	SSF	15 FPU/gDM	12	72	49.34	85%	94%	80%	(He et al., 2014)
WS30	30%	SHF	20 mg/g	72	60	81.9	80%	98%	78%	This study
WS40	40%	SSF	7 FPU/gDM	96	48	47	37%	90%	33%	(Jørgensen et al., 2007)
CS45	45%	SHF	20 mg/g	48	96	115.9	80%	98%	78%	This study

Table 3. List of assumptions used in this study.

Type	Assumption
Capacity	60,000 dry MT/year
Biomass cost	\$80/ dry MT
Gypsum cost	\$30/MT
Ethanol cost	\$0.95/kg
Enzymes cost	\$0.517/kg
Sulfuric acid cost	\$35/MT
Electricity cost	\$0.07/kW-h
Gasoline cost	\$0.8/kg
Discount rate	2%
Annual hours	7920 h
Depreciation method	Straight line
Salvage value	5%
Depreciation years	10 years

Table 4. A number of different raw materials used in this study.

	Unit (MT)							
	WS19	WS20	BG20	EC20	CS25	WS30	WS40	CS45
Water	267,877	285,832	148,361	191,913	237,886	279,920	296,584	280,333
Gasoline	146	113	148	140	140	150	70	157
Calcium hydroxide	1,557	1,466	1,806	1,925	1,293	1,477	1,933	1,272
Sulfuric acid	2,273	2,140	2,636	2,809	1,880	2,155	2,821	1,856
Biomass (dry MT)	60,000	60,000	60,000	60,000	60,000	60,000	60,000	60,000
Cellulase	3,957	1,979	4,259	3,800	6,307	3,957	1,979	4,205
DAP	20	20	20	20	20	20	20	20
Yeast	50	50	50	50	50	50	50	50

**The values mentioned above were rounded off to the nearest metric tons.*

Table 5. List of different utilities consumed for different solids loading and biomass.

	WS19	WS20	BG20	EC20	CS25	WS30	WS40	CS45
Power Consumption (kW-h)	(3,909,000)	(3,869,000)	(6,864,000)	(6,251,000)	(3,653,000)	(3,866,000)	(4,777,000)	(4,180,000)
Power Production (KW-h)	15,379,000	16,481,000	12,714,000	17,983,000	12,942,000	14,669,000	18,276,000	12,511,000
Steam (MT)	(145,000)	(127,000)	(157,000)	(163,000)	(109,000)	(85,000)	(46,000)	(52,000)
Cooling Water (MT)	9,244,000	7,790,000	9,689,000	9,583,000	7,822,000	7,108,000	3,726,000	5,996,000
Chilled Water (MT)	1,508,000	1,684,000	1,345,000	1,111,000	1,372,000	1,524,000	1,847,000	1,337,000
CT Water (MT)	6,467,000	5,864,000	6,592,000	6,773,000	6,249,000	5,868,000	6,044,000	5,799,000
Steam High P (MT)	(22,000)	(21,000)	(26,000)	(28,000)	(18,000)	(21,000)	(21,000)	(18,000)

**The value inside the parentheses indicates that it was produced onsite.*

Effect of solids loading on ethanol production: Experimental, Economic and Environmental analysis.

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Highlights:

- Hydrolysis and fermentation were conducted at 19%, 30%, and 45% solids
- Corn stover at 45% solids loading released 205±25.8 g/L glucose and resulted in 115.9±6.7 g/L ethanol after 60h of fermentation
- Techno-economic analysis revealed an ROI of 8% at 45% solids loading