LIGNIN MAXIMIZATION:

ANALYZING THE IMPACT OF DIFFERENT FEEDSTOCKS AND FEEDSTOCK RATIOS USING ORGANOSOLV FRACTIONATION

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ABSTRACT

Over-exploitation of fossil fuels coupled with increasing pressure to reduce carbon emissions are prompting a transition from conventional petrochemical feedstocks to sustainable and renewable sourced carbon. The use of lignocellulosic biomass as a feedstock for integrated biorefining is of current high interest, as separation into its component parts affords process streams of cellulose, hemicellulose and lignin, each of which can serve as a starting point for the production of biobased chemicals and fuels. Given the large number of potential sources of lignocellulosic feedstocks, the biorefinery will need to adapt to the supplies available over a normal growing season. Of particular importance is the lignin fraction, as its conversion to chemicals and materials to allow economic viability of the operation.

Previous work has demonstrated that organosolv fractionation effectively separates lignocellulosic biomass into its component parts. In this project, we investigated the use of organosolv technology for separating mixtures of lignocellulosic feedstocks to isolate pure lignin. Mixtures of switchgrass (*Panicum virgatum*), southern yellow pine (*Pinus taeda L.*), and hybrid poplar (*Populus spp.*) were separated using organosolv fractionation. Experiments were performed by heating the feedstock mixtures at 150^{o}C in a 3.5 L flow-through reactor with a ternary, one-phase solvent mixture of methyl isobutylketone (MIBK), ethanol (EtOH) and water (H_2O) in a wt% ratio of 16/34/50, and containing sulfuric acid as a catalyst. The impact of different process variables was examined by

experimental design (‘Design of Experiments’) to minimize the number of experimental runs using a balanced approach in the response surface to maximize inference. The process variables included two different runtimes (60, 120 min), two different sulfuric acid levels (0.05, 0.15 M), and four different wt% feedstock ratios for switchgrass/pine/poplar ([10/10/80], [10/80/10], [80/10/10], [33/33/33]). After completion of the initial experimental matrix, four additional center-points were carried out using a 90 min runtime, and 0.1 M acid level to validate the results for each of the four feedstock ratios. The dependent factors were lignin yield, lignin purity, and cellulose purity. Response surface methodology (RSM) was used to evaluate the impact of the process variables and to determine optimization settings for the process.

TABLE OF CONTENTS

Chapter One Introduction and General Information 1
Research Hypothesis 5
Objectives 6
Thesis Organization 6
Chapter Two Literature Review 8
Integrated Biorefinery 8
Feedstock 10
Lignocellulosic Biomass 12
Cellulose 14
Hemicellulose 15
Lignin 16
Principle of Pretreatment 17
Pretreatment Technologies 18
Steam explosion 18
Ammonia Fiber Expansion (AFEX) 19
Dilute Acid Pretreatment 19
Alkaline Pretreatment Technology 20
Organosolv Pretreatment 20
Experimental Design 22
Chapter Three Materials and Methods 28

Experimental Design 28
Feedstock 30
Solvent mixture 32
Operation 34
Processing of soluble and insoluble fractions 36
Chapter Four Results and Discussion 40
Total Lignin Analysis 40
Probability Density Function of the Data 41
Lignin Yield 45
Response Surface Design 45
Maximizing Lignin Yield 49
Prediction Validation 50
Impact of Feedstock Ratio on Lignin Yield 52
Impact of Acid Level on Lignin Yield 54
Impact of Run Time on Lignin Yield 55
Lignin Purity 56
Response Surface Design 56
Maximizing Lignin Purity 57
Prediction Formula Validation 60
Impact of Feedstock Ratio on Lignin Purity 61
Impact of Acid Level on Lignin Purity 63
Impact of Run Time on Lignin Purity 64

Cellulose Purity 65
Response Surface Design 65
Maximizing Cellulose Purity 67
Prediction Validation 68
Impact of Feedstock Ratio on Cellulose Purity 70
Impact of Acid Level on Cellulose Purity 71
Impact of Run Time on Cellulose Purity 71
Maximizing Multiple Responses 71
Chapter Five Conclusions and Recommendations 75
List of References 78
Appendix 84
Vita 87

LIST OF TABLES

Table 1. Design Selection Guideline (NIST, 2016).....25

Table 2. Continuous factors analyzed on their impact on lignin yield, lignin and cellulose purity. 28

Table 3. Categorical (or nominal) factors analyzed on the impact on lignin yield, lignin and cellulose purity. 29

Table 4. Experimental runs used in the RSM. 29

Table 5. Compositional Analysis of Feedstocks (in %). 30

Table 6. Feedstock mixtures in wet weight [ratio %]. 32

Table 7. Maximum available lignin for each mixture based on feedstock composition.....33

Table 8. Feedstock ratio broken down in wet weight [%], wet weight [g], and dry weight [g]. 33

Table 9. Lignin yield, lignin purity, and cellulose purity results presented in percent by run settings and feedstock mixture and combined severity. 42

Table 10. Data used for further analysis: recovered lignin by impure, pure, and compositional lignin content. 43

Table 11. Analysis of Variance (Response Lignin Yield). 46

Table 12. Effect Tests (Response Lignin Yield). 46

Table 13. Combined severity at 150°C for each used factor setting, ordered from low to high combined severity. 48

Table 14. Predicted versus Actual Lignin Yield data for Validation, including the specific standard deviation as Root Mean Squared Error (RMSE). 51
Table 15. Analysis of Variance (Response Lignin Purity). 58
Table 16. Effect Tests (Response Lignin Purity). 58
Table 17. Alternative effects test (Response Lignin Purity). 58
Table 18. Predicted versus Actual Lignin Purity data for Validation, including the specific standard deviation as Root Mean Squared Error (RMSE). 60
Table 19. Analysis of Variance (Response Cellulose Purity). 66
Table 20. Effects Tests (Response Cellulose Purity). 66
Table 21. Predicted versus Actual Cellulose Purity Data for Validation. 69

LIST OF FIGURES

Figure 1. The three stages of a biorefinery operation (Bozell, 2008). 9

Figure 2. Cut-away drawing of the cell wall, middle lamella (ML), primary wall (P), and secondary cell wall (S1, S2, S3) (Rowell, 2013). 14

Figure 3. Chemical structure of cellobiose (Rowell, 2013). 15

Figure 4. Chemical structure of lignin precursors, p-coumaryl alcohol (1), coniferyl alcohol (2), and sinapyl alcohol (3) (Rowell, 2013). 16

Figure 5. Schematic of pretreatment to disrupt the physical structure of biomass (Mosier et al., 2005b). 18

Figure 6. General process or system model (Antony, 2014). 24

Figure 7. Three different types of central composite designs (NIST, 2016). 26

Figure 8. Switchgrass (*Panicum virgatum*) used for organosolv fractionation (mesh sizes 20-4). 31

Figure 9. Hybrid poplar (*Populus spp*.) used for organosolv fractionation (mesh sizes 20-4). 31

Figure 10. Loblolly Pine (*Pinus taeda L*.) used for organosolv fractionation (mesh sizes 20-4). 31

Figure 11. Standard solvent composition: 50% water, 34% ethanol, 16% MIBK.34

Figure 12. Reactor flow diagram and layout (Astner et al., 2015). 35

Figure 13. Recovered organic lignin fraction.....39

Figure 14. Recovered aqueous lignin fraction. 39

Figure 15. Recovered milled solid fraction (mainly cellulose). 39
Figure 16 (a) and (b). Histogram, boxplot, and normal quantile plot suggesting normal distribution for lignin yield (a.) and purity (b.) data, ...44
Figure 17 (a.) and (b.). Histogram, boxplot, and normal quantile plot suggesting normal distribution of cellulose yield (a.) and purity data (a.), furthermore for a confidence interval of 95%, three outliers were detected (cellulose yield (a.) of run 9, 23, and 24). 45
Figure 18. Response surface design of lignin yield by combined severity and feedstock ratio. 48
Figure 19. Prediction profiler recommending maximizing settings for lignin yield by runtime, acid level, and feedstock ratio.....49
Figure 20. Simplex plot for lignin yield training data set, by x1 (poplar), x2 (switchgrass), and x3 (pine) percentage. 51
Figure 21. Box and whisker plots including training (grey points) and validation data (black triangles) points. 52
Figure 22. One-way ANOVA for mean lignin yield between the four different feedstock ratios expressed in percent and mean comparison by Student's t. 53
Figure 23. One-way ANOVA for mean lignin yield of 0.05, 0.1, and 0.15 Mole Acid Level expressed in percent and mean comparison by Student's t. 55
Figure 24. One-way ANOVA for mean lignin yield between 60, 90, and 120 minutes expressed in percent and mean comparison by Student's t. 56

Figure 25. Response surface design of lignin purity by combined severity and feedstock ratio. 59
Figure 26. Prediction profiler recommending maximizing feedstock ratio and combined severity. 59
Figure 27. Lignin Purity by Combined severity, linear line in red indicates the prediction formula. 61
Figure 28. One-way ANOVA for mean lignin purity between the four different feedstock ratios expressed in percent and mean comparison by Student's t. 62
Figure 29. One-way ANOVA for mean lignin purity between 0.05, 0.1, and 0.15 Mole Acid Level expressed in percent and mean comparison by Student's t. 63
Figure 30. One-way ANOVA for mean lignin purity between 60, 90, and 120 minutes expressed in percent and mean comparison by Student's t. 64
Figure 31. Response surface design of cellulose purity by combined severity and feedstock ratio. 65
Figure 32. Prediction profiler recommending maximizing settings for cellulose purity combined severity and feedstock ratio. 67
Figure 33. Box and whisker plots including training (grey points) and validation data sets (black triangles) by Feedstock Ratio for Cellulose Purity. 68
Figure 34. Simplex plot of cellulose purity by x1 (poplar), x2 (switchgrass), and x3 (pine) in percentage. 69

Figure 35. One-way ANOVA for mean cellulose purity between the four different feedstock ratios expressed in percent and mean comparison by Student's t. 70
Figure 36. One-way ANOVA for mean cellulose purity between 0.05, 0.1, and 0.15 Mole Acid Level expressed in percent and mean comparison by Student's t. 72
Figure 37. One-way ANOVA for mean cellulose purity between 60, 90, and 120 minutes expressed in percent and mean comparison by Student's t. 72
Figure 38. Prediction profiler plot, maximized desirability was found at a feedstock ratio using high switchgrass at a medium severity (2.26). 74

CHAPTER ONE
INTRODUCTION AND GENERAL INFORMATION

Worldwide energy demand has steadily risen since the first industrial revolution. Fossil fuels are the main source of energy, and consequently, release significant amounts of greenhouse gases (GHG). Transportation, which relies primarily on liquid fuels, as well as plastics, chemicals, and other synthetic materials are produced from crude oil (EIA, 2012). To decrease the negative environmental effects of man-made GHG emissions, a reduction of oil dependency and a shift from finite resources to alternative, sustainable sources is paramount (EIA, 2016).

Biomass (plants, plant derived materials and animal manure) has not only the potential to provide energy and fuel but also has the ability to produce products otherwise made from crude oil based precursors. To promote biofuels and energy security, the US congress passed the "Energy Independence and Security Act of 2007" (EISA) that targets a biofuel production of 36 billion gallons/year by the year 2022, incorporating 16 billion gallons/year from cellulosic biomass. The "Corporate Average Fuel Standard" demands an average fleet fuel consumption of 54.5 mpg for cars and light trucks by 2025, which will most efficiently be met, by decreasing vehicle weight. Low cost carbon fibers derived from lignocellulosic biomass, for example, could be used in the automotive industry to reduce fuel consumption (Baker and Rials, 2013). Currently, the majority of renewable transportation fuels are known as first generation biofuels which are manufactured from starch-based

crops. In the US, fuel ethanol is mostly derived from corn starch and blended with gasoline. However, skepticism about the sustainability of further extending first generation biofuel production amounts has risen due to competition for arable land between biofuel and food crops. This re-dedication of arable land, to produce crops for fuel instead of food, is partially cited as a cause of food crises in 2007 (Abbott, 2009).

The constraints of first generation biofuel has created greater interest in biofuels from non-food crops, also known as second generation biofuels (Sims et al., 2010). In particular, lignocellulosic biomass is presented as a low cost and potentially available feedstock (Perlack et al., 2005). Currently, biorefineries mainly focus on biofuel production, and therefore, have limited opportunities for profitability. However, lignocellulosic biomass has the potential to be a raw material for a wide variety of bio-based products comparable to those derived from crude oil (Bozell, 2008). A new generation of integrated biorefineries will produce low value fuels and integrate the production of high value chemicals to maximize profitability. If applied, dependency on oil imports and oil in general could be reduced, as targeted by the EISA, and could revitalize rural areas (Cherubini, 2010). Lignin, a biopolymer found in lignocellulosic cell walls and one of the major components of lignocellulosic biomass, is currently being explored as a source for valuable aromatic chemicals (Jarrell et al., 2014). Furthermore, lignin is under investigation as a low-cost alternative to crude oil derived precursors for carbon fibers (Baker and Rials, 2013). To gain access to lignin, a pretreatment method

has to be applied. Today's pretreatments, however, aim specifically at making carbohydrates accessible for enzymatic hydrolysis and fermentation to produce biofuel (Bozell et al., 2011a). Selective pretreatments are necessary to allow lignin refining that leads to higher initial costs, however, eventual biorefineries will enable more profitability by producing additional high value products.

In the Center for Renewable Carbon (CRC) at the University of Tennessee, an advanced organosolv pretreatment method is applied to fractionate lignocellulosic biomass into its major components. Organosolv fractionation is able to treat lignocellulosic biomass using a ternary solvent mixture of water, ethanol (EtOH), methylisobutyl ketone (MIBK) and sulfuric acid (H_2SO_4) as a catalyst in a closed, heated, and pressurized flow through reactor. In general, the reactor process treats the lignocellulosic biomass by removing lignin and hemicellulose as a dissolved liquid fraction (black liquor), while the majority of cellulose stays in the reactor in its solid state. Subsequently, hemicellulose and lignin can be fractionated to gain a high purity lignin suitable for further refining.

Since the lignocellulosic structural components vary in their amount and chemical structure, the feedstock for the process impacts recovered lignin yield and purity. One major constraint of lignocellulosic biorefining is maintaining a steady feedstock supply which varies due to natural growing and harvesting seasons. An attempt to remove this constraint is presented in this study, by utilization of a variety of feedstocks in different mixtures. Promising sustainable feedstocks in the Southern US were used to conduct this research, namely,

switchgrass (*Panicum virgatum*), hybrid poplar (*Populus spp.*), and loblolly pine (*Pinus taeda L.*).

Earlier studies at the CRC have established a foundation that identifies significant factors impacting lignin yield and purity using feedstock mixtures of switchgrass and tulip poplar (*Liriodendron tulipifera*). Astner (2012) assessed factors impacting variation in lignin yield and purity and proposed that feedstock surface reduction prior to treatment could improve the solvent penetration during the organosolv process. Maraun (2013) consequently researched the influence of different particle sizes and feedstock ratios using a Taguchi robust experimental design methodology. Maraun's (2013) simulation indicated that the small size of tulip poplar (*Liriodendron tulipifera*) particles had the potential to positively influence lignin yield. Maraun (2013) also found that solvents containing high amounts of MIBK (62%) have significant influence on maximizing lignin purity, but not lignin yield. His research suggested that high MIBK dissolved more biomass than low MIBK solvents during the process; however, this significantly adds to the process cost (Maraun, 2013). Runtime of the process and acid amount in the solvent were not found to be significant, but were not tested for loblolly pine and hybrid poplar, which might have an important impact due to the feedstock's different chemical compositions (Maraun 2013).

This study applied response surface experimental design to enhance inference of this complex process. The common practice known as 'One-Variable-At-a-Time' is to change one variable at a time which leads to unnecessary resource

use, more experimental runs, inability to detect interactions of factors, and limits inference. Statistical methods improve the understanding of variance and allow for improved understanding of how variance effects experimental outcomes. Experimental design or ‘DOE’ is useful since several independent variables (factors) that potentially influence the response are studied using a proven methodology which enables a researcher to draw valid, reliable, and sound conclusions in an effective, efficient and economical manner (Clements and Kean, 1995). Feedstock ratio, run time, and acid level were used as predictor variables (X’s) and the resulting responses (y) of lignin yield, lignin purity, were used as dependent variables in the analysis in the response surface method (RSM) experimental design.

Research Hypothesis

An integrated biorefinery using organosolv fractionation is able to process softwood, hardwood and herbeacous grasses simultaneously and still separate lignocellulosic biomass into pure cellulose and lignin fractions. Based on previous research as discussed in the Introduction, pure fractions of cellulose and lignin were attainable from hardwood and herbaceous feedstocks by applying organosolv fractionation, however, these did not include softwoods. The most significant reactor factors found in the results from Astner (2012) and Maraun´s (2013) research were applied in this study to predict lignin yields, affiliated purities, and cellulose purity as a function of runtime, acid level, and specifically, feedstock mixture ratio. Subsequently, the hypothesis is that organosolv fractionation fed by

a different feedstock mixture will not affect the yield and purity of the lignin and cellulose fraction as found in previous results.

Objectives

Organosolv fractionation runs using lignocellulosic biomass will be performed to maximize lignin yields and purities. The following objectives were evaluated in the context of experimental deign to test the research hypothesis:

- Develop a RSM experimental design to reduce numbers of runs and resources used;
- Apply different mixed feedstock (hybrid poplar, switchgrass, and loblolly pine) ratios by varying reactor runtime and acid levels;
- Determine the influence of runtime and acid levels on lignin and cellulose yields and purities from the different feedstock ratios;
- Train the RSM model to predict results of lignin and cellulose fractionation using a given set of parameters and validate model performance.

Thesis Organization

The literature review in Chapter Two describes the necessity for integrated biorefineries in the future. Biorefineries are characterized regarding feedstock input and applied pretreatment technologies. An overview of fuels, chemicals, and biobased products is presented. The materials and methods used for the study are described in Chapter Three and include the feedstock, experimental design,

the organosolv fractionation process, and statistical analyses. Results and Discussion are given in Chapter Four and contain the gained insights from the research. Conclusions are given in Chapter Five with a discussion on future directions.

CHAPTER TWO
LITERATURE REVIEW

Integrated Biorefinery

Establishing integrated biorefineries has been identified as the most promising route to develop the US bio-industry (Kokossis et al., 2015). Integrated biorefineries are capable of producing a broad variety of products from biomass, such as biofuels, biopower, and biomaterials (DOE, 2013). The “Biomass R&D Technological Advisory Committee” (BTAC), a committee consisting of leading industrial company representatives, established targets for the US economy, regarding bioenergy, biofuels, and bioproducts production until 2030. BTACs current targets are five quads of electricity and heat (5%), 9.5 quads of transportation fuels (20%), and substitution of 25% of current chemical production with biobased equivalents (BTAC, 2005).

Integrated biorefineries are built based on the ‘biorefinery concept’, a collection of methods and techniques which produce comparable products to crude oil-based refineries from organic material. Methods and techniques used in a biorefinery aim to separate biomass into its building blocks, such as carbohydrates or aromatics, which are applied as precursors for conversion to products like fuels, chemicals, and/or biobased goods. Output products such as furfural and butadiene can be further converted into products such as nylon fibers and synthetic rubbers (Figure 1) (Bozell, 2008). The main promoting factor, however, is the growing demand for renewable fuel in the steadily growing transportation sector. Co-

products benefit with the production of new innovative products which may have beneficial effects both economically and environmentally (Cherubini, 2010).

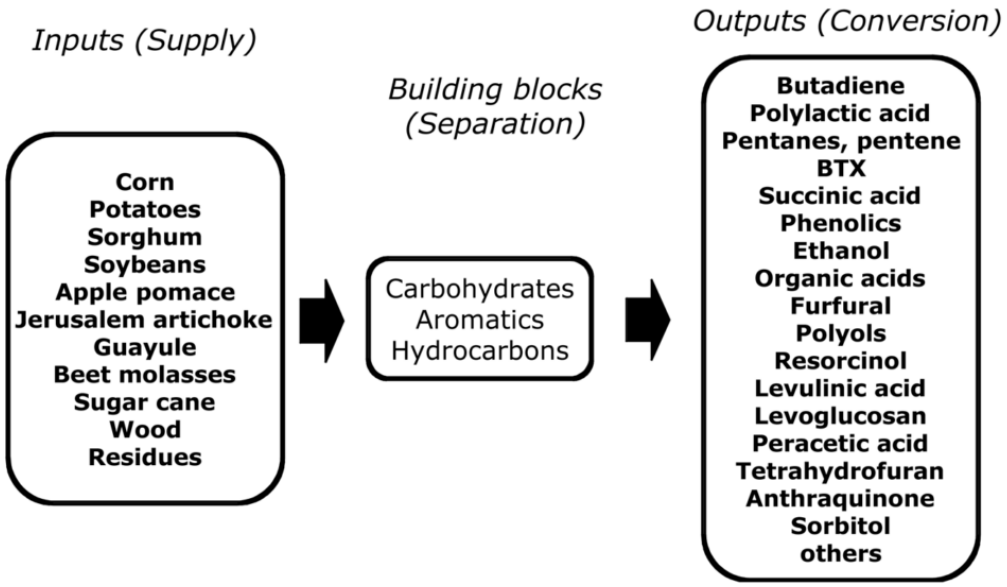


Figure 1. The three stages of a biorefinery operation (Bozell, 2008).

Increased interest in biorefining, to be used in chemical research, has begun to narrow the technological gap in crude oil refining. The lack of available technologies is due to the focus on crude oil derived chemicals, fuels, and materials. Recent research has led to innovative separation methods and techniques to convert the biorefinery building blocks into valuable output products. Major interest in a wider range of building blocks and use of novel conversion methods to reach a larger variety of marketable products with high profit margins, as discussed in this study, will make biorefining more economically feasible, due

to more efficient processes and greater amount of output products which are convertible and comparable to their crude oil based counterpart (Bozell, 2008).

Integrated biorefineries are broken down by their raw materials –whole-crop, green feedstock, and lignocellulosic feedstock (LCF). Whole-crop biorefineries process cereals, corn, etc., green biorefineries process naturally wet biomass like green grass, clover, etc., while LCF biorefineries process naturally dry biomass, such as cellulose containing plants and waste (Kamm and Kamm, 2004). The LCF biorefinery has several advantages, specifically including a vast availability of feedstock and a wide range of conversion products. In the US, approximately 368 million dry tons of biomass per year can be extracted from forestland and an additional 998 million dry tons per year, can be taken from agricultural land without impacting other industries. It is feasible that one-third of the US transportation fuel can be produced from forest and agricultural-derived lignocellulosic biomass feedstocks (Perlack et al., 2005).

Feedstock

Feedstock consists of the raw materials used in biorefineries. Four different sectors will provide lignocellulosic feedstocks for biorefineries in the future: agriculture (dedicated crops and residues), forestry, process residues and leftovers from industries and household waste, as well as algae and seaweed derived from aquaculture (Cherubini, 2010). Lignocellulosic plants, with higher biomass yields per acre, are applied in this study, since they were found more effective than conventional biofuel produced from first generation feedstocks for

biofuel (Kamm et al., 2007, Katzen and Schell, 2008). This work focuses on lignocellulosic biomass including an herbaceous plant, hardwood, and softwood, namely, switchgrass (*Panicum virgatum*), hybrid poplar (*Populus spp.*) and loblolly pine (*Pinus taeda L.).* Further, to simulate the different biomass availability throughout a year, the three feedstocks were mixed in various ratios.

Switchgrass (*Panicum virgatum*) is a hardy, deep-rooted, warm-season, perennial grass, which can grow to a height of 2.5m and produce between two and four dry tons of biomass per acre and year (Uchytil, 1993). Switchgrass, a non-food biomass source, has a broad growing range across the US and southern parts of Canada. The deep roots of switchgrass bind to soil and block erosion, eliminating the effects of run off and loss of nutrients. Because of this, switchgrass is considered a low effort plant as it needs less fertilizer and can be harvested for up to 30 years before requiring rotation (Adler et al., 2006).

Hybrid poplars (*Populus spp.*), belonging to the hardwood family, are the result of crossing of poplar species. Improvements in growth rate, form, adaptability, and disease resistance are being made to meet rising forest resource demands (Kretschmann, 1999). Hybrid poplars are often grown in fast-rotation at tree plantations for lumber production and for the pulp and paper industry. The achieved biomass yield depends highly on the regional climate. In Tennessee, up to nine tons per acre for three to five year rotations have been harvested (IBSS, 2015).

Belonging to the softwood family, loblolly pine (*Pinus taeda L*.), also called Southern yellow pine, is the most widely used commercial tree species, grown on more than 28.9 million acres in the US (Baker and Rials, 2013). Furthermore, loblolly pine is used worldwide for timber and pulpwood and is grown extensively on plantations with more than 741,310,000 acres, accounting for about 18% of the world's industrial round wood supply (Wegrzyn et al., 2014). Loblolly pine reproduces and grows rapidly on diverse sites where it provides large yields of biomass per acre. During each year in the southeastern US, yield maximums range between 3.8 unfertilized to 5.2 fertilized dry tons per acre (Perlack et al., 2005).

Lignocellulosic Biomass

The three biomasses studied in this research, switchgrass, hybrid poplar, and loblolly pine, have a lignocellulosic structure which consists primarily of carbohydrates (cellulose and hemicellulose) and aromatics (lignin).

Trees are broadly defined as softwoods or hardwoods, a botanical description that is determined by how they seed. Softwoods seed from gymnosperms, a Greek term referring to naked seeds, e.g., the coniferous portion of pine species. In contrast, hardwoods come from angiosperms (enclosed seeds) which are flowering plants. As the definition refers to gymnosperms and angiosperms, the names softwood and hardwood are misnomers as not all softwoods are actually considered soft or lightweight and not all hardwoods are considered hard and heavy. However, hardwoods have greater structural

complexity since they have a greater number of cell types and a far greater degree of variability within cell types. The greatest difference is the presence of the characteristic vessel element in hardwoods, which does not exist in softwoods (Rowell, 2013).

Lignin in secondary walls and pectin in primary walls work as a matrix for cellulosic material (Panshin and De Zeeuw, 1980). Between the cell walls is the middle lamella (ML). On the inside of the ML, the primary wall (P) is characterized by cellulose microfibrils in random orientation, which has a major impact on the mechanical properties of the wood cell. Microfibrils, which contribute to the high tensile strength of wood, are formed by bundles of cellulose molecules which synthesize into longer and stronger, thread-like macromolecules (Bruce and Palfreyman, 1998). The secondary wall, made up of three layers, called S1, S2, and S3 (from P inwards), are present in all wood cells as well as in many non-woody plants and plant parts. S1 is a thin layer with a large microfibril angle of 50 to 70° from the long axis of the cell, while S2 is a thick secondary wall attributing to its importance regarding the overall extractable, chemical properties of the cell wall. Characteristics include a low lignin content and a low microfibril angle of 5 to 30°. The microfibril angle of S2 has a strong relationship with the macroscopic wood properties. However, this relationship is not fully understood and is an active area of research. The thin S3 is located on the interior of S2 with a relatively high microfibril angle of ≥70° and contains the lowest percentage of lignin (Figure 2) (Rowell, 2013). The lignin content is of special interest for a biorefinery since lignin

covers the cellulose in the woody structure, halting fermentation during the refining process.

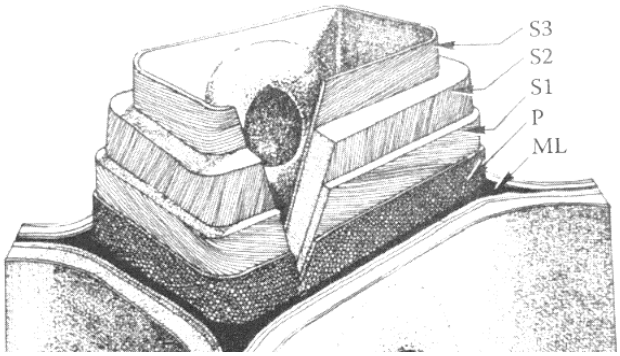


Figure 2. Cut-away drawing of the cell wall, middle lamella (ML), primary wall (P), and secondary cell wall (S1, S2, S3) (Rowell, 2013).

Cellulose

Cellulose, a glucan polymer of D-glucopyranose units, is the most abundant polymer on earth and the main part of a plant cell. The two-sugar repeating unit in cellulose is known as cellobiose (Figure 3) with an average degree of polymerization, the number of glucose units in a cellulose molecule (DP), at 9000 to 10000 DP (Rowell, 2013) Wood contains cellulose, with different structural order, categorized in crystalline or non-crystalline and further as enzymatic accessible or non-accessible. Cellulose has the tendency to form intra- and intermolecular hydrogen bonds, which leads to higher crystallinity which refers to the degree of structural order in solids. The wood surface is accessible to water

and microorganisms, important for degradation products like ethanol fuel, however, lignin and hemicellulose covered cellulose is inaccessible. Further, if highly crystalline regions occur in water and microorganism accessible cellulose, only the surface is accessible. Non-crystalline cellulose is mostly accessible; except for lignin and hemicellulose covered cellulose (Rowell, 2013).

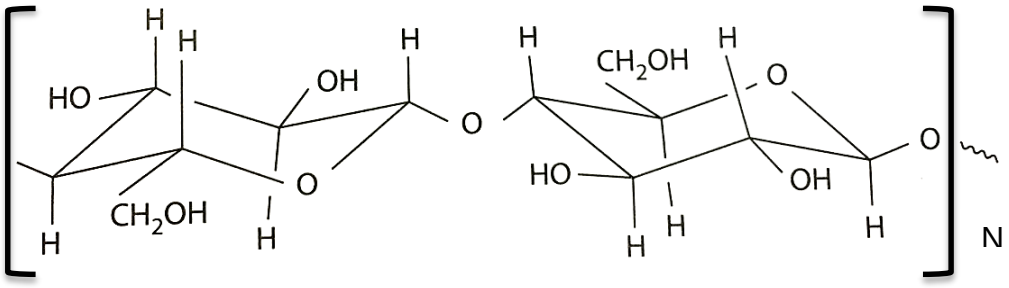


Figure 3. Chemical structure of cellobiose (Rowell, 2013).

Hemicellulose

Hemicellulose is a heterogeneous, polysaccharide made up of hexose sugars (IEA, 2008), such as glucose, galactose, mannose, xylose, and/or other sugars (Bruce and Palfreyman, 1998). The major hemicelluloses in hardwoods are glucuronoxylan (15 to 30% in wood) and glucomannan (2 to 5% in wood), while the major hemicellulose in softwood is galactoglucomannan (15 to 23% in wood) followed by arabinoglucuronoxylan and arabinogalactan. Most wood hemicellulose structures have not been determined, but the sugar ratios of the polysaccharides are known (Rowell, 2013).

Lignin

Lignins are amorphous, highly complex, polymers of non-repeating phenylpropane units, and provide the structural integrity of lignocellulosic plants (Rowell, 2013). Behind cellulose, lignin is the second most abundant organic substance on earth (Northey et al., 2000). Lignin biosynthesis uses three primary precursors, p-coumaryl, coniferyl, and sinapyl alcohol, which compare with the aromatic constituents p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) of lignin. For softwood lignin, coniferyl alcohol (2.) is the dominant precursor, while both coniferyl alcohol (2.) and sinapyl alcohol (3.) are used in hardwood biosynthesis. P-coumaryl alcohol (1.) is a minor precursor for both and is also found extensively in herbaceous feedstocks (Figure 4).

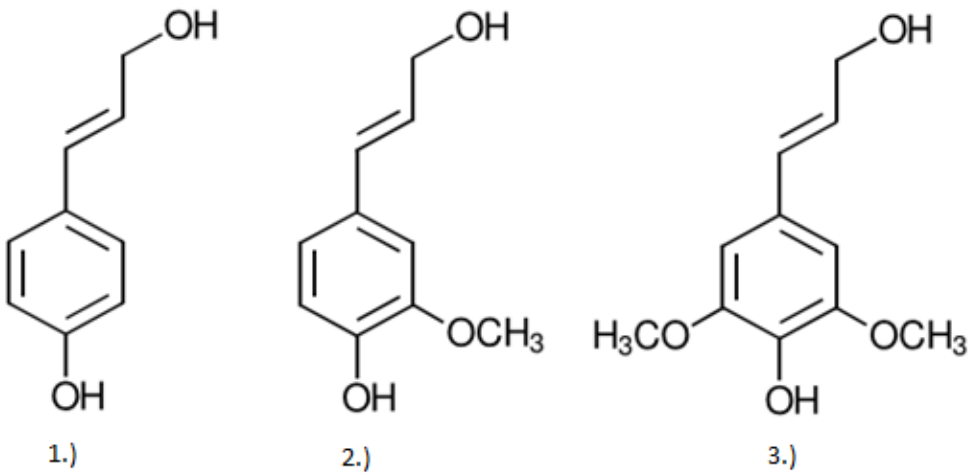


Figure 4. Chemical structure of lignin precursors, p-coumaryl alcohol (1), coniferyl alcohol (2), and sinapyl alcohol (3) (Rowell, 2013).

Principle of Pretreatment

Lignocellulosic biomass, is the most abundant organic material worldwide, and can be converted to ethanol and other fuels (Zhao et al., 2009, Claassen et al., 1999). A integrated biorefinery, producing biofuels, chemicals, and biomaterials from lignocellulosic biomass requires a pretreatment to increase the enzymatic accessibility of cellulose but also to fractionate the biomass into highly pure streams (Figure 5) (Sims et al., 2010).

However, most pretreatments available today aim at the production of biofuel to increase the enzymatic accessibility of cellulose for hydrolysis. Hydrolysis breaks the β-1,4-glycosidic bonds of cellulose and the gained glucose is further fermented to ethanol, used as biofuel (Gupta, 2010). Hydrolysis of lignocellulosic biomass without pretreatment leads to sugar yields under 20%, but the application of a pretreatment allows sugar yields over 90% (Hamelinck et al., 2005). Pretreating lignocellulosic biomass is one of the most cost intensive steps of biorefining (Eggeman and Elander, 2005). For that reason, biorefineries that only produce fuel are often not cost effective and require substitutions. Furthermore, the fractionation efficiency varies between feedstocks which means the final product must be taken into consideration prior to selecting an applicable pretreatment technology (Kumar et al., 2009). This study is using organosolv fractionation, a pretreatment technology making the lignin and cellulose components available in fractions with little cross-contamination. The focus of this study is on the fractionation effectiveness using mixed feedstocks.

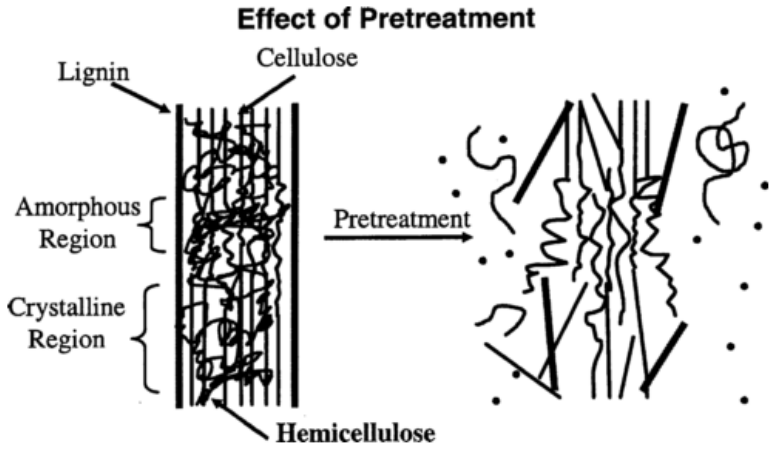


Figure 5. Schematic of pretreatment to disrupt the physical structure of biomass (Mosier et al., 2005b).

Pretreatment Technologies

Lignocellulosic biomass may be pretreated with various different processes, but only a few are promising to suit the biorefinery concept, including steam explosion, ammonia fiber expansion (AFEX), dilute acid pretreatment, alkali pretreatment technology including lime pretreatment (Mosier et al., 2005a, Wyman et al., 2005), and organosolv fractionation.

Steam explosion

Un-catalyzed steam explosion is commercially applied and refers to a pretreatment process that rapidly heats lignocellulosic biomass by high pressured saturated steam which is held for a short time period (seconds to minutes) before the pressure is explosively decompressed (Brownell and Saddler, 1987). While the process opens up the particulate biomass, reduces particle size, and increases the

pore volume, the major effect is attributed to the removal of the hemicellulose, which enhances the enzymatic accessibility (Brownell et al., 1986).

Ammonia Fiber Expansion (AFEX)

AFEX, a physio-chemical pretreatment method, uses liquid ammonia at temperatures between 160 and 180°C in a pressure reactor, similar to steam explosion, to depolymerize lignin, de-crystallize cellulose, and split linkages between lignin and carbohydrates (Kim et al., 2003). However, this method works well for herbaceous and agricultural residues, but only partially on hardwoods and with even less efficiency for softwoods. A major cost factor of this process is ammonia and the ammonia recovery (Mosier et al., 2005a).

Dilute Acid Pretreatment

The dilute acid pretreatment disrupts the lignocellulosic structure, primarily by hydrolyzing hemicelluloses to enhance digestibility of the residual solids. Most frequently studied is the dilute sulfuric acid pretreatment, which was found to effectively remove hemicelluloses for various feedstocks, such as hardwoods, grasses, and agricultural residues (Mosier et al., 2005a). Further, hydrochloric acid, phosphoric acid, and nitric acid were tested. The process can be carried out by adding acid to the biomass and introducing the hydrolysis by indirectly heating the vessel, or directly heating by steam injection (Mosier et al., 2005a).

Alkaline Pretreatment Technology

Alkaline pretreatment methods employ bases, such as calcium, sodium, potassium, and ammonia hydroxide or aqueous ammonia, primarily to remove lignin from the biomass which improves enzymatic accessibility to hemicellulose and cellulose (Mosier et al., 2005a). The process can be carried out at ambient temperature, which results in a process time of hours or days, and biomass contamination as the alkaline material is converted to salts (Lin et al., 1981).

Lime pretreatment uses calcium hydroxide and is a specific example of alkali pretreatments. The process is carried out at ambient temperatures by spreading aqueous lime on the biomass, and storing the biomass as a pile for hours or days (Mosier et al., 2005b). However, higher temperatures can reduce the process time, e.g. 6 h for poplar wood at 150°C, and 2 h for switchgrass at 100°C (Chang et al., 1998).

Organosolv Pretreatment

In the 1970s, due to air and water pollutions caused by kraft and sulfite pulping processes, pretreating lignocellulosic biomass with organic solvents found interest (Zhao et al., 2009). Organosolv pretreatments apply a mixture of organic solvent and water, to remove lignin and hemicellulose from the cellulose and offer a selective and effective fractionation of lignocellulosic biomass. Compared to other pretreatments, organic solvents are costly, however, most used solvents can be recovered and recycled. Further, the selectivity of the fractionation provides separate cellulose, hemicellulose, and lignin fractions, promising for integrated

biorefineries. Preferred organic solvents for this process are ethanol or methanol, but also solvents with higher boiling points like ethylene glycol or glycerol, that allow process temperatures up to 250°C, have beem investigated (Zhao et al., 2009). Mineral acids, such as hydrochloric, sulfuric, or phosphoric acids, are applied as a catalysts to improve delignification (Zhao et al., 2009).

For this study an advanced organosolv fractionation method, using a ternary solvent mixture of water, ethanol, MIBK and sulfuric acid as a catalyst was applied, to fractionate mixed feedstocks as explained in the Materials and Methods chapter. Compared to the earlier mentioned pretreatment methods, which primarily target the enzymatic accessibility of cellulose for EtOH production, solvent fractionation offers higher selectivity to separate the three primary lignocellulosic components (Bozell et al., 2011a). Since cellulose purities of over 95% are achievable, by applying high acid concentrations, solvent fractionation derived cellulose could be an alternative to high pure cellulose derived from conventional energy and chemical intense sulfite and pre-hydrolysis kraft pulping, which achieves purities of over 97% (Bozell et al., 2011a, NREL, 2001). Highly pure cellulose is a commercial precursor for a variety of products such as chemicals, plastics, food additives, fibers, and textiles. Further, during this commercial pulping/pretreatment process, lignin and hemicellulose are highly cross-contaminated and are mainly used to produce thermal heat by combustion (NREL, 2001). Due to the highly selectivity of solvent fractionation, hemicellulose and high pure lignin are applicable for high-value downstream

conversion products such as chemicals or bio-materials (Bozell et al., 2011a). In conclusion, solvent fractionation yields of hemicellulose along with highly pure cellulose and lignin fractions, can be used to produce not only low value fuel, but high value chemicals and bio-products, enabling bio-refining as a cost-effective alternative to conventional crude oil refining in the future.

Experimental Design

The goal of this study is to determine if the lignin yield and purity gained from the organosolv fractionation process from different mixed feedstocks can be maximized. The experiments follow an experimental design matrix that finds similar application in industrial experiments. Industrial experiments are usually executed using deductive reasoning in the following order (Montgomery and Buchanan, 2001):

1. **Hypothesis** Prior knowledge of a phenomenon leads to a hypothesis of the experimenter, that motivates the experiment
2. **Experiment** Series of tests to investigate the hypothesis
3. **Analysis** Understanding the nature of the data and perform statistical analyses
4. **Interpretation** Understanding the experimental analysis results
5. **Conclusion** Stating if hypothesis is true or false. If the hypothesis is true further experiments have to be conducted to validate the results, if not a new hypothesis is established

According to Snee (1990), it is important to develop a ‘statistical thinking’ philosophy of learning and action based on three key principles (Snee, 1990):

- All work occurs in a system of interconnected processes
- Variation exists in all processes
- Understanding and reducing variation are the key to success

‘Statistical thinking’ is derived from Demings’ key principle of quality: ‘Reduce variation and you improve quality’ (ASQ, 1996).

During designed experiments input variables (factors) are intentionally changed to receive corresponding output changes (Montgomery and Buchanan, 2001). A general overview of a system model is shown in Figure 6. The performance is characterized by the output(s) *Y* and are measured to assess the product/process performance. The controllable factors X_n are varied and are considered to be important variables defining the process (e.g., temperature, acid level, etc.). Controllable factors can be continuous (e.g., temperature, moisture, etc.) or categorical (e.g., acid type, feedstock type, etc.) Other factors Z_n or ‘covariates’ (e.g., bulk density, ambient temperature, ambient humidity, etc.) are not considered controllable and may induce variation in the response or *Y*. The influence of these uncontrollable variables (sometimes also called ‘noise’) in the presence of factors (X_n) that can be controlled are fundamental to robust product design, i.e., a product at optimal settings of the *X’s* that is not influenced by uncontrollable factors or ‘noise’, e.g., room temperature differences (Roy, 2001).

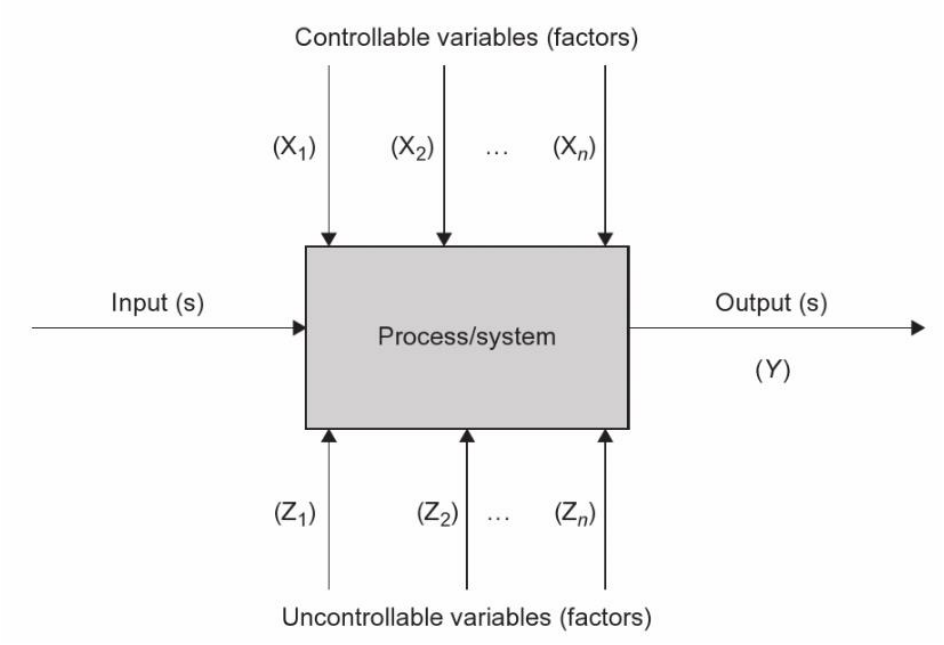


Figure 6. General process or system model (Antony, 2014).

To reduce experimental bias, randomization, replication, and blocking are crucial in Design of Experiments (DOE). A particular experimental design is chosen depending on the objectives of the study (e.g., screening design, full factorial design, RSM, etc.) and financial budget for the research. Table 1 presents a guideline used to choose a suitable experimental design. The primary goal of a 'comparative objective' is to find the most influential factors. To select or screen out the important main effects of an experiment, the 'screening objective' is given. For this study, the factors with the highest impact were defined from previous research (Maraun, 2013, Astner, 2012) and a RSM was selected for the purpose of developing a predictive model across the response surface of the response variables. The RSM allows for estimation of the interactions between factors and

a model is developed in the context of center points and a quadratic equation. RSM was used to detect issues and weak points, improve process settings, and make the process more robust against external and non-controllable influences (NIST, 2016).

Table 1. Design Selection Guideline (NIST, 2016).

Number of Factors	**Comparative Objective**	**Screening Objective**	**Response Surface Objective**
1	One-factor completely randomized design	-	-
2 - 4	Randomized block design	Full or fractional factorial	Central composite or Box-Behnken
5 or more	Randomized block design	Fractional factorial or Plackett-Burman	Screen first to reduce number of factors

RSM is a designed regression analysis that uses systematic level changes of feedstock ratio, run time, and acid level as predictor or independent variables (*Xs*) for the dependent or response variables (*Ys*) of lignin yield, lignin purity, and cellulose purity. RSM is distinguished as first and second order designs. First order (linear) designs are able to point in the optimum direction, but cannot pinpoint it. Second order (quadratic) models allow, as long as the optimum is in range, to pin point the optimum direction. Central composite RSM designs and Box-Behnken RSM designs are the most popular second order models and can be distinguished

on how they achieve multiple levels of prediction variables. There are three different types of central composite (CC) designs (Figure 7). CC inscribed (CCI) handles the limits as fixed and stays within the boundaries (smallest range). CC circumscribed (CCC) extends its range by establishing new extreme points (biggest experimental range). The CC-face centered (CCF) has levels on the faces which are centered and require only three levels instead of five. For this study, a central composite face centered design was applied since the factor range was determined from earlier studies Astner (2012) and Mauran (2013) (NIST, 2016). Both previous studies applied the Taguchi Robust Design, a screening design allowing to examine various factors simultaneously, which recommended significant factors studied in this work (feedstock ratios, acid level, and runtime).

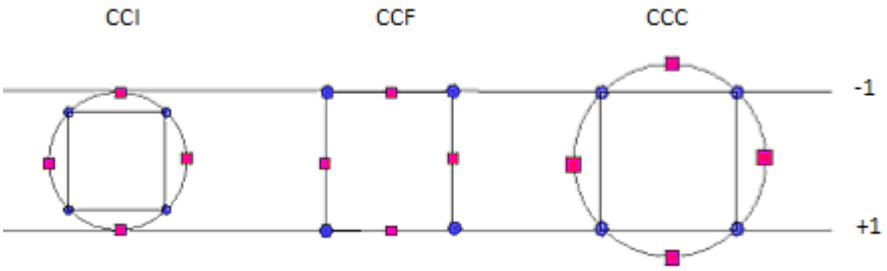


Figure 7. Three different types of central composite designs (NIST, 2016).

To describe the predictive model of the optimization study, a second order polynomial equation is used:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (1)$$

y describes the quality measure unit (for example, in this study lignin yield in percent), X_i is the level of experimental variable *ith* and β_j represents the coefficients values calculated in the regression analysis. Standard model-selection regression techniques can be used to ensure only significant effects (p-value ≤ 0.05) are included in the model. When the regression model fits well, the prediction is mostly accurate in the range of the studied levels. However, it is not possible to tell at which point the prediction leaves the model after the minimum and maximum of the explored range (between high and low levels of the experimental factor (*X*)).

The impact of the different factor levels was visually displayed in box plots to gain insight into the distribution of the collected data. The hypothesis was tested and interpreted by one-way ANOVA (Summary of Fit and Analysis of Variance) and each pair was set side-by-side using the Student's t test.

CHAPTER THREE
MATERIALS AND METHODS

Experimental Design

To determine the impact primarily on lignin yield and secondarily on lignin and cellulose purity, responses to following factors were analyzed: runtime (60 and 120 min), acid level (0.05 and 0.15 M), and feedstock ratio (two feedstocks low (10%) and one feedstock high (80%)). The response surface design contained four center point runs (90 min; 0.10 M; equal amounts of each feedstock Table 2 and Table 3). Feedstock ratios were chosen, based on Astner (2012) and Maraun (2013) studies that applied 10% as the low factor. Since three factors were applied, the high factor was chosen to be 80%. Also, high runtime and acid level were applied, since pine was found to be hardly fractionated by organic solvents.

The response surface design was created using JMP 12.0.1, a statistical computer program (http://www.jmp.com/en_us/home.html, accessed August 10, 2016). All experiments were carried out randomly in order to reduce bias caused by uncontrolled variables. Table 4 displays runs from low to high reactor settings.

Table 2. Continuous factors analyzed on their impact on lignin yield, lignin and cellulose purity.

Continuous Factors		**Levels**	
	-1	Center	+1
Runtime (X_1)	60 min	90 min	120 min
Acid Level (X_2)	0.05 M	0.1 M	0.15 M

Table 3. Categorical (or nominal) factors analyzed on the impact on lignin yield, lignin and cellulose purity.

Nominal Factor	**Levels (in wt %)**			
	1	2	3	4
Feedstock Ratio (X_3)	High switchgrass (10/10/80)	High poplar (10/80/10)	High loblolly pine (80/10/10)	Center (33/33/33)

Table 4. Experimental runs used in the RSM.

Run #	**Run Time (min)**	**Acid Level (Mole)**	**Feedstock Ratio (%)**
1	60	0.05	10/80/10
2	60	0.05	10/80/10
3	60	0.05	33/33/33
4	60	0.05	80/10/10
5	60	0.05	80/10/10
6	60	0.15	10/10/80
7	60	0.15	10/10/80
8	60	0.15	33/33/33
9	60	0.15	33/33/33
10	60	0.15	80/10/10
11	90	0.10	10/10/80
12	90	0.10	10/80/10
13	90	0.10	33/33/33
14	90	0.10	80/10/10
15	120	0.05	10/10/80
16	120	0.05	10/10/80
17	120	0.05	10/80/10
18	120	0.05	33/33/33
19	120	0.05	33/33/33
20	120	0.05	80/10/10
21	120	0.05	80/10/10
22	120	0.15	10/10/80
23	120	0.15	10/80/10
24	120	0.15	10/80/10
25	120	0.15	33/33/33
26	120	0.15	33/33/33
27	120	0.15	80/10/10
28	120	0.15	80/10/10

Feedstock

Feedstocks, made up of loblolly pine (*Pinus taeda L.)*, hybrid poplar (*Populus spp.),* and switchgrass (*Panicum virgatum*), were mixed in various ratios to test their impact regarding lignin and cellulose yield and purity resulting from organosolv fractionation. The mixture ratios were determined from previous research conducted by Astner (2012) and Maraun (2013). Loblolly pine (Auburn University) and dried hybrid poplar (TennEra) were dried to a moisture level of 8±1 % in the form of pulp grade chips, approximately 4 cm^2 and 0.5 cm in thickness. Switchgrass (provided by TennEra) was harvested in East Tennessee and was dried to 8 ± 1%, and then knife milled to an average length of 1 to 2 inches (25.4 mm to 50.8 mm). For the experiment, all feedstocks were hammer-milled and sieved to a mesh size between 20 (0.841 mm) and 4 (4.76 mm) (Figure 8, Figure 9, and Figure 10). Each feedstock type was stored in sealed, labeled buckets to maintain a stable moisture content (8 ± 1%).

Table 5 shows the structural components of each feedstock obtained by compositional analysis for each feedstock.

Table 5. Compositional Analysis of Feedstocks (in %).

Feedstock	Cellulose	Hemicellulose	Lignin	Ash	Extractives
Hybrid Pop.	44.1	17.7	28.7	1.0	4.4
Loblolly Pine	42.2	19.3	32.9	0.5	2.9
Switchgrass	36.4	28.9	20.9	1.9	9.1



Figure 8. Switchgrass (*Panicum virgatum*) used for organosolv fractionation (mesh sizes 20-4).



Figure 9. Hybrid poplar (*Populus spp*.) used for organosolv fractionation (mesh sizes 20-4).



Figure 10. Loblolly Pine (*Pinus taeda L*.) used for organosolv fractionation (mesh sizes 20-4).

Prior to use, a 400 gram (g) total of each feedstock was hand stirred to create an even distribution. Feedstock mixtures were labeled according to their ratios in the order pine/poplar/switchgrass, e.g. Mix 1 (10/10/80), and were referred to by their primary feedstock as “high”, e.g. high switchgrass, as shown in Table 6.

Table 6. Feedstock mixtures in wet weight [ratio %].

Feedstock	Loblolly Pine	Hybrid Pop.	Switchgrass
Mix 1 (High Switchgrass)	10	10	80
Mix 2 (High Poplar)	10	80	10
Mix 3 (High Pine)	80	10	10
Mix 4 (Center)	33	33	33

Mixed feedstocks, due to their different composition, had different total amounts of lignin available. The total potential lignin for each feedstock mixture was determined based on the compositional analysis of the individual feedstocks in the mixture (Table 7). The feedstock components per reactor run for each mixture are also presented as wet weight in %, wet weight in g, and corresponding dry weight in g (Table 8).

Solvent mixture

The solvent used for the fractionation consists of MIBK, 190 proof ethanol (EtOH), and deionized water (H_2O) in a 16/34/50 wt% ratio of MIBK, EtOH, and respectively (Figure 11). This solvent mixture in previous studies has shown positive fractionation results without vast amounts of dissolved cellulose.

Table 7. Maximum available lignin for each mixture based on feedstock composition.

Feedstock	**Lignin content [g]**			**Maximum attainable lignin [g]**
	Loblolly Pine	Hybrid Poplar	Switchgrass	
Mix 1 (High Switchgrass)	11.91	10.83	62.16	84.90
Mix 2 (High Poplar)	11.91	86.65	7.77	106.33
Mix 3 (High Pine)	95.26	10.83	7.77	113.86
Mix 4 (Center)	39.69	36.11	25.90	101.70

Table 8. Feedstock ratio broken down in wet weight [%], wet weight [g], and dry weight [g].

	Feedstock (1. wet weight [%] / 2. wet weight [g] / 3. dry weight [g])								
	Loblolly Pine			**Hybrid Pop.**			**Switchgrass**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Mix 1 (High Switchgrass)	10	40	36.2	10	40	37.7	80	320	197.4
Mix 2 (High Poplar)	10	40	36.2	80	320	301.9	10	40	37.2
Mix 3 (High Pine)	80	320	289.6	10	40	37.7	10	40	37.2
Mix 4 (Center)	33	133.3	120.6	33	133.3	125.8	33	133.3	123.9

The process is catalyzed with 95% sulfuric acid at three different levels (0.05, 0.1, and 0.15 M).

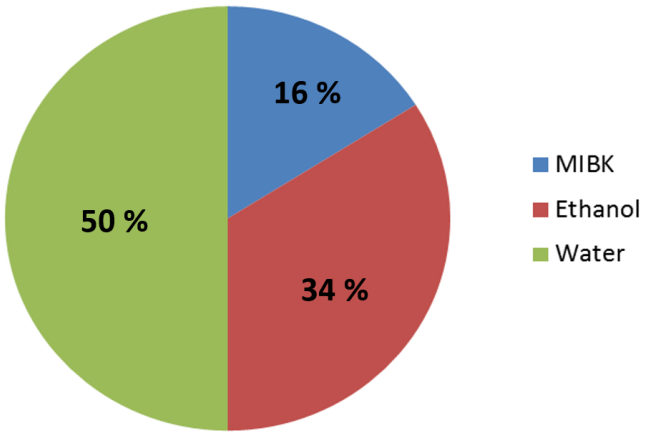


Figure 11. Standard solvent composition: 50% water, 34% ethanol, 16% MIBK.

Operation

Experiments fractionated mixed feedstocks into building blocks to target maximum lignin yields and purities, and cellulose purity during the organosolv fractionation process as earlier described (Astner et al., 2015, Bozell et al., 2011a, Bozell et al., 2011b).

In general, 400 g of the feedstock mixture was placed in a Teflon sock supported by a perforated Teflon basket. The Teflon basket was placed in a Hastelloy C276 flow-through pressure reactor. After sealing the reactor, vacuum (- 10 ± 1 psi) was run for 20 minutes to remove excess air and allow better penetration of the solvent into the feedstock. Subsequently, the vacuum is used to

pull the solvent into the reactor. When filled, electrical heaters were used to bring the reactor to a 150°C process temperature. As the process temperature and correlating pressure (115 psi) in the reactor were reached, the solvent flow was started. Additional pressure during the reaction resulted in solvent flow and triggered the output valve (at +0.15 psi) to stabilize internal pressure and release excess liquid, known as black liquor. Once the solvent flow began, the runtime (60, 90, and 120 min) was started. As the runtime ran out, the heaters were turned off and remaining black liquor collected. Once the reactor cooled, the remaining solid fraction was removed. Reactor temperature and pressure were monitored and controlled by Lab-VIEW 8.6 software (Figure 12).

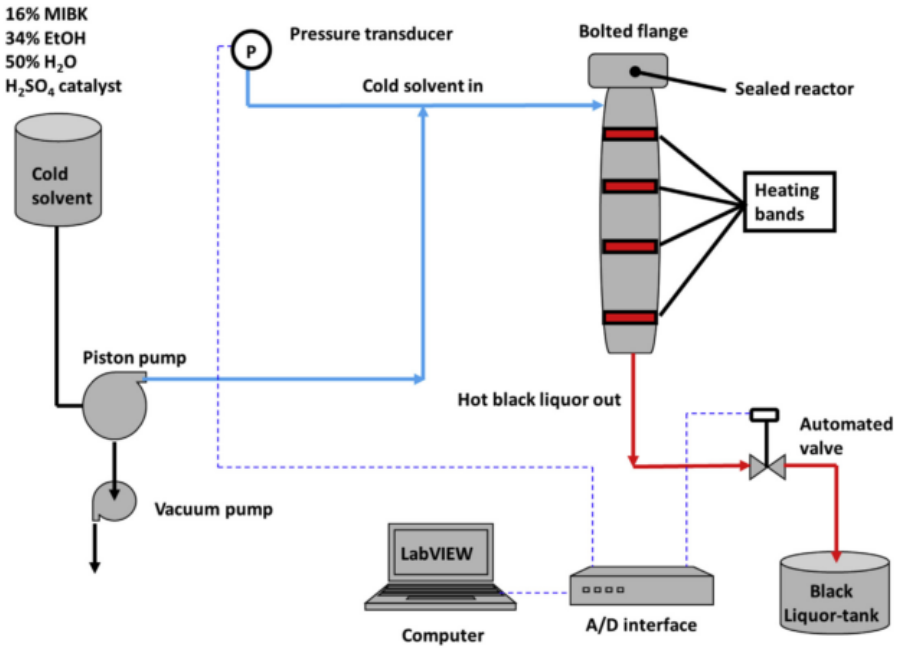


Figure 12. Reactor flow diagram and layout (Astner et al., 2015).

Processing of soluble and insoluble fractions

In order to obtain individual fractions of cellulose, lignin, and hemicellulose, the black liquor (BL) was filtered to eliminate small amounts of insoluble cross-contamination. Filtered BL was then transferred to two to four 4000 ml (dependent on run time) separatory funnels (3000 ml each + one funnel for the remaining). To achieve a phase separation into aqueous and organic phase, 30% of DI-H_2O based on the amount of BL present was added (BL [ml] x 0.3 = added DI-H_2O [ml]), and separated into two primary phases within 30 minutes. The dark upper fraction of the BL consisted of mainly organic solvents (MIBK and EtOH) and lignin called the “organic fraction” (ORG). A lower fraction in the black liquor was light brown consisting of primarily DI-H_2O, hemicellulose and lignin, and is known as the “aqueous fraction” (AQU). A murky intermediate fraction between AQU and ORG generally appeared during the separation and was collected with the AQU fraction. Two additional water washes were conducted on the ORG-fraction in order to reduce ethanol content in the ORG phase to accelerate further treatments. The ORG-phase was then treated in a rotary evaporator using a 50°C water bath at 60 revolutions/minute for 45 minutes to remove solvent. The initial “sticky lignin” was further treated twice on the rotary evaporator by adding 500 ml of H_2O and stirring at a lower temperature (40°C) for 45 minutes, with the purpose of dissolving and removing extractives and sugars by decantation.

The AQU-fraction was processed on a large rotary evaporator (20 liter) at 50°C, with a vacuum of 130 mbar, for 2 hours to remove excess solvent and

precipitate additional lignin. Both fractions (ORG and AQU) were filtered under vacuum for 12 hours. To generate a dry, powdery lignin, the filtered ORG and AQU fractions were dried in a vacuum oven at 80°C for 12 hours. After drying, the AQU and ORG fractions both were separately homogenized using mortar and pestle, to produce a powdery lignin as shown in Figure 13 and Figure 14. The recovered solid fraction (mainly cellulose) was dispersed in deionized water for at least 12 hours to remove residual solvent. The solid fraction was then washed continuously with water in a Büchner funnel with a polypropylene filter as described in previous studies, to remove excess solvent (Maraun, 2013, Astner, 2012). Afterwards, residual water was pressed from the cellulose using a latex membrane under vacuum. The weight of the cellulose was determined, three samples taken for dry weight and further purity analysis, and the remaining cellulose was packaged, labelled, and frozen. A dried and milled cellulose sample is presented in Figure 15. Klason lignin analysis for acid soluble (ASL) and acid insoluble (AIL) lignin was conducted following protocol NREL/TP-510-4268 to determine total lignin in all collected cellulose and lignin fractions. The AIL was determined by near infrared spectroscopy by applying 205 nm as a peak, which was recommended for mixed feedstocks. For the analysis, the weights of AQU and ORG lignins as well as percentages of purity were added together for the statistical analysis. The calculation of the total lignin yield for each organosolv run was carried out using the following formula:

$$Lignin\ Yield\ [\%] = \frac{ASL + AIL\ Lignin\ after\ pretreatment\ [g]}{Klason\ lignin\ in\ biomass\ [g]} \times 100 \qquad (2)$$



Figure 13. Recovered organic lignin fraction



Figure 14. Recovered aqueous lignin fraction.



Figure 15. Recovered milled solid fraction (mainly cellulose).

CHAPTER FOUR
RESULTS AND DISCUSSION

The results of the total lignin analysis, which includes acid insoluble lignin (AIL) and acid soluble lignin (ASL), are presented in this chapter. After organosolv fractionation, the total lignin analysis was applied to determine the purity of the recovered lignin and cellulose. The second section of the chapter analyzes the probability density function (or 'distribution') of the data. Sections three through five of the chapter quantifies the lignin maximization from the RSM for lignin yield, lignin purity, and cellulose purity. In the last section of the chapter, the three lignin maximization responses in the context cellulose yield is given.

Total Lignin Analysis

The results of the total lignin analysis indicate that the organosolv fractionation resulted in a highly pure lignin fraction, with little cross-contamination. Specific results suggest that for mixed feedstocks, a mean lignin purity of 95.47% and a mean cellulose purity of 86.42% were attainable. The mean lignin yield was found to be 64.80%. However, yields varied and were dependent on feedstock ratio and are discussed in detail in the lignin yield section of this chapter.

The mean unextracted lignin yield was 13.58%. Unextracted lignin was not removed from the solid cellulose fraction and another minor part was lost during processing of the black liquor. The total lignin of cellulose ('cellulose purity') was determined using the assumption that lignin residues are present in the solid fraction. Lignin yield, lignin purity, and cellulose purity results are given in Table 9.

The data used for lignin purity and yield, displayed in Table 9, are based on the derived data for impure recovered lignin, pure recovered lignin, and the compositional lignin content for each feedstock mixture (Table 10). The impure recovered lignin represents the dry weight of collected lignin after black liquor processing, as explained in the Materials and Methods Chapter. The pure recovered lignin displays the actual lignin of the recovered lignin fractions by total lignin analysis (ASL+AIL). Feedstock lignin content represents the maximum attainable lignin yield, i.e., the pure recovered lignin divided by the feedstock lignin content as seen in equation [1] of the Materials and Methods Chapter and is presented in Table 9.

Probability Density Function of the Data

The data were found to be normally (Gaussian) distributed, which is important for further statistical analyses of lignin and cellulose yields and purities (Figure 16 and Figure 17). Since a key statistical assumption of analysis of variance (ANOVA) is that the data are normally distributed, a ‘Goodness of Fit’ for a normal distribution was used with the Shapiro-Wilk W Test. Numbers close to one indicate normally distributed data, while closer to zero suggests a departure from normality (Shapiro and Wilk, 1965). The Shapiro-Wilk W tests for lignin yield was $W = 0.98$ ($p = 0.9477$); and for lignin purity was $W = 0.94$ ($p = 0.1299$).

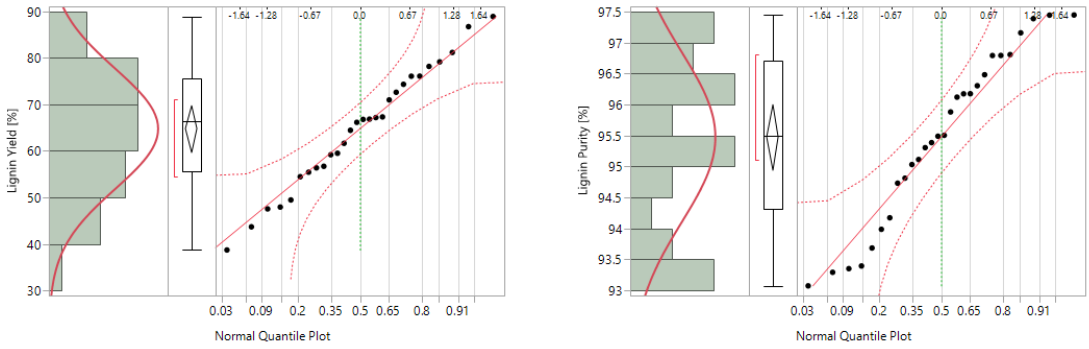
Table 9. Lignin yield, lignin purity, and cellulose purity results presented in percent by run settings and feedstock mixture and combined severity.

Run #	Run Time [min]	Acid Level [M]	Feedstock Ratio [PINE/POP/ SG]	Combined Severity	Lignin Yield in [%]	Lignin Purity ASL+AIL in [%]	Cellulose Purity in [%]
1	60	0.05	10/80/10	1.90	79.18	93.69	91.55
2	60	0.05	10/80/10	1.90	59.21	95.49	84.53
3	60	0.05	33/33/33	1.90	59.54	95.03	86.11
4	60	0.05	80/10/10	1.90	38.73	93.08	77.60
5	60	0.05	80/10/10	1.90	43.70	94.81	75.43
6	60	0.15	10/10/80	2.30	76.10	96.17	92.43
7	60	0.15	10/10/80	2.30	86.75	93.35	91.44
8	60	0.15	33/33/33	2.30	56.72	96.12	85.24
9	60	0.15	33/33/33	2.30	74.36	96.81	85.56
10	60	0.15	80/10/10	2.30	47.96	96.48	77.91
11	90	0.10	10/10/80	2.33	88.93	95.88	92.74
12	90	0.10	10/80/10	2.33	67.35	93.29	92.94
13	90	0.10	33/33/33	2.33	64.47	96.79	86.76
14	90	0.10	80/10/10	2.33	49.49	95.11	78.74
15	120	0.05	10/10/80	2.20	72.64	94.73	89.25
16	120	0.05	10/10/80	2.20	71.00	94.17	90.79
17	120	0.05	10/80/10	2.20	78.20	95.39	94.50
18	120	0.05	33/33/33	2.20	66.81	93.99	90.98
19	120	0.05	33/33/33	2.20	66.87	93.40	90.15
20	120	0.05	80/10/10	2.20	55.45	96.79	81.38
21	120	0.05	80/10/10	2.20	54.49	95.50	81.46
22	120	0.15	80/10/10	2.60	81.20	97.44	92.56
23	120	0.15	10/80/10	2.60	76.11	97.16	94.08
24	120	0.15	10/80/10	2.60	66.18	96.30	94.97
25	120	0.15	33/33/33	2.60	61.67	97.44	87.91
26	120	0.15	33/33/33	2.60	67.20	95.31	88.68
27	120	0.15	80/10/10	2.60	56.39	96.17	75.57
28	120	0.15	80/10/10	2.60	47.56	97.38	68.54
Validation Runs							
V1	90	0.1	10/10/80	2.33	76.56	96.97	94.18
V2	90	0.1	10/80/10	2.33	65.67	97.06	92.98
V3	90	0.1	10/10/80	2.33	56.99	96.91	79.88
V4	90	0.1	80/10/10	2.33	57.47	95.87	84.30

Table 10. Data used for further analysis: recovered lignin by impure, pure, and compositional lignin content.

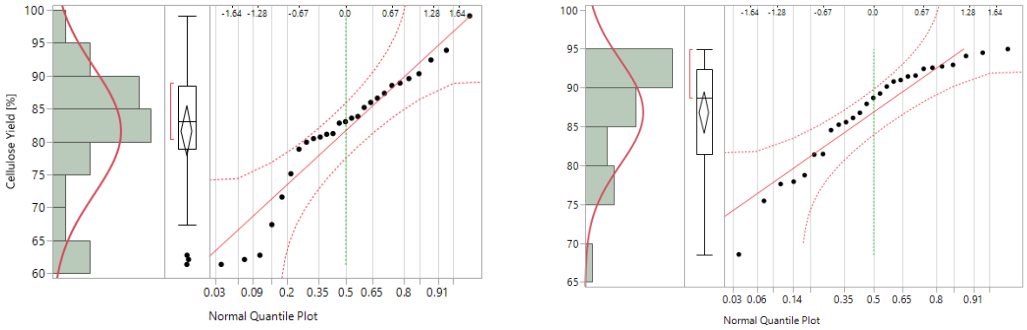
Run #	Run Time [min]	Acid Level [M]	Feedstock Ratio [PINE/POP/SG]	Impure recovered Lignin [g]	Pure recovered Lignin [g]	Feedstock Lignin Content [g]
1	60	0.05	10/80/10	89.87	84.20	106.33
2	60	0.05	10/80/10	65.93	62.96	106.33
3	60	0.05	33/33/33	63.71	60.55	101.70
4	60	0.05	80/10/10	47.38	44.10	113.86
5	60	0.05	80/10/10	52.49	49.76	113.86
6	60	0.15	10/10/80	67.18	64.61	84.90
7	60	0.15	10/10/80	78.90	73.65	84.90
8	60	0.15	33/33/33	60.01	57.68	101.70
9	60	0.15	33/33/33	78.11	75.62	101.70
10	60	0.15	80/10/10	56.60	54.61	113.86
11	90	0.10	10/10/80	78.75	75.51	84.90
12	90	0.10	10/80/10	76.76	71.61	106.33
13	90	0.10	33/33/33	67.74	65.57	101.70
14	90	0.10	80/10/10	59.25	56.35	113.86
15	120	0.05	10/10/80	65.10	61.67	84.90
16	120	0.05	10/10/80	64.01	60.28	84.90
17	120	0.05	10/80/10	87.17	83.15	106.33
18	120	0.05	33/33/33	72.29	67.94	101.70
19	120	0.05	33/33/33	72.81	68.00	101.70
20	120	0.05	80/10/10	65.24	63.14	113.86
21	120	0.05	80/10/10	64.96	62.04	113.86
22	120	0.15	10/10/80	70.75	68.94	84.90
23	120	0.15	10/80/10	83.30	80.93	106.33
24	120	0.15	10/80/10	73.07	70.37	106.33
25	120	0.15	33/33/33	64.36	62.72	101.70
26	120	0.15	33/33/33	71.71	68.34	101.70
27	120	0.15	80/10/10	66.76	64.20	113.86
28	120	0.15	80/10/10	55.61	54.16	113.86
Validation Runs						
V1	90	0.1	10/10/80	67.03	65.00	84.90
V2	90	0.1	10/80/10	71.95	69.83	106.33
V3	90	0.1	10/10/80	66.96	64.89	113.86
V4	90	0.1	80/10/10	60.96	58.44	101.70

The data distribution for cellulose yield indicates a slight skew to the left, indicating asymmetry of the collected data and detection of three possible outliers. Possible outliers of cellulose yield were found for runs 9 (center point run), 23 and 24 (high poplar run), with their settings displayed in Table 4. There was no indication during the experimental runs or laboratory analyses that anything unusual occurred or that an error occurred associated with the identification of an outlier. However, the influence of these three runs using the Shapiro-Wilk W Test were not significant enough to suggest departure from normality for both cellulose yield and purity, i.e., $W = 0.93$ ($p = 0.0695$) and $W = 0.91$ ($p = 0.0218$), respectively.



a. b.

Figure 16 (a) and (b). Histogram, boxplot, and normal quantile plot suggesting normal distribution for lignin yield (a.) and purity (b.) data,



a. b.

Figure 17 (a.) and (b.). Histogram, boxplot, and normal quantile plot suggesting normal distribution of cellulose yield (a.) and purity data (a.), furthermore for a confidence interval of 95%, three outliers were detected (cellulose yield (a.) of run 9, 23, and 24).

Lignin Yield

Response Surface Design

Analysis of Variance (ANOVA), indicated that one or more of the factors studied were significant (p = 0.0016) (Table 11). Feedstock ratio was highly significant for lignin yield (p = 0.0003). Softwood lignin structure has higher amounts of stronger carbon-carbon linkages compared to hardwood lignin. Because pine derived, softwood lignin contains high amounts of guaiacyl lignin, the lignin yield was significantly affected due to the difficulty of removing softwood lignin from lignocellulosic biomass. For an α = 0.05, all other factors and interactions were not statistically significant (Table 12).

Runtime and acid level were used with run temperature to generate a combined process severity (CS) index (Goh et al., 2011). CS is commonly used to describe the intensity of process conditions for pretreatment and pulping, indicating

Table 11. Analysis of Variance (Response Lignin Yield).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	15	3997.4886	266.499	6.0364	0.0016
Error	12	529.7867	44.149		
C. Total	27	4527.2753			

Table 12. Effect Tests (Response Lignin Yield).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Run Time(60,120)	1	1	0.2453	0.0056	0.9418
Acid Level(0.05,0.15)	1	1	48.0707	1.0888	0.3173
Feedstock Ratio	3	3	1886.8886	14.2464	0.0003*
Run Time*Acid Level	1	1	39.9446	0.9048	0.3603
Run Time*Feedstock Ratio	3	3	208.6777	1.5756	0.2466
Acid Level*Feedstock Ratio	3	3	82.2383	0.6209	0.6148
Run Time*Acid Level*Feedstock Ratio	3	3	174.1259	1.3147	0.3151

a correlation between CS and dissolution of polymers during pretreatments, as documented by Chum et al. (1990). CS was estimated since data suggested positive effects of both higher runtime and acid levels on lignin yields. The logarithmic combined severity formula included runtime (t = 60, 90, and 120 min), acid level (by solvent pH), and run temperature (T_r = 150°C), with a base temperature of 100°C (T_b) is:

$$CS = log\left(\int_0^t exp\left[\frac{T_r - T_b}{14.75}\right] dt\right) - pH \quad (3)$$

Combined severities for each factor combination using a 150°C runtime temperature are given in Table 13. Prior research shows that high severities create a pseudo-lignin fraction from carbohydrate and lignin degradation products (Sannigrahi et al., 2011). Research in this study revealed similar lignin purities between low and high severities, as pointed out in the following section, which led to the assumption of non-significant creation of pseudo lignin. To fully support this hypothesis, further examination of the lignin quality is recommended. Acid level and runtime factors were substituted in the model with the continuous combined severity factor. The new model applying CS indicated that the process intensity has no significant influence on lignin yield. The RSM design visualized in Figure 18 displays the influence of high severity on lignin yield from feedstock ratios and CS. feedstock ratios given in increasing percentage ratios for pine, poplar, and switchgrass. The impact of 80% pine mixture on the lignin yield is shown in the

Table 13. Combined severity at 150°C for each used factor setting, ordered from low to high combined severity.

Combined Severity	Runtime	Acid Level
1.90	60	0.05
2.08	90	0.05
2.15	60	0.1
2.20	120	0.05
2.30	60	0.15
2.33	90	0.1
2.45	120	0.1
2.48	90	0.15
2.60	120	0.15

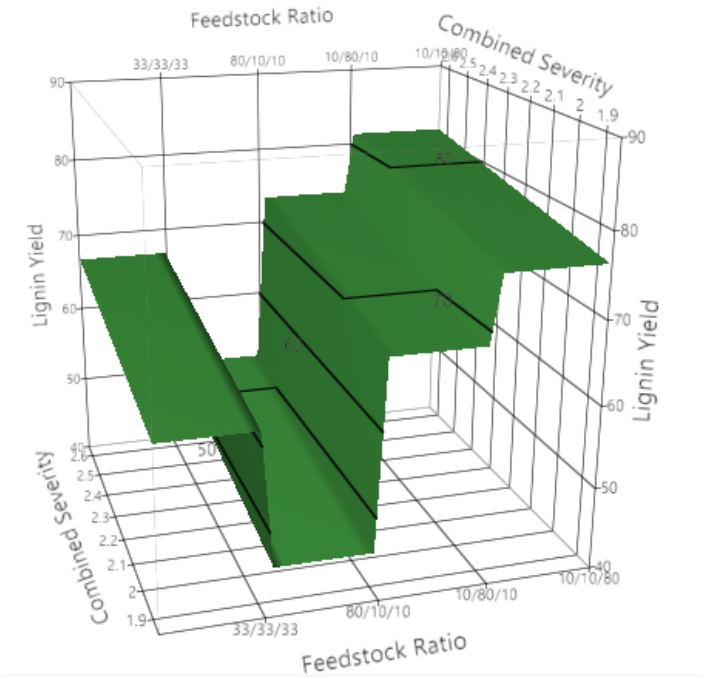


Figure 18. Response surface design of lignin yield by combined severity and feedstock ratio.

response surface graph (Figure 18). The response surface suggests a tendency towards slightly higher lignin yields if higher CSs were applied.

Maximizing Lignin Yield

The 'Prediction Profiler' from JMP 12.0.1 software of the SAS Institute (http://www.jmp.com/en_us/home.html) was used to visualize the importance of factors on the predictions of lignin yield. Figure 19 displays a maximization using the levels or settings of the factors from the data presented in Table 9. The highest predicted lignin yield is 81.7% for 120 min runtime, 0.15 M acid level, and a feedstock mixture that consists of 10% loblolly pine, 10% hybrid poplar, and 80% switchgrass (Figure 19). As mentioned earlier, hardwood lignins (poplar) and herbeacous plants (switchgrass) consist of guaiacyl and syringyl monolignols,

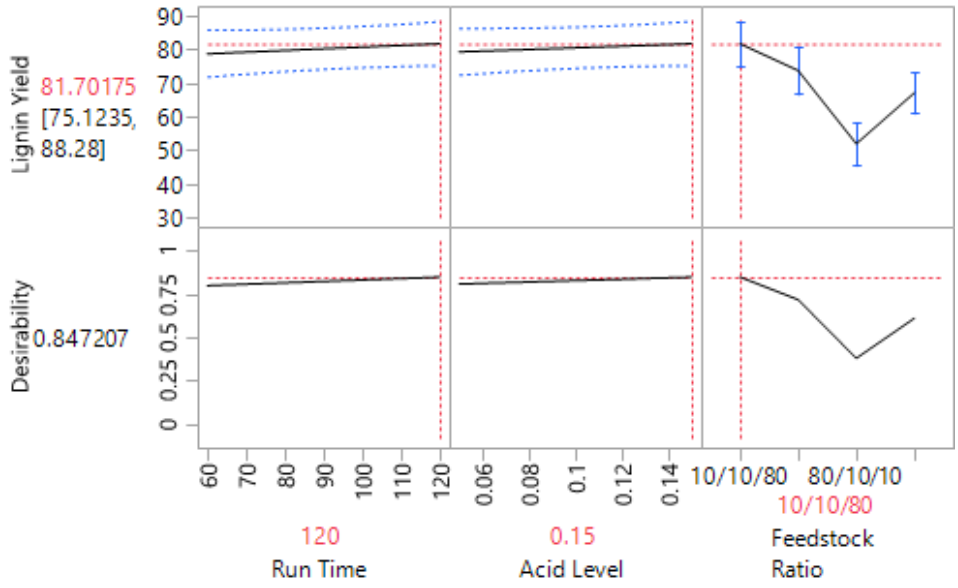


Figure 19. Prediction profiler recommending maximizing settings for lignin yield by runtime, acid level, and feedstock ratio.

while softwood (pine) consists mainly of guaiacyl lignin with more C-C linkages. Presumably, this chemical difference in the complex lignin structure led to significantly lower lignin yields for high pine mixtures. The settings maximizing lignin yield, translate to a high combined severity of 2.60 (Table 13). However, the shallow slope of the linear trend between levels of the factors illustrates the insignificance of runtime (p = 0.9418) and acid level (p = 0.3171) (Table 12).

Prediction Validation

For lignin yield prediction, runtime, and acid level were not found to be significant. Subsequently, only the feedstock ratios, expressed as considered in the prediction of lignin yield (Figure 20).

To validate the prediction formula of lignin yield, four additional runs were carried out for each feedstock ratio using center point settings for runtime (90 min) and acid level (0.1 M), v1-v4 in Table 9. The validation compares the predicted data with the additional collected data (validation set). Precisely, the mean squared prediction error (MSPR) were calculated (Kutner et al., 2004):

$$MSPR = \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{n} \quad (3)$$

While Y_i represents the actual response for *i*-th validation case, e.g., Y_1. High switchgrass, 71.13%. $\hat{Y}_i$ is the predicted data for the *i*-th validation run, e.g., $\hat{Y}_1$ High switchgrass, 79.54% (Table 14). Kutner (2004) states, if the MSPR is fairly close to the mean squared error (MSE), the validation set is not significantly biased and suggests predictive abilities (Kutner et al., 2004). The training set for lignin

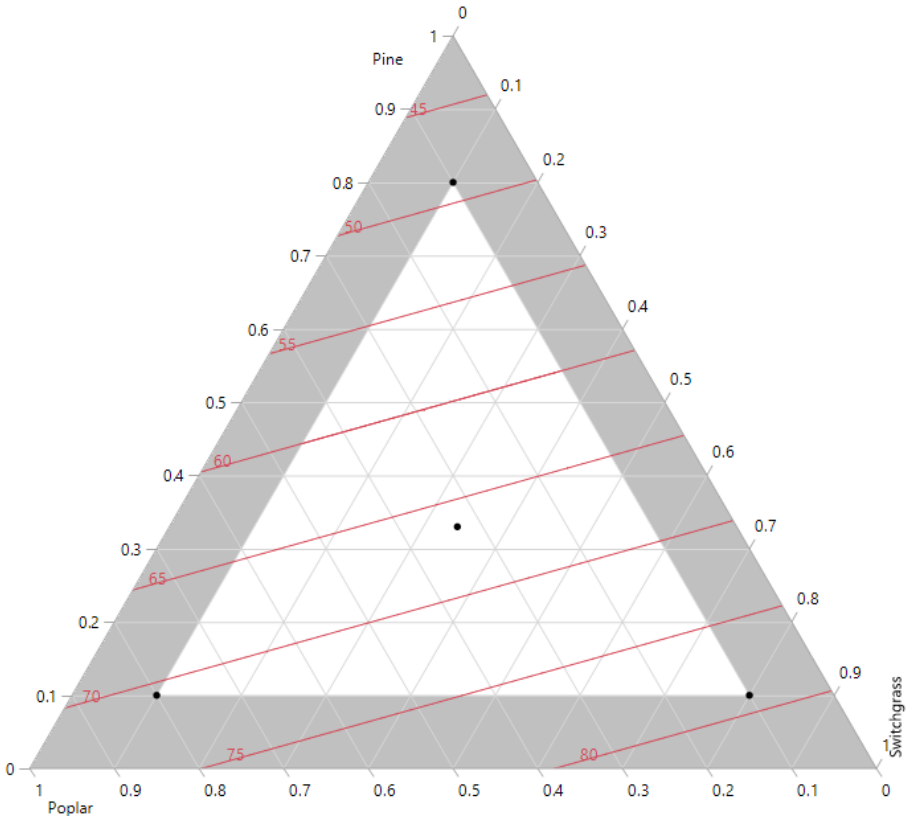


Figure 20. Simplex plot for lignin yield training data set, by x1 (poplar), x2 (switchgrass), and x3 (pine) percentage.

Table 14. Predicted versus Actual Lignin Yield data for Validation, including the specific standard deviation as Root Mean Squared Error (RMSE).

Run	Predicted Lignin Yield [%] RMSE 6.64	Actual Lignin Yield [%] RMSE 7.91
V1	79.06	76.56
V2	70.66	65.67
V3	48.94	57.47
V4	66.39	54.23

yield provided a MSE of 44.15. The MSPR of 62.58 indicated a close fit that validates the applicability of the lignin yield prediction. Furthermore, the visualization of the training and validation data sets, shown as Box and whisker plots in Figure 21, support the validity of the prediction.

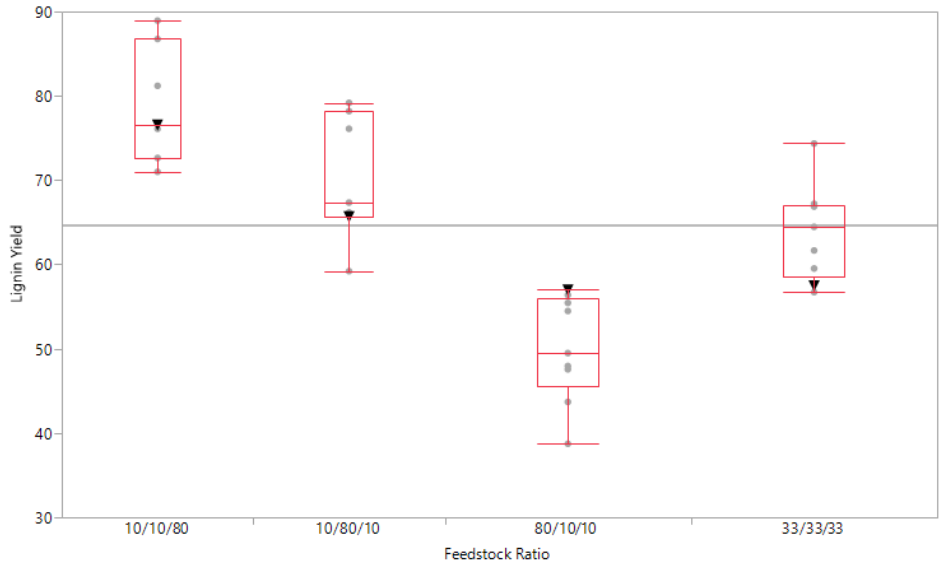


Figure 21. Box and whisker plots including training (grey points) and validation data (black triangles) points.

Impact of Feedstock Ratio on Lignin Yield

A one-way ANOVA was conducted to detect statistical differences at an $\alpha = 0.05$ between the mean lignin yield and the four feedstock ratios. To compare factors and to test if the null hypothesis is supported, a “Student’s t test” was applied. Each pair of box plot groupings is visualized by circles using the t-test. The bigger the diameter, the larger is the range for the collected data. The distance

between circles indicates statistical differences between data set grouping (Figure 22).

The regression model regarding feedstock ratio has a good validation fit ($R^2 = 0.7644$) and is significant overall ($p = 0.0001$). There is a significant difference between each feedstock ratio ($p \leq 0.0001$ to 0.0391). The high switchgrass proportion [10/10/80] achieved a significantly higher mean lignin yield of 79.44 wt% relative to the "high poplar" proportion [10/80/10] ($p = 0.0391$) of 71.038 wt% and to the "high pine" proportion [10/10/80] ($p \leq 0.0001$) of 49.22 wt% (Figure 22). Increased lignin yields, using higher amounts of switchgrass reinforces Astner's (2012) findings that lower density of switchgrass promotes solvent penetration, compared to poplar where higher feedstock density might hinder proper penetration. However, Maraun (2013) found higher lignin yields for tulip poplar

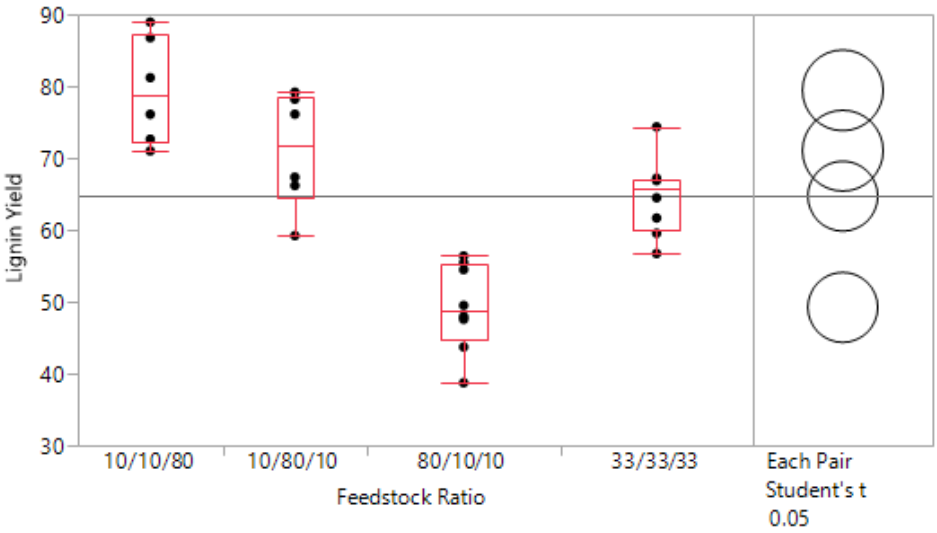


Figure 22. One-way ANOVA for mean lignin yield between the four different feedstock ratios expressed in percent and mean comparison by Student's t.

relative to switchgrass which suggests that hybrid poplar used in this study displays different organosolv fractionation properties, due to the different lignocellulosic composition. The high pine content with a mean lignin yield of 49.22 wt% suggests that the structural differences between soft and hardwood, as explained in the previous section, has a major effect regarding organosolv fractionation abilities. The mean lignin yield of the three “one high feedstock” (66.56 wt%) has no statistical difference compared to the center point run with an equal feedstock mixture [33/33/33] (64.70 wt%).

Impact of Acid Level on Lignin Yield

A simple regression model of acid level predicted a poorly correlated lignin purity of $R^2 = 0.0253$. An ANOVA indicated no statistical significance between high and low acid levels for this model ($p = 0.4192$). With a maximum lignin yield of 66.51 wt%, the acid level of 0.15 M provided a slightly higher mean lignin yield than the 0.05 M run (62.15 wt%) ($p = 0.4266$) (Figure 23). The lack of significance between acid levels of 0.05 and 0.1 M is supported by Maraun (2013); however, a significant difference was found between 0.25 and 0.05 M which resulted in a lignin yield gain of 7.33 wt%. Higher acid levels were found to increase the rate of delignification, but represent a higher cost solution and may accelerate deterioration of equipment (Maraun, 2013, Astner, 2012). The results of the three studies suggest an acceptable acid level of 0.05 M for the lignin yield maximization.

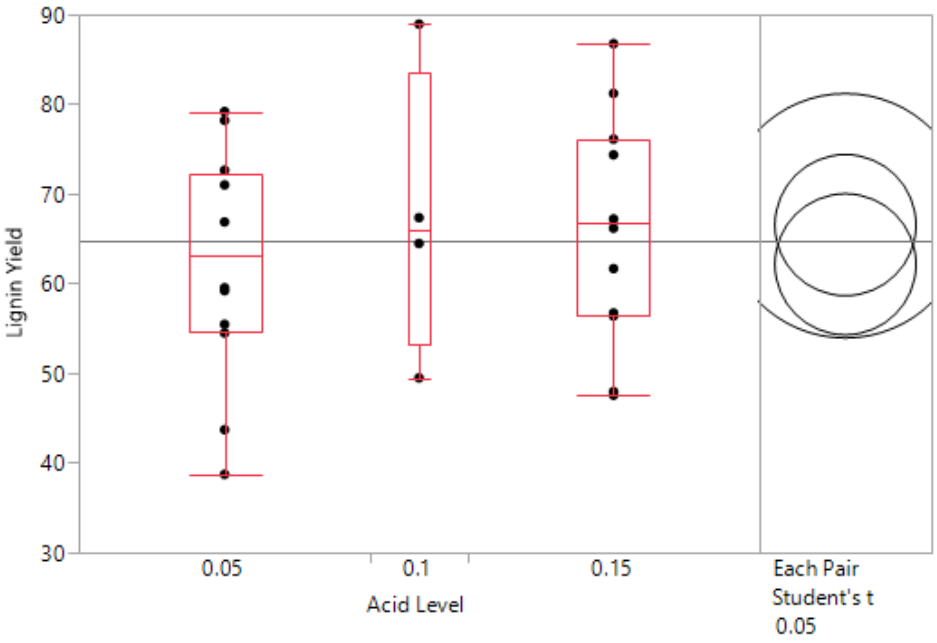


Figure 23. One-way ANOVA for mean lignin yield of 0.05, 0.1, and 0.15 Mole Acid Level expressed in percent and mean comparison by Student's t.

Impact of Run Time on Lignin Yield

A simple regression model of runtime predicted a poorly correlated lignin purity of R^2 = 0.0153. A one-way ANOVA indicated no statistical significance between long (120 min) and short (60 min) runtimes for this model (p=0.5302). With a maximum lignin yield of 65.84 wt%, the run time of 120 minutes provided a slightly higher mean lignin yield than the 60 minute run (62.23 wt%) (p=0.5172) (Figure 24). Astner (2012) applied 56 and 90 min and did not find significant different between those, which supports this finding (Astner, 2012). However, Maraun (2013) found significant differences between 56 and 90 min runtime, as longer runtimes resulted in a 5.39% higher mean lignin yield for tulip poplar and

switchgrass mixtures. Presumably, the fractionation abilities of pine impacted the runtime significance in this study.

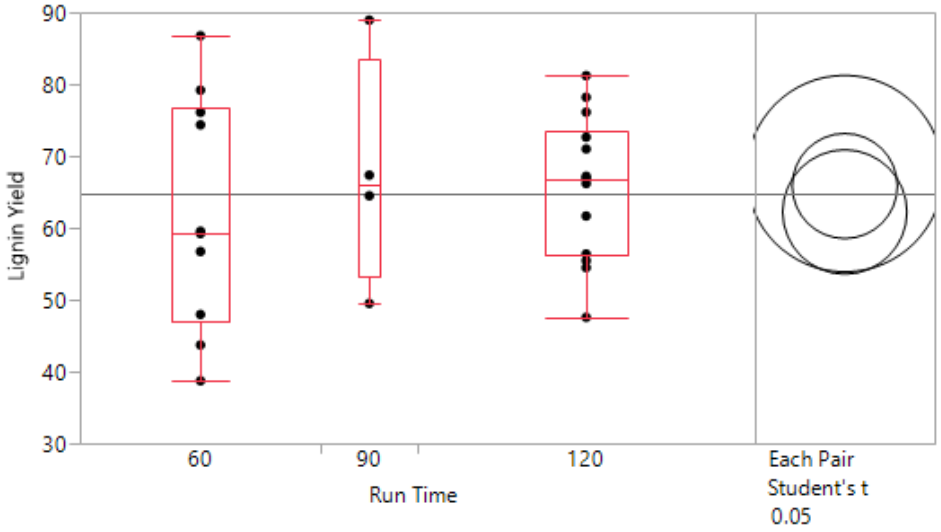


Figure 24. One-way ANOVA for mean lignin yield between 60, 90, and 120 minutes expressed in percent and mean comparison by Student's t.

Lignin Purity

Response Surface Design

The ANOVA suggested a weak significance of p = 0.1039 (Table 15). The interaction between runtime and acid level had a p = 0.0753 (Table 16). Consequently, a model using combined severity was analyzed to ensure the impact of runtime and acid level on lignin purity (Table 17). The new model had a R^2 = 0.386, which indicates factors (noise) other than acid level, runtime, and

feedstock ratio were responsible for the variance in lignin purity, such as the natural variance in feedstocks or black liquor processing. Future investigation should take processing black liquor into account when generating a designed experiment, since stickiness of the product influences recovery during certain steps (e.g. separation or rotary evaporation). The combined severity was found significant for the lignin purity prediction (p = 0.0012) (Table 17), while the interaction between feedstock ratio and combined severity was not significant (p = 0.6601).

The response surface displays the lignin purity by combined severity and feedstock mixtures, indicating a significant gain in lignin purity for higher severity (Figure 25). The assumption that pseudo-lignin was created while applying higher combined severities, could not be eliminated for this study. Subsequently, the occurrence of pseudo lignin may elevate purity levels at high severities.

Maximizing Lignin Purity

An attainable lignin purity of 97.43% was predicted at an $\alpha = 0.05$ for a feedstock ratio of 10% pine, 10% poplar and 80% switchgrass at a high combined severity of 2.60. The high combined severity factor of 2.60 translates into 120 min runtime and a 0.15 M acid level, at a run temperature of 150°C. The steepness of the combined severity (CS) line visually indicates the strong influence of CS (Figure 26).

Table 15. Analysis of Variance (Response Lignin Purity).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	15	36.456989	2.43047	2.0780	0.1039
Error	12	14.035182	1.16960		
C. Total	27	50.492171			

Table 16. Effect Tests (Response Lignin Purity).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Run Time(60,120)	1	1	3.1008579	2.6512	0.1294
Acid Level(0.05,0.15)	1	1	0.0279406	0.0239	0.8797
Feedstock Ratio	3	3	5.4644281	1.5574	0.2508
Run Time*Acid Level	1	1	4.4357540	3.7925	0.0753
Run Time*Feedstock Ratio	3	3	8.2982053	2.3650	0.1223
Acid Level*Feedstock Ratio	3	3	4.9001251	1.3965	0.2916
Run Time*Acid Level*Feedstock Ratio	3	3	7.5557008	2.1534	0.1467

Table 17. Alternative effects test (Response Lignin Purity).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Feedstock Ratio	3	3	1.478010	0.3657	0.7784
Combined Severity	1	1	18.470914	13.7096	0.0012
Feedstock Ratio*Combined Severity	3	3	2.323788	0.5405	0.6601

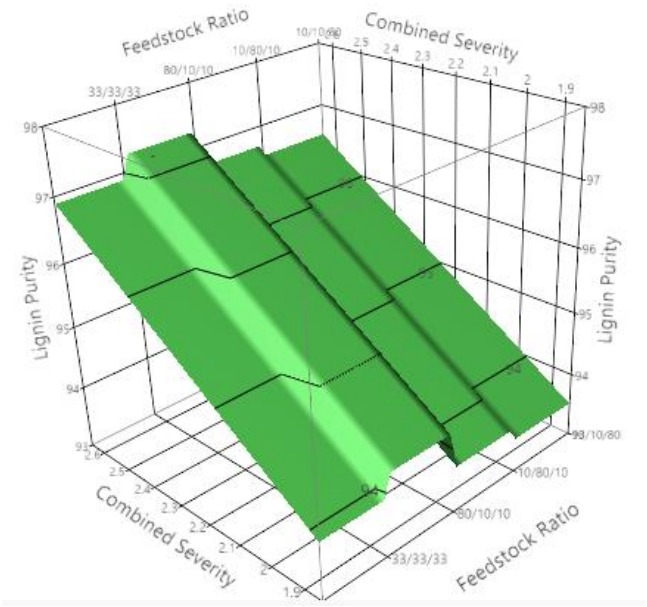


Figure 25. Response surface design of lignin purity by combined severity and feedstock ratio.

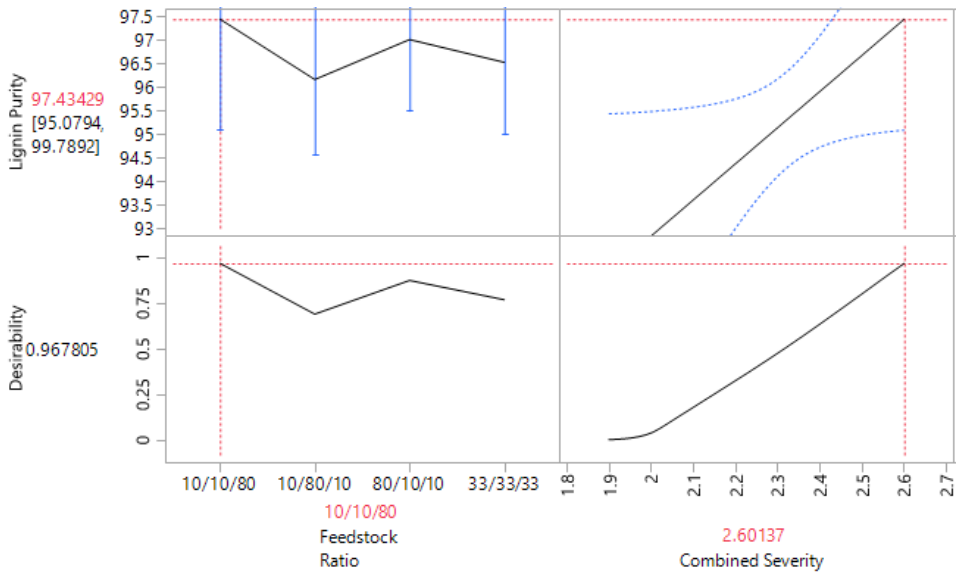


Figure 26. Prediction profiler recommending maximizing feedstock ratio and combined severity.

Prediction Formula Validation

The following graph fits lignin purity by combined severity, across all feedstock ratios (Figure 27). The graph suggests higher lignin purities with higher severities and expresses the fitted line to the training data set as follows ($R^2 = 0.36$, $p = 0.0008$):

$$Lignin\ Purity\ =\ 87.553021\ +\ 3.4696943 * Combined\ Severity + \varepsilon \quad (4)$$

The MSPR was found at 1.39, which compared well to a MSE of 1.16 (Table 18), and indicates valid predictive abilities for the lignin purity of this study (Table 14). However, due to the weak correlation of $R^2 = 0.36$, the formula does not explain the majority of the lignin purity variance and strengthens the earlier recommendation to examine variation before and after the reactor run to gain a stronger prediction of the lignin purity.

Table 18. Predicted versus Actual Lignin Purity data for Validation, including the specific standard deviation as Root Mean Squared Error (RMSE).

Run	Predicted Lignin Purity [%]	Actual Lignin Purity [%]
	RMSE 1.08	**RMSE 1.18**
V1	95.625	96.97
V2	95.625	97.06
V3	95.625	96.91
V4	95.625	95.87

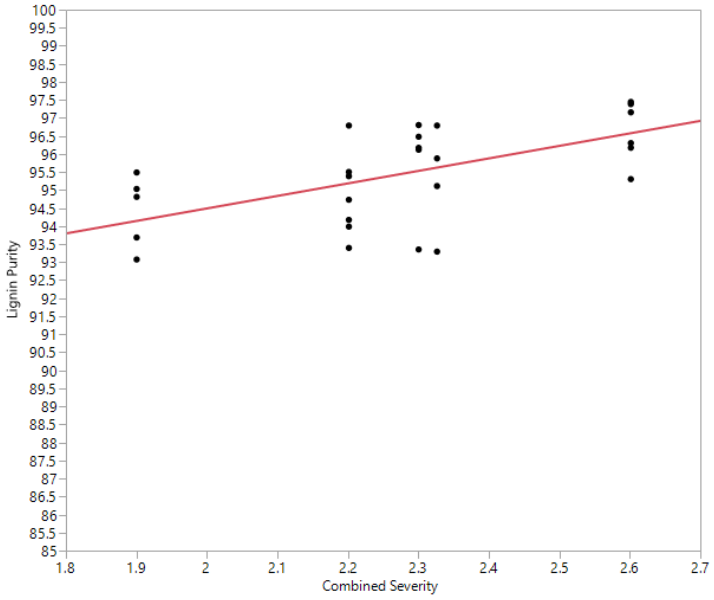


Figure 27. Lignin Purity by Combined severity, linear line in red indicates the prediction formula.

Impact of Feedstock Ratio on Lignin Purity

The linear regression model regarding feedstock ratio is too weak (R^2 = 0.0205) to predict lignin purity which is confirmed by a non-significant ANOVA (p = 0.9175). The mean lignin purity was found to be 95.29% (Figure 28) indicating that the lignin purity was not impacted by the lignin yield nor by the various feedstocks (softwood, hardwood, and herbaceous). Furthermore, the black liquor processing to powdery lignin only used water, instead of salt and ether as applied in previous studies (Maraun, 2013, Astner, 2012), which successfully produced a high pure lignin fraction. The reduction of resources necessary to an up-scaled biorefinery process will lower the cost and cross-contamination of the lignin fraction, which will be important to cost effectiveness and conversion of lignin into

high value chemicals and biomaterials. The high pine mixture displayed a slightly higher lignin purity when compared to the high poplar. However, the lignin yield and cellulose purity (as pointed out in the following section) of high pine mixtures, indicate residual lignin especially in the cellulose fraction. Presumably, guaiacyl lignin was extracted from the particle surface, but the solvent penetration into the particle core was insufficient. In order to increase lignin yield and cellulose purity further, the particle size of the feedstock must be decreased for deeper penetration of solvent.

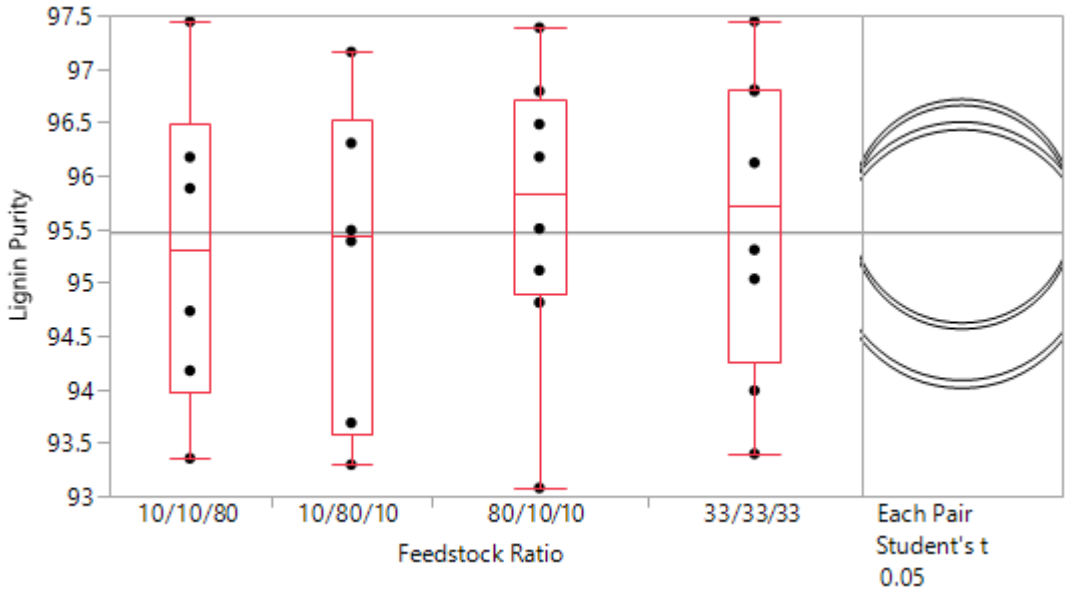


Figure 28. One-way ANOVA for mean lignin purity between the four different feedstock ratios expressed in percent and mean comparison by Student's t.

Impact of Acid Level on Lignin Purity

The simple regression model for modeling lignin purity as a function of acid level has a R^2 = 0.3364. The ANOVA had a p =0.0059 indicating lignin purity is influenced by acid level (Figure 29). Furthermore, an outlier for the lignin purity of Run 7 was found for 0.15 M (60 min runtime). With a maximum lignin purity of 96.35%, the acid level of 0.15 M provides a significantly higher mean lignin purity (p = 0.0016) than the 0.05 M run with a mean lignin purity of 94.67% (Figure 29). The importance of the acid level supports the significance of the combined severity, as discussed earlier. However, the profitability of applying high acid concentrations (0.15 M) to gain a mean lignin purity of 1.68% will be dependent on the downstream product requirements of the up-scaled biorefinery.

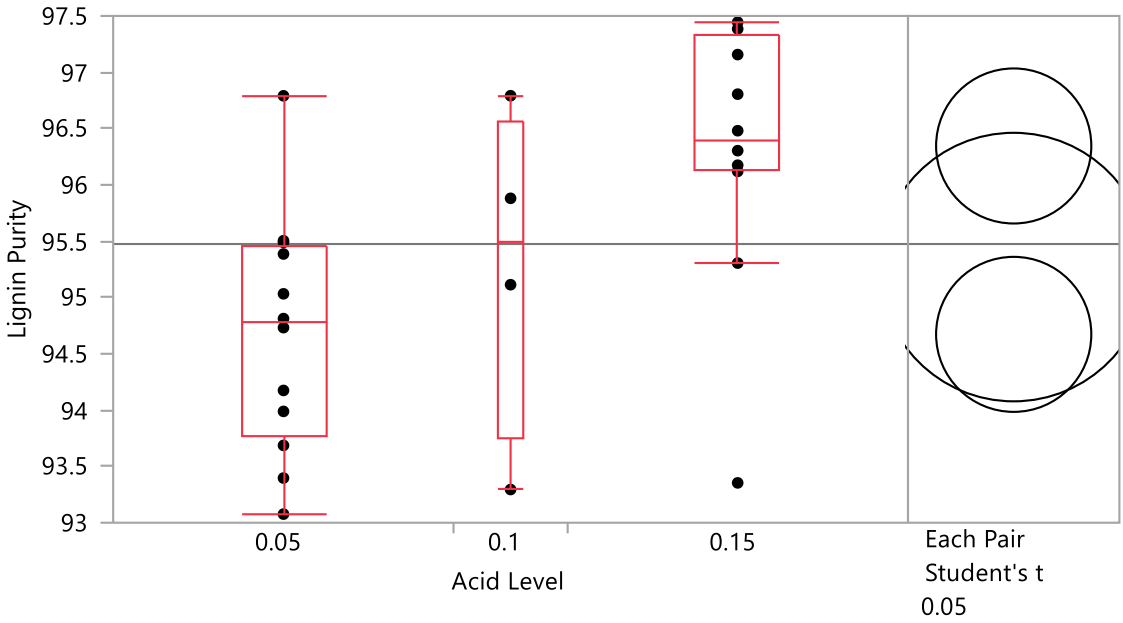


Figure 29. One-way ANOVA for mean lignin purity between 0.05, 0.1, and 0.15 Mole Acid Level expressed in percent and mean comparison by Student's t.

Impact of Run Time on Lignin Purity

A linear regression model of acid level used to predict the lignin purity indicates a poor correlation (R^2= 0.0598). An ANOVA indicated no statistical significance between longer and shorter runtimes (p = 0.9175). With a maximum mean lignin purity of 95.80%, the run time of 120 minutes provided only a slightly higher mean lignin purity than the 60-minute of 95.10% (p=0.2340) (Figure 30). An upscaled biorefinery, based on this study, may increase cost efficiency by decreasing runtimes.

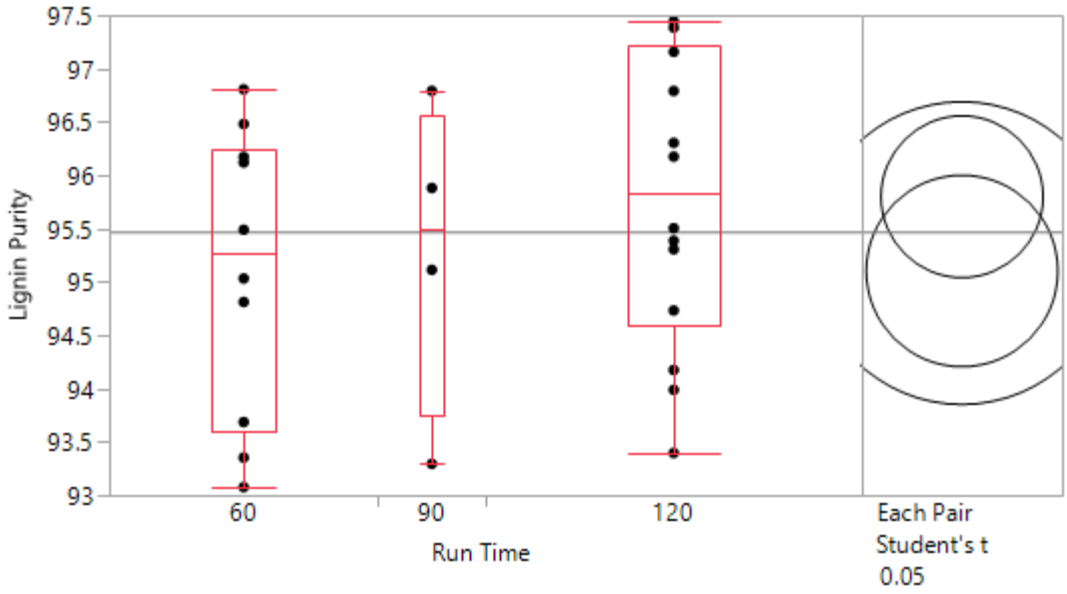


Figure 30. One-way ANOVA for mean lignin purity between 60, 90, and 120 minutes expressed in percent and mean comparison by Student's t.

Cellulose Purity

Response Surface Design

The cellulose purity was evaluated by total Klason lignin analysis, since prior studies indicated residual lignin to be in the solid fraction (Astner, 2012, Maraun, 2013). The ANOVA indicated statistical significance of one or more factors ($p < 0.0001$) (Table 19).

The response surface displays a significant drop in cellulose purities for mixtures using 80% pine, which explains the lower lignin yields (Figure 31). Slightly higher purities were achieved with higher combined severity settings.

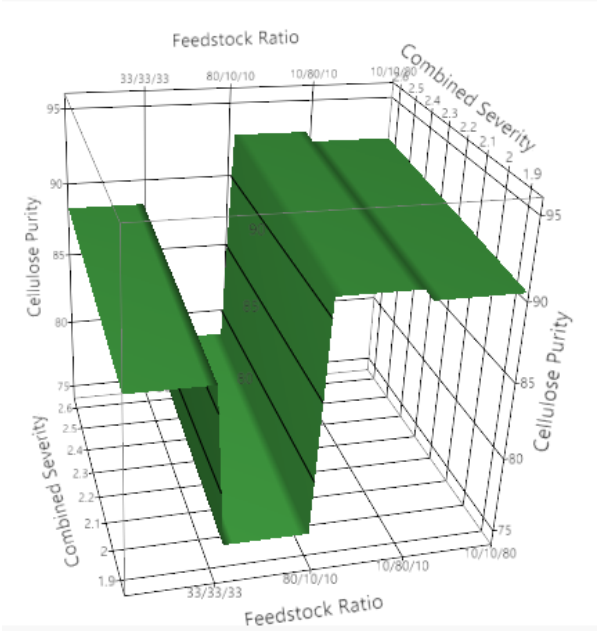


Figure 31. Response surface design of cellulose purity by combined severity and feedstock ratio.

Table 19. Analysis of Variance (Response Cellulose Purity).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	15	1239.5727	82.6382	17.1666	<.0001
Error	12	57.7666	4.8139		
C. Total	27	1297.3392			

Table 20. Effects Tests (Response Cellulose Purity).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Run Time(60,120)	1	1	0.95065	0.1975	0.6647
Acid Level(0.05,0.15)	1	1	0.72749	0.1511	0.7043
Feedstock Ratio	3	3	584.05250	40.4423	<.0001*
Run Time*Acid Level	1	1	3.28093	0.6816	0.4252
Run Time*Feedstock Ratio	3	3	18.82536	1.3035	0.3184
Acid Level*Feedstock Ratio	3	3	11.34499	0.7856	0.5247
Run Time*Acid Level*Feedstock Ratio	3	3	25.82090	1.7879	0.2030

Maximizing Cellulose Purity

The prediction profiler predicts a maximum cellulose purity at 120 min, 0.05 M, applying a feedstock ratio of 10% pine, 80% poplar, and 10% switchgrass. However, as described earlier, the runtime and acid level were not found to be statistically significant (Figure 32). This result contradicts with the findings of maximum lignin yields for high switchgrass mixtures, since it was assumed that missing lignin yields would be found in the cellulose fraction. However, the difference between cellulose purity between high poplar and high switchgrass mixtures is not significant (p = 0.7603), as pointed out in the following one-way ANOVA. But, lignin yields between high poplar (79.44%) and high switchgrass (71.04%) mixtures were found significant (p = 0.0001), a possible explanation

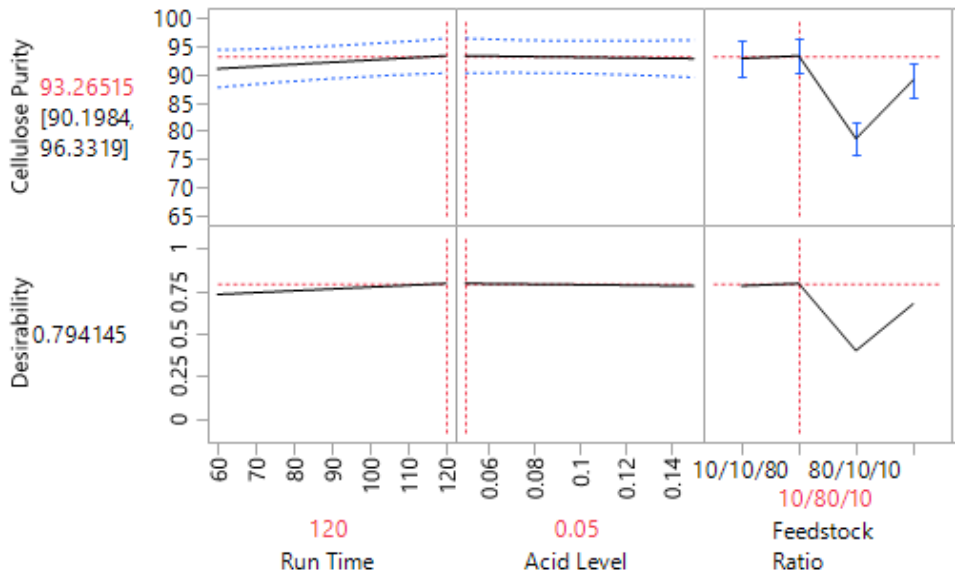


Figure 32. Prediction profiler recommending maximizing settings for cellulose purity combined severity and feedstock ratio.

would be higher loss during the processing of high switchgrass mixtures, due to higher stickiness and smaller particle size of herbaceous lignin. Further, as suggested earlier the creation of pseudo-lignin was not absolutely eliminated, which makes a further lignin quality determination important.

Prediction Validation

Similar to the response for the lignin yield, runtime and acid level were not found to be significant for predicting cellulose purity. Therefore, the prediction formula only considers the feedstock ratio. A simplex plot to visualize and predict cellulose purity by the training data set, was developed Figure 34. MSPR and MSE were calculated and compared to evaluate the predictive abilities of the plot, applying the data displayed in Table 21. The MSPR was 5.54, which with a MSE

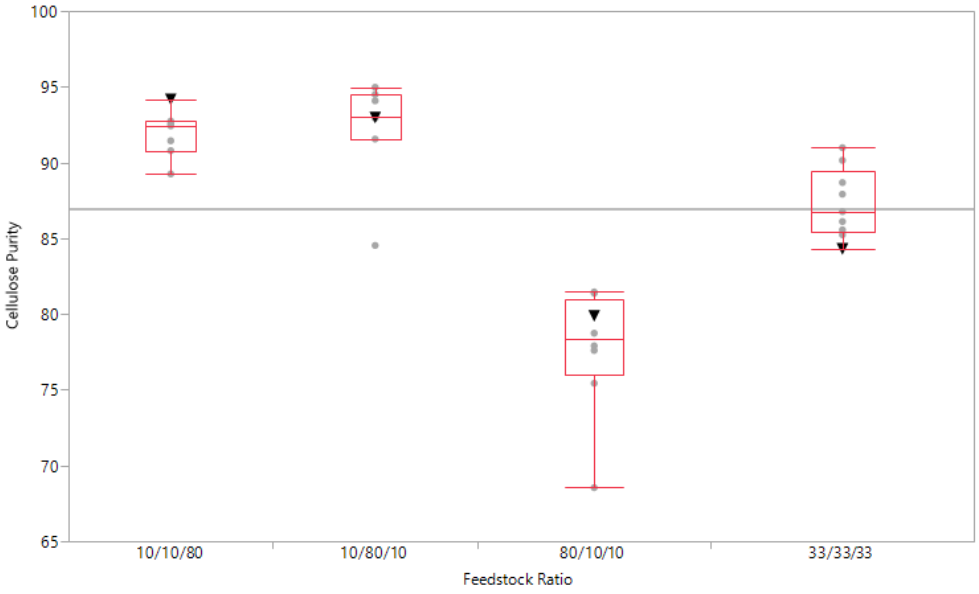


Figure 33. Box and whisker plots including training (grey points) and validation data sets (black triangles) by Feedstock Ratio for Cellulose Purity.

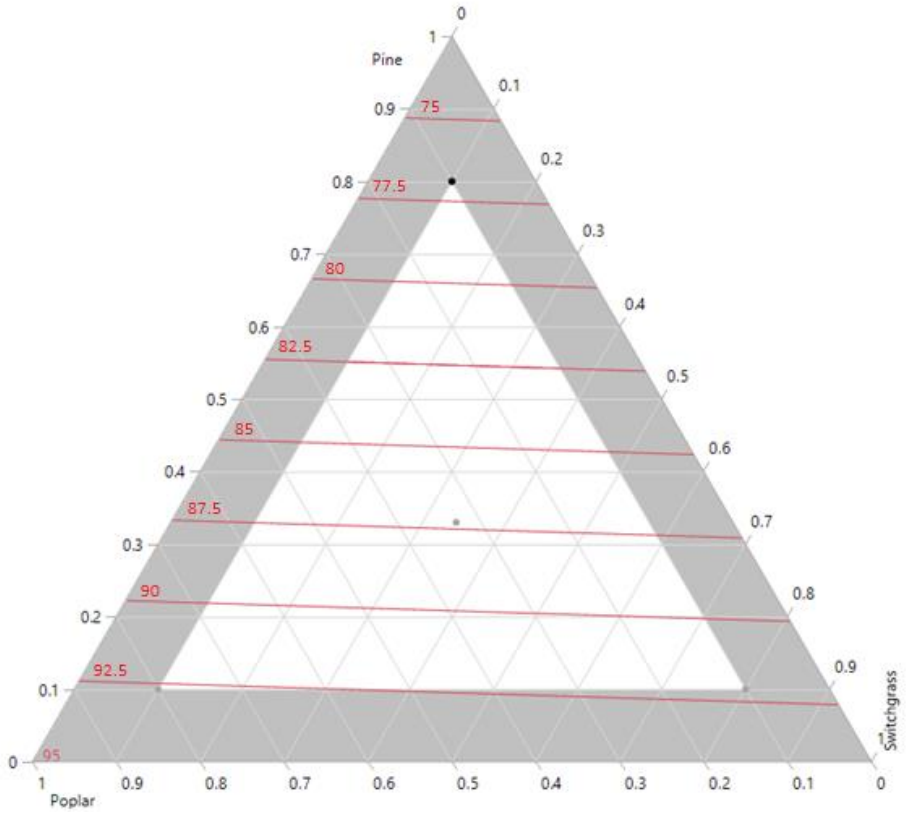


Figure 34. Simplex plot of cellulose purity by x1 (poplar), x2 (switchgrass), and x3 (pine) in percentage.

Table 21. Predicted versus Actual Cellulose Purity Data for Validation.

Run	Predicted Cellulose Purity [%] RMSE 2.19	Actual Cellulose Purity [%] RMSE 2.35
V1	92.12	94.18
V2	92.68	92.98
V3	76.89	79.87
V4	87.29	84.30

of 4.81. The prediction appears to have good validation. The visualization fitting training and validation data, as provided in Figure 33, supports this predictive claim.

Impact of Feedstock Ratio on Cellulose Purity

The linear regression model predicting cellulose purity as a function of feedstock ratio has an R^2 = 0.81777. The ANOVA of this relationship was significant (p = 0.0001). As earlier mentioned the mean cellulose purity of 92.09%, the feedstock ratio [10/80/10] (high poplar) provided no statistically significant higher purity than [10/10/80] (high switchgrass) showing a mean cellulose purity of

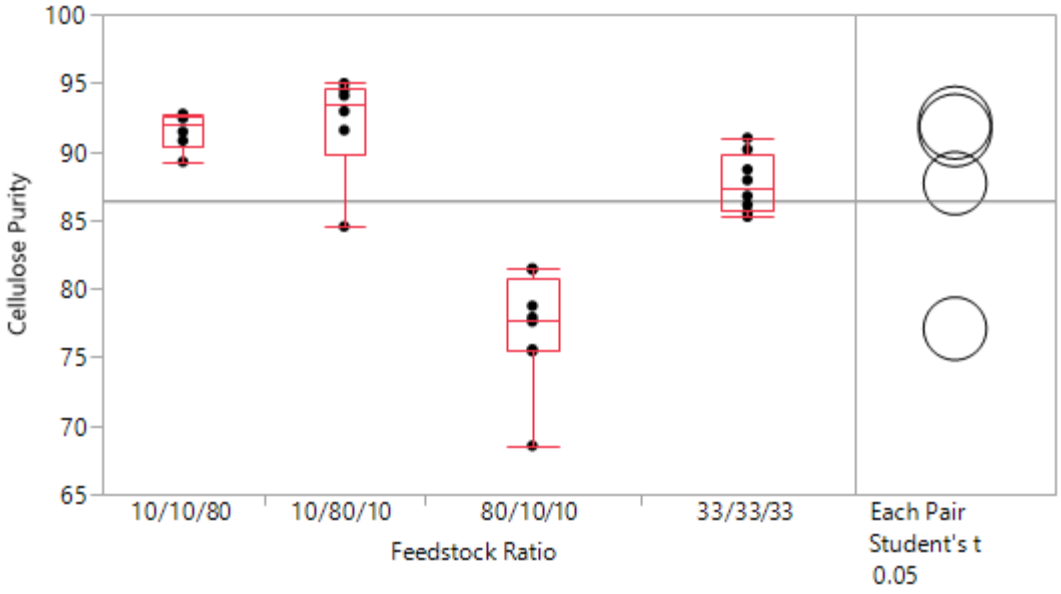


Figure 35. One-way ANOVA for mean cellulose purity between the four different feedstock ratios expressed in percent and mean comparison by Student's t.

91.54% (p = 0.7603) (Figure 35). However, the [80/10/10] high pine is statistically

significant with a mean cellulose purity of 77.08% ($p \leq 0.0001$). A mean cellulose purity of [33/33/33] of 87.68% compares well with the mean of response 86.42%

Impact of Acid Level on Cellulose Purity

A simple regression model of acid level to predict the cellulose purity indicated a poor correlation $R^2 = 0.0068$. An ANOVA indicated no statistical significance between a high and low acid levels for this model ($p = 0.9180$). With a maximum cellulose purity of 87.79 % the acid level of 0.15 M provided statistically the same mean lignin purity compared to 0.05 M with a mean cellulose purity of 86.14 % ($p = 0.9739$) (Figure 36).

Impact of Run Time on Cellulose Purity

A simple regression model of the run time to predict the cellulose purity indicated a poor correlation $R^2 = 0.03316$. An ANOVA indicated no statistical significance between long and short runtimes ($p = 0.6560$). Mean cellulose purity for a runtime of 120 minutes is 87.20%, for 60 minutes 84.78% (Figure 37).

Maximizing Multiple Responses

The impacts of the model were described with two factors, feedstock ratio and combined severity, since the acid level and runtime factors were found mainly not significant. Feedstock ratio and severity settings were used to predict the outcome of lignin yield and purity, as well as cellulose yield and purity (responses). The underlying data for cellulose yields is provided in the appendix, including

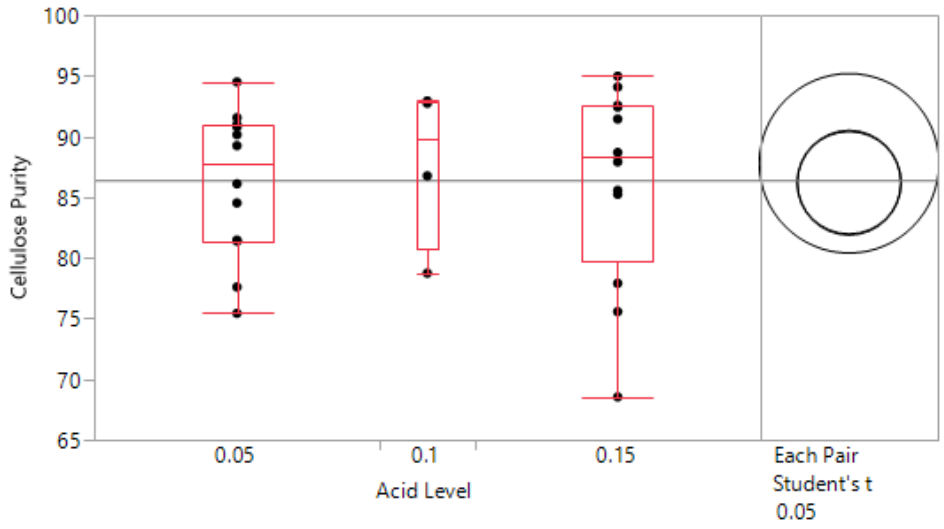


Figure 36. One-way ANOVA for mean cellulose purity between 0.05, 0.1, and 0.15 Mole Acid Level expressed in percent and mean comparison by Student's t.

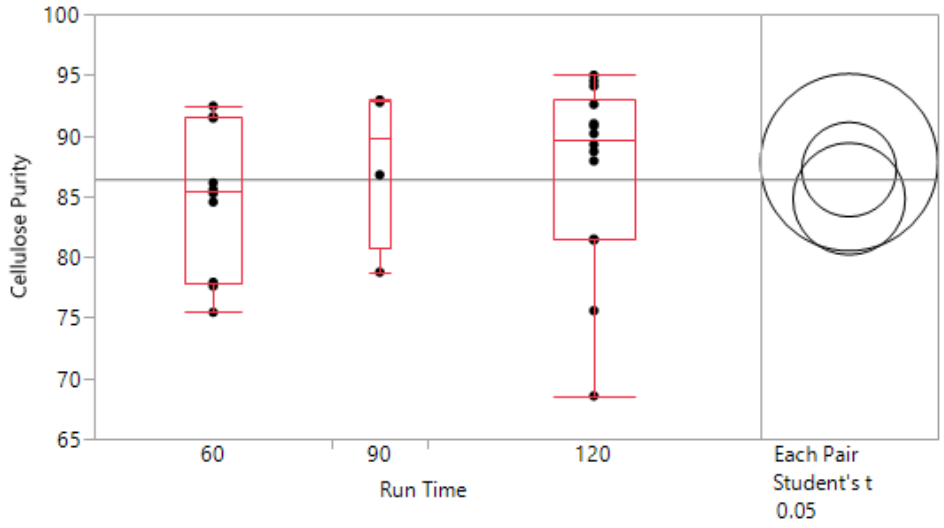


Figure 37. One-way ANOVA for mean cellulose purity between 60, 90, and 120 minutes expressed in percent and mean comparison by Student's t.

cellulose yields in percentages, ANOVA, and effects test. As mentioned in chapter two, the concept of a biorefinery, as described in this study, is to produce separate fractions from lignocellulosic biomass mixtures for further convertion to high value chemicals and biomaterials. Higher severities were found beneficial, to maximize lignin yield, purity, and cellulose purity. Cellulose yield was included to further optimize the integrated biorefinery concept, which droped significantly by higher severities. The study suggests a medium severity (2.26) to maximize all four responses (Figure 38) and an application of 120 min at 0.05 M or 60 min at 0.15 M is preferable (Table 13). However, the prediction profiler used in this study allows to change individual factors as well as responses, allowing a future biorefinery operator to customize output yields and purities dependent on available feedstocks, runtime preference, marketprice of sulfuric acid and desired outcome (Figure 38).

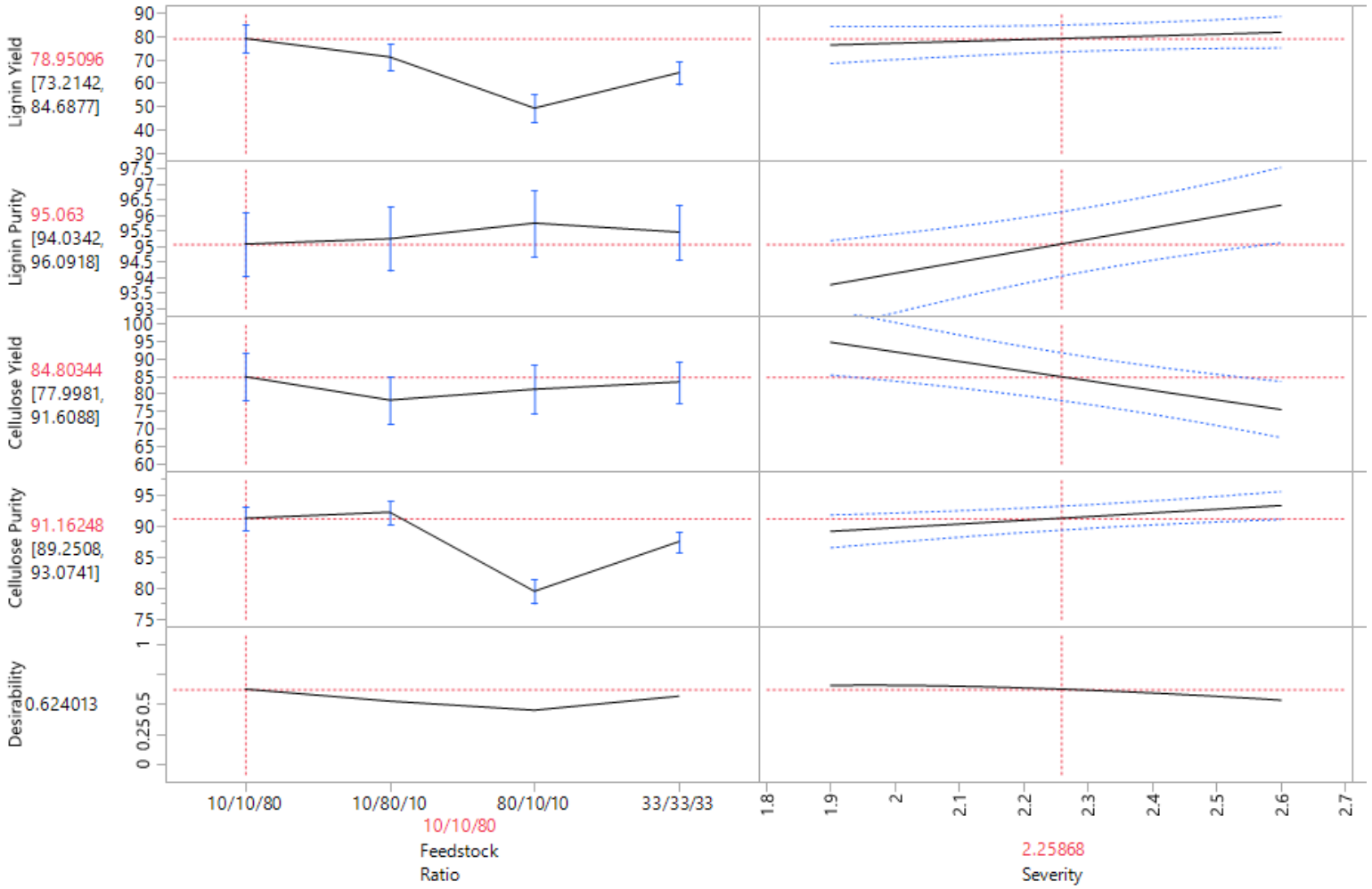


Figure 38. Prediction profiler plot, maximized desirability was found at a feedstock ratio using high switchgrass at a medium severity (2.26).

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

The study applied organosolv fractionation with the goal of maximizing lignin yield, purity and cellulose purity by using different feedstock mixtures of switchgrass (*Panicum virgatum*), hyprid poplar (*hyprids spp.*), and Loblolly Pine (*Pinus taeda L.*). Response surface methodology (RSM) was applied to identify significant factors in the model. RSM with three independent factors was used to maximize the response of three dependent variables. The independent variables were: runtime (60 minutes, 120 minutes); acid level (0.05 mol/L, 0.15 mol/L); and feedstock ratios (loblolly pine/tulip poplar/switchgrass of 10%/10%/80%, 10%/80%/10%, 80%/10%/10%, 33%/33%/33%), while the responses were lignin yield, lignin purity, and cellulose purity. Additionally, four center point runs were carried out, with the settings: runtime of 90 minutes, acid level of 0.1 mol/L for each earlier presented feedstock ratio.

For high switchgrass (10%/10%/80%) the highest mean lignin yield of 79.44% was found, followed by high poplar (10%/80%/10%) with 71.33%, and high pine (80%/10%/10%) with only 66.56%. It was found, that guaiacyl lignin was successfully extracted from pine wood, but presumably only from the particle surface. To avoid the lack of solvent penetration for softwood, single feedstock pine runs should be conducted. To optimize the softwood lignin extraction even smaller particle sizes (< meshsize 20) and eventually higher MIBK solvents should be considered.

The lignin purities were statistically not different from each other, while a mean lignin purity of 95.47% was achieved. The combined severity had the highest statistical impact on the purity, but could only give insight in a fraction of the occurring variance. Following, this unexplained variation (noise) has to occur before or after the organosolv pretreatment, such as natural variation in feedstocks or during the processing of the black liquor to powder. Further investigations, while generating a designed experiment, should consider to incorporate factors like stickiness during processing and/or water separation abilities into the design. Additionally, the lignin quality might be determined to exclude the assumption of pseudo-lignin creation.

Cellulose purities of high switchgrass (10%/10%/80%) (91.54%) and high poplar (10%/80%/10%) (92.02%) were statistically not different, but high pine (80%/10%/10%) displayed significant difference and achieved only a cellulose purity of 77.08%. The cellulose purity did not as assumed correlate equally to the lignin yield. An assumption is, that during the black liquor processing unequal amounts, due to different separation properties, got lost.

Further research should be conducted on the lignin and cellulose quality, to determine their applicability for downstream processing. Also, varying levels of high pine feedstock mixtures should be taken into account to achieve higher lignin and cellulose yields. Mixture models using the simplex method should be explored within the subspace of exiting feedstock ratios to determine a new global maximum. The process settings for runtime, temperature, acid level, etc., should

be held at the optimum settings learned from the previous research during the mixture modeling.

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APPENDIX

Cellulose Yield results presented in percent by run settings, feedstock mixture and combined severity.

Run #	Run Time [min]	Acid Level [M]	Feedstock Ratio [PINE/POP/SG]	Combined Severity	Cellulose Yield [%]
1	60	0.05	10/80/10	1.90	88.51
2	60	0.05	10/80/10	1.90	82.79
3	60	0.05	33/33/33	1.90	92.38
4	60	0.05	80/10/10	1.90	89.55
5	60	0.05	80/10/10	1.90	93.86
6	60	0.15	10/10/80	2.30	90.29
7	60	0.15	10/10/80	2.30	99.07
8	60	0.15	33/33/33	2.30	87.32
9	60	0.15	33/33/33	2.30	62.09
10	60	0.15	80/10/10	2.30	79.89
11	90	0.10	10/10/80	2.33	80.68
12	90	0.10	10/80/10	2.33	85.17
13	90	0.10	33/33/33	2.33	83.01
14	90	0.10	80/10/10	2.33	81.10
15	120	0.05	10/10/80	2.20	75.11
16	120	0.05	10/10/80	2.20	85.93
17	120	0.05	10/80/10	2.20	88.87
18	120	0.05	33/33/33	2.20	86.58
19	120	0.05	33/33/33	2.20	83.55
20	120	0.05	80/10/10	2.20	83.80
21	120	0.05	80/10/10	2.20	78.84
22	120	0.15	10/10/80	2.60	67.38
23	120	0.15	10/80/10	2.60	62.73
24	120	0.15	10/80/10	2.60	61.34
25	120	0.15	33/33/33	2.60	81.20
26	120	0.15	33/33/33	2.60	80.44
27	120	0.15	80/10/10	2.60	74.90
28	120	0.15	80/10/10	2.60	71.57

Analysis of Variance (Response Cellulose Yield)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	7	1218.99	174.68	2.81	0.0331
Error	20	1241.55	46.48		
C. Total	27	2460.55			

Effect Tests (Response Cellulose Yield)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Feedstock Ratio	3	3	165.76	0.8901	0.4633
Combined Severity	1	1	807.56	13.0090	0.0018*
Feedstock Ratio*Combined Severity	3	3	88.03347	0.4727	0.7047

VITA

Marc Banholzer is from Aachen, Germany, were he attended Mies-van-der-Rohe High school, before he completed a carpentry apprenticeship in the Black forest, Germany. After completion he studied three semesters of civil engineering with the concentration of wood constructions at the University for Applied Sciences and Art in Hildesheim, Germany, before he changed to the Salzburg University of Applied Sciences, Austria, were he pursued studies in Forest Product Technologies and received his B.S. in 2014. During his Bachelor studies he carried out an exchange semester at the Seinäjoki University of Applied Sciences, Finland, an internship at the Center for Renewable Carbon at the University of Tennessee, USA, as well as an internship at Green Resources Ltd., a plantation and sawmilling company, Tanzania. After his B.S. he continued with his Master studies at the Salzburg University of Applied Sciences and completes currently a double degree program in collaboration with the University of Tennessee under Dr. Joseph J. Bozell and Dr. Timothy M. Young. Marc plans to graduate with a Master of Science degree in Wood Science and Biomaterials in December 2016