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**Lignin Yield Maximization of Lignocellulosic Biomass by Taguchi Robust
Product Design using Organosolv Fractionation**

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Anton Friedrich Astner

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Dedication

I would like to dedicate this work to my family for their unconditional love, mental aid, and supportive prayers, and never-ending faith in me. In particular, I would like to express my sincere gratitude to my mom, Maria Astner, for her continuous moral support during the times of my study. Furthermore I want to thank my sisters Maria and Elisabeth and my brothers Josef and Hans for their long lasting conversations and support.

I needed you all the most during this challenging journey.

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ABSTRACT

Lignin, a byproduct of the organosolv pretreatment process using lignocellulosic biomass from switchgrass (*Panicum virgatum*) and tulip poplar (*Liriodendron tulipifera*) is currently being explored for its potential use in the production of value-added chemicals and biobased polymers. Pretreatment is one of the most expensive processing steps in cellulosic biomass conversion. Optimization of the process is one of the major goals of the biomass-to-ethanol conversion process. Taguchi Robust Product Design (TRPD) provides an effective engineering experimental design method for optimizing a system and designing products that are robust to process variations.

Given the results of several preliminary studies of the organosolv pretreatment process, four controllable design factors (inner array) were used in the TRPD: process temperature (120°C, 140°C, 160°C), fractionation time (56 minutes, 90 minutes), sulfuric acid concentration (0.025 M, 0.05 M, 0.1 M), and feedstock ratio (switchgrass/tulip poplar ratios of 10%/90%, 50%/50%, 90%/10%, based on both mass and volume of feedstock). Process noise was induced in the experiment by using either the mass-based or volume-based feedstock charges of switchgrass and tulip poplar.

A maximum mean lignin yield of 78.63 wt% was found in the study. Optimum conditions for maximum lignin yield were found at a 90 minute runtime, 160°C process temperature, 0.1 M sulfuric acid concentration, and a feedstock composition of 90% switchgrass and 10% tulip poplar. The most statistically significant factor influencing lignin yield was process temperature. There was statistical evidence that lignin yield increased after 120°C for both feedstock charges of switchgrass and tulip poplar (p-value < 0.0001 for mass-based, p-value < 0.0001 for volume-based). The variance in lignin yield declined as the proportion of switchgrass increased (p-value = 0.0346 for mass-based and p-value = 0.0678

for volume-based). The finding of a local maximum for lignin yield for process temperature at 160°C suggests that high processing temperatures are required to receive high lignin yields. The finding that the variance in lignin yield declined as the switchgrass percentage of feedstock increased may provide a pathway for other researchers interested in maximizing switchgrass use in the pretreatment process.

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CHAPTER 1 INTRODUCTION

Potential fossil energy shortages, worldwide energy demands, and greenhouse gas emissions have increased the level of scientific interest in alternative energy research. This has led to a new focus on alternative sources of energy such as solar, wind, hydropower, and biomass (Tan *et al.* 2008). Ethanol, derived from biobased materials is considered a promising renewable source for fuel because of its sustainability, and reduction in NO_x and greenhouse gas emissions (Demirbas 2007; Prasad *et al.* 2007).

In recent years, a growing number of bio-ethanol plants worldwide have been constructed. This substitution away from petroleum products is envisioned to grow as the exploration for new petroleum resources becomes increasingly sophisticated and expensive (Ozcimen and Karaosmanoglu 2004; Jefferson 2006). The substitution away from petroleum fuels to biofuels derived from sustainable feedstocks offers potential economic advantages. Bioenergy and biochemical products offer sustainable solutions, energy security, economic development opportunities, environmental wellness, and possible socioeconomic benefits to rural economies. This is exemplified by recent innovations and technologies that have produced biofuels that are cost competitive with fossil fuels (Demirbas *et al.* 2000). Bio-based materials also offer significant CO₂ emission sequestration from the atmosphere compared to petroleum-based transportation fuels (Figure 1).

Currently, the concept of the integrated biorefinery offers conversion technologies that utilize renewable biomass feedstocks to produce both biofuels and biochemicals. The general idea of the concept is to convert renewable biomass feedstocks into a variety of high-value products and byproducts which can serve as a basis for further downstream processing and additional value-added products. However, most of the current conversion technologies are focused on ethanol production and disregard incorporation of chemical by-products

created during the conversion process of biorefinery operations. Abundant lignocellulosic feedstocks exist for conversion into biochemical products (Sanchez and Cardona 2008).

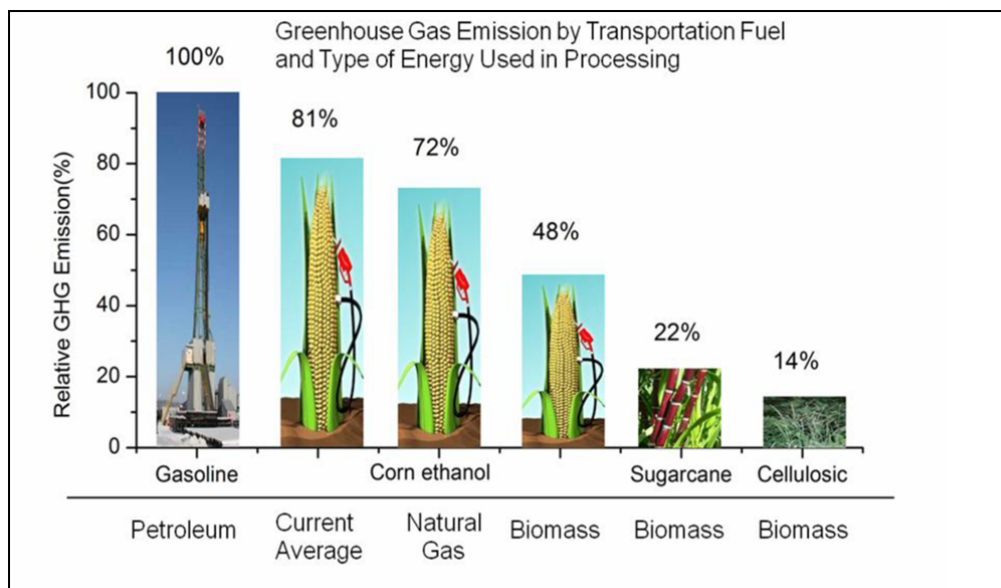


Figure 1. Potential of reduction of GHG-emissions based on different feedstocks (Wang *et al.* 2007).

The need for new production biochemical and biofuel technologies arises from the steadily increasing global energy demand and greenhouse gas emissions created from the burning of fossil fuels (Ragauskas *et al.* 2006; Pu *et al.* 2008; Sannigrahi *et al.* 2010). To meet this increasing energy demand, additional research in biofuels, biochemical, and bioproducts is needed. To date, most of this research has been conducted in the United States and Europe (Wright 2006; Galik *et al.* 2009).

The conversion of biomass into chemicals and ethanol involves three major processes: pretreatment, hydrolysis, and fermentation. Efficient pretreatment technologies are required to alter biomass in macroscopic and microscopic size and its structure, as well as in the

submicroscopic structural and chemical composition allowing for the efficient conversion of carbohydrates to fermentable sugars (Brownell *et al.* 1986). Pretreatment provides the breakdown of lignocellulosic materials into its main components in order to allow hydrolysis and subsequent fermentation. The pretreatment stage is generally considered to be the most costly process and has a significant impact on the efficiency of enzymatic hydrolysis following conversion steps. Continuous improvement of pretreatment technologies is important. This continuous improvement of pretreatment technologies includes high efficiency enzymes, development of better fermentation processes, and employment of technologies from other disciplines such as genetic modification of lignocellulosic biomass (Vogel and Jung 2001; Sarath *et al.* 2011). A better understanding about the relationship and interactions between pretreatment and subsequent downstream processing is also required.

Recently, a novel modified organosolv fractionation process has been developed and implemented at the Center for Renewable Carbon at the University of Tennessee as an improved method for pretreating biomass. This process treats lignocellulosic biomass with a ternary solvent mixture to isolate cellulose, hemicellulose, and lignin from biomass (Bozell *et al.* 2011a). This process provides separate fractions of high quality cellulose, hemicellulose, and lignin for further downstream processing for the production of bio-based chemicals (Figure 2). More than 120 reactor runs have been performed using this modified fractionation process under varying operational conditions. However, an improved quantification of the process and product outcomes from this modified fractionation process is desired and is the rationale for this study. Designed experimentation is the next logical step to improve this modified fractionation process to reduce variation and maximize co-products for the greatest value in an integrated biorefinery.

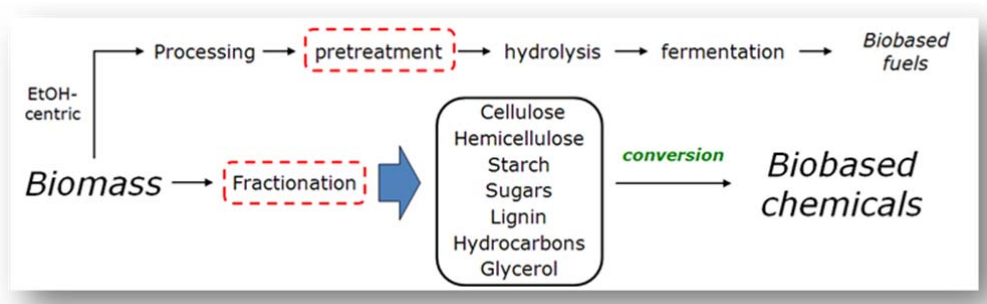


Figure 2. Comparison of fractionation and pretreatment (Bozell *et al.* 2011a).

The present thesis addresses the improvement of this fractionation process with the goal of maximizing the yield of lignin and minimizing lignin yield variation using the robust product experimental design methodology of Taguchi (Taguchi 1993). Based on preliminary studies, controllable input factors selected for testing in the experimental design were temperature, runtime, solvent acid concentration, and feedstock type. Following the Taguchi protocol, these factors were combined with uncontrollable noise factors such as the mass and volume of feedstock inputs (switchgrass - *Panicum virgatum* and tulip poplar - *Liriodendron tulipifera*). The robust product design was intended to maximize lignin yield in the presence of controlled manipulation of operational parameters associated with the organosolv fractionation process.

1.1 Research Hypothesis

Based on the knowledge from preliminary studies of fractionation, a significant outcome of this research will be the ability to predict lignin yields as a function of feedstock type, process temperature, acid concentration, and runtime. The research hypothesis aims to determine if formal experimental design in a controlled laboratory setting can accurately estimate lignin yields during fractionation.

1.2 Thesis Objectives

Based on the research hypothesis, lignin yield maximization from lignocellulosic biomass was performed using the organosolv fractionation process. The goal of this research was to identify the most significant and robust factors by using the statistical method of Taguchi. To achieve this goal, the following objectives were evaluated:

- Perform various organosolv fractionation runs with mixed switchgrass and poplar feedstocks under controlled variations of operational conditions to estimate the lignin yield;
- Compare lignin yield between mass-based and volume-based feedstock loadings;
- Use designed experimentation to determine if maximal lignin yield is attainable;
- Develop a statistical simulation of lignin yield based on experimental results;
- Prepare recommendations for future directions of research for students and scientists.

1.3 Thesis Organization

Chapter 2 is a review of the literature of lignocellulosic biomass conversion, current leading pretreatment technologies, and their importance in context of the integrated biorefinery. This chapter also gives a brief description of value-added utilization of lignin. Chapter 3 describes the materials and methods of this research. Chapter 4 is the results and discussion of the designed experimentation. Chapter 5 provides conclusions and proposes steps for future research.

The contribution of this thesis is to designed experimentation of the organosolv fractionation process. Significant factors influencing lignin yield are also a contribution of the work. In this study, the Taguchi engineering philosophy using Robust Product Design may provide a pathway for larger scale investigations.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Energy from non-fossil sources such as cellulosic feedstocks is an emerging area of research in the 21st century. Energy from cellulosic biomass research is largely driven by the global demand for petroleum-based energy that is predicted to be 40% higher by 2020 (Energy Information Administration 2008). Key sources of petroleum are located in complex geopolitical environments that increase risk to global economies (Goldemberg 2007).¹ Since the 1970s, macroeconomists have viewed changes in the price of oil as an important source of economic fluctuations, as well as a paradigm for global shock, likely to affect many economies simultaneously (Blanchard and Gali 2007). The supply of oil is predicted to be insufficient in the long term to meet global demand (Ragauskas *et al.* 2006; Lange 2007).

Fossil fuels have a detrimental effect on the environment, primarily from emissions that contribute to greenhouse gases. Significant changes in the equilibrium thermal conditions in the atmosphere are based on the emissions originating from combustion of fossil fuels. Research is needed on alternative sources of energy that are economically sustainable and that will also alleviate environmental degradation (Hill *et al.* 2006; Goldemberg 2007; Lynd *et al.* 2008). A recent study from the US Department of Agriculture (USDA) and Department of Energy (DOE) in the United States indicated that over 1.3 billion dry tons of lignocellulosic biomass could be available annually for the production of ethanol and other derived products (Perlack and Stokes 2011). This could result in 65 billion gallons² of bio-

¹About 59% of current U.S. oil use is imported, with approximately 20% coming from the Persian Gulf (Caputo 2009).

²Based on well-developed conversion technologies, a yield of 50-100 gallons of ethanol per dry ton can be estimated.

ethanol made from renewable cellulosic biomass which is equivalent to about one-third of the entire U.S. gasoline consumption (Perlack and Stokes 2011).

In previous decades, the low cost and abundant supplies of crude oil, natural gas, and coal were combined with modern organic chemistry technologies to create an efficient and successful petrochemical industry which offers thousands of products to the marketplace. However, low cost and abundant oil is no longer a valid assumption for long-term sustainable energy. Production of chemicals and fuels from renewable cellulosic feedstocks offers an avenue towards sustainable and environmentally friendly energy. This will also provide opportunities for expanded employment from “*green jobs*” and may strengthen the agricultural sector by providing alternative crops on marginal lands, *i.e.*, *switchgrass*.

2.2 Importance of Renewable Materials

Lignocellulosic materials are promising sources of energy because they are the most abundant form of biomass on earth and they are a renewable resource created by photosynthesis (Pu *et al.* 2008). Biologically-convertible cellulosic materials for ethanol and value-added chemicals are abundant in nature. In recent years, the conversion of lignocellulosic biomass into second generation bio-ethanol has attracted much interest. However, the overall processing efficiency and cost-effectiveness of specific conversion techniques remain a challenge. Commercialization of bio-ethanol depends on the efficiency of processing conversion rates and low cost inputs (Himmel *et al.* 1999; Wyman *et al.* 2007). As a result, the development of an integrated biorefinery using sources of renewable carbon (*e.g.*, *forest resources and dedicated energy crops*) as feedstocks is widely recognized as a feasible solution to transform biomass into the intermediate building blocks and ultimately into both biochemicals and biofuels. Currently only around two percent of chemicals and fuels in the U.S. are derived from biomass (Petersen and McLaren 2000).

2.3 Integrated Biorefinery

An integrated biorefinery is a processing facility that includes processes and technical equipment to produce chemicals, fuels, and power derived from biomass. Efficient conversion of renewable lignocellulosic feedstocks into fuels and value added chemicals is the goal of the biorefining industry (Bozell and Petersen 2010). The principle is analogous to petroleum refineries, which produce fuels, power, and chemicals from crude oil.

The U.S. Biomass Program,³ initiated and driven by the Department of Energy, supports the concept of the integrated biorefinery with the focus on examining value-added products from all lignocellulosic biomass components which could serve as the economic driver to accomplish a profitable integrated biorefinery. Production of petroleum-based fuels and chemicals are dominated by conversion of crude oil into thousands of chemicals and materials from only a few primary building blocks. However, the utilization and conversion of renewable materials offers a new combination of building blocks such as carbohydrates, hemicelluloses, and aromatics in the form of lignin. For sustainable valorization of biomass resources in the future, the concept of the integrated biorefinery is a suitable model that aims to produce primary bio-based products (*e.g., chemicals and materials*) and secondary energy carriers (*e.g., fuels, power, and heat*) analogous to the oil refinery concept (Kamm *et al.* 2006).

The output of low-volume, high-value chemical products and low-value, high-volume liquid transportation fuels can contribute to enhanced economic development and can contribute to lower greenhouse gas emissions. Two strategic goals are important for the developing a biorefinery: substitution away from fossil fuel and production of high-value chemicals (

³ <http://www1.eere.energy.gov/biomass/index.html> Accessed August 14, 2012.

Figure 3).

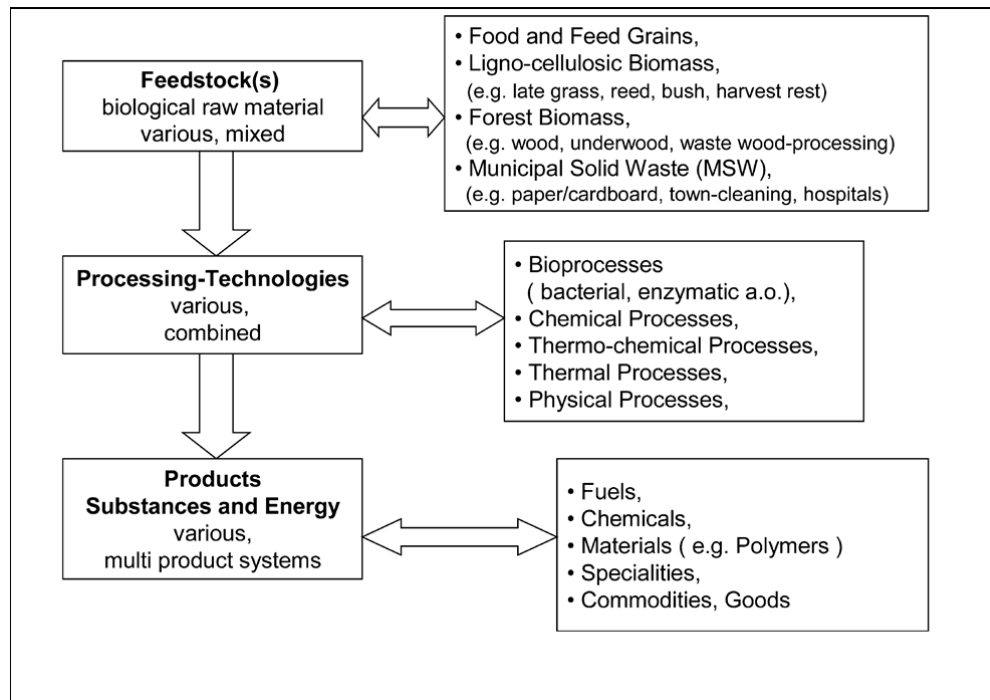


Figure 3. Flow-diagram of a biorefinery (Kamm 2004)

Pulp and paper mills are examples of biorefineries, where pure cellulose and by-products are produced for food, feed, power, and consumer products (Bozell and Petersen 2010). However, the raw material supply for a biorefinery is variable coming from a range of sources from the forest, agricultural materials, and residue streams from timber or food production (*e.g., wood, corn stover, and perennial feedstocks*) to crops such as sugarcane and beet molasses. The development of efficient conversion technologies that have the capacity to produce useful biochemicals and biomaterials from this diversity of feedstocks is fundamental to the biorefinery concept (Bozell 2008).

Previous research has been focused on understanding the nature of lignin and hemicellulose in plant cell walls and in developing effective methods of pretreatment to remove or modify them (Kong *et al.* 1992; Montane *et al.* 1998) as a means to maximize fermentable sugar production for conversion to ethanol. More recently however, research has been directed toward the investigation of pure hemicellulose and lignin fractions that are created downstream in the biorefinery process as starting materials for the production of biobased chemicals. These steps are closely related to the applied pretreatment technologies for ethanol production (Sun and Cheng 2002; Mosier *et al.* 2005a).

2.4 Mixed Feedstocks for Biorefineries

This study examines the use of organosolv fractionation as a means to pretreat and separate mixed bioenergy feedstocks and the improvement of these separations through the use of experimental design. Mixed feedstock streams for biorefinery raw materials are of interest because a wide variety of feedstocks within an economically feasible transportation distance can be utilized. The use of perennial (herbaceous) and perpetual (woody) biomass mixed feedstocks can tolerate variations in weather conditions (*e.g., drought*) that other annual agricultural crops such as corn cannot. Perennial (herbaceous) and perpetual (woody) sources of biomass offer mixed feedstock solutions that are beneficial to the sustainable supply required by a viable biorefinery.

Supply logistics associated with a biorefinery may be a limitation at present. Biomass from mixed feedstocks may require longer transportation distances from source to the plant gate and therefore incur more cost. A recent study (Sultana and Kumar 2011) was conducted to assess the delivery cost of raw materials for biorefineries. Wheat straw, corn stover, and forest biomass were evaluated. They found that the delivered cost was lower when wood and herbaceous biomass were combined when compared to single feedstock only. The optimum

ratio of mixed feedstocks for a biorefinery with an annual capacity of 5000 dry tons per day was estimated to be 30% herbaceous biomass (in the form of bales) and 70% wood chips. This study supports the importance of conducting research on mixed feedstocks as a solution to supplying the biorefinery.

2.5 Lignin Utilization from Biomass

An additional focus of this work is the use of experimental design to maximize the yield of lignin from an organosolv fractionation process. Lignin is a byproduct of various biomass pretreatment and fractionation processes of lignocellulosic materials and is traditionally underutilized. Most commercially produced lignin is burned for energy purposes and only one percent is used for the production of chemical products such as natural filler in polymer matrices or as an additive for concrete mixtures and pavement (Alexy *et al.* 2004). One study by Cetin and Ozmen (2002) described organosolv-derived lignin as an adhesive component for particleboard production. Cetin and Ozmen (2002) indicated that organosolv-derived lignin in particleboard exhibited comparable results in the strength and stiffness, and improved tensile strength when compared to particleboard without lignin. Cetin and Ozmen (2002) further reported that adding lignin to particleboard offset phenol-based resin additions in the particleboard manufacturing process by 30%.

In another study (Baumberger *et al.* 1998), lignin generated from the kraft process was mixed with wheat starch (30% lignin) at varying humidity levels for the extrusion of films. Tests on water sorption and dissolution indicated a reduction in the water affinity of the films. In a study by Kubo and Kadla (2005), carbon fibers were blended with a mixture of kraft-derived lignin, polyethylene terephthalate (PET), and polypropylene (Kubo and Kadla 2005). The lignin was blended with the two polymers and spun into fibers with a minimum diameter greater than 30 μm . Thermal stabilization was applied to avoid fusion of individual

fibers. The mechanical properties of the spun fibers increased and had feasible yields of up to 50%. This study also advanced the concept of large scale fiber production using lignin.

Lignin is currently being studied at the University of Tennessee's Center for Renewable Carbon for the production of valuable chemicals and biobased polymers. The use of lignin in the production of high-valued products, such as carbon fiber and foams, from a biorefinery could significantly improve the cost structure of a biorefinery. The literature suggests that lignin used as a precursor material for carbon fiber production is feasible and offers potential for the product stream originating from a biorefinery.

2.6 Lignocellulosic Biomass

The challenges of separating biomass into its individual components are illustrated with an examination of the general structural characteristics of lignocellulosic materials as illustrated by the structure of wood. A single plant cell wall in wood is composed of the primary (P) and secondary (SW) walls and middle lamella (ML). The primary cell wall is a thin layer (0.1 – 1 μm) and comprises a randomly arranged matrix of cellulose microfibrils (Sticklen 2006). The secondary cell wall is thicker (10 – 20 μm) and is composed of the sub-layers S1 (outer), S2 and S3 (inner) with different orientation of the microfibrils for each of the layers. In S1, the microfibrils are oriented in a cross-helical structure (S- and Z helix). The thickest of the layers, S2, has a relatively consistent orientation of microfibrils. The secondary cell wall contains the major portion of cellulose (Figure 4). The middle lamella binds the neighbored cells together and contains the major portion of lignin (Pandey *et al.* 2009).

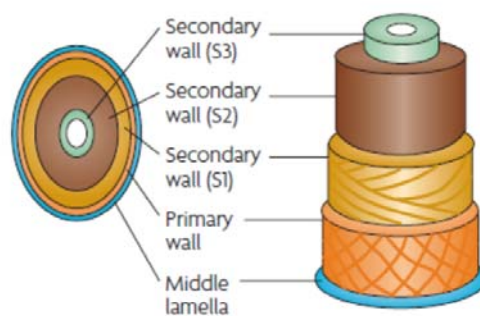
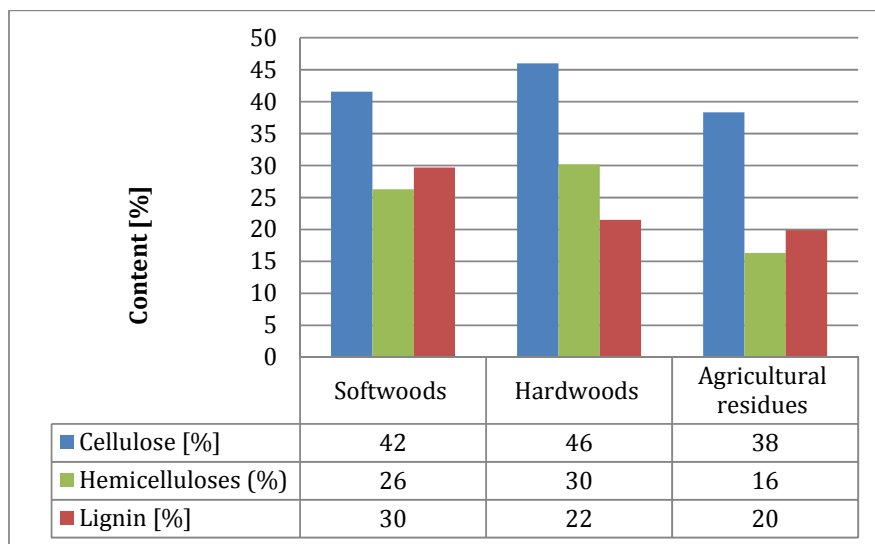


Figure 4. Schematic representation of plant cell wall (Sticklen 2006)

2.7 Chemical Structure of Lignocellulosic Biomass

Lignocellulosic biomass is composed of a ternary matrix consisting of cellulose, hemicelluloses, and lignin and smaller amounts of ash and extractives. The components are interlinked and form a complex and rigid structure (Fengel and Bocher 1984; Kumar *et al.* 2009). Because of its unique structure, biomass is recalcitrant to biological and chemical degradation. Distribution of the three major biopolymers found in hardwood, softwood, and agricultural residue species are given in Table 1.

Table 1. Average cell wall composition of various lignocellulosic species (Saha 2003; Ragauskas et al. 2006).



Depending on the species, lignocellulosic biomass is composed of 40-50% cellulose, 15-30% hemicellulose, and 15-30% lignin with the remaining portion comprised of extractives (2-5%) (Knauf and Moniruzzaman 2004).

2.7.1 Lignin

Lignin, after cellulose is the second most abundant bio-polymeric organic natural product on earth. Lignin is covalently linked with cellulose among hemicelluloses and pectin (Puls 1997; Abreu *et al.* 1999; Pu *et al.* 2008; Bozell *et al.* 2011b). Lignin is an amorphous, cross-linked phenolic macromolecule with relatively high molecular masses (Figure 5) (Boerjan *et al.* 2003). Lignin is an amorphous, cross-linked phenolic macromolecule with relatively high molecular masses (

Figure 5), which is composed of three different phenylpropanoid monolignol monomers with increasing methoxylation: p-hydroxyphenyl (H), guaiacyl (G), and syringyl

(S), respectively (Bomati and Noel 2005). This biopolymer provides structural rigidity and supports the plant cell walls to resist against compression and bending.

Lignin gives plants mechanical strength by covalently linking with hemicelluloses and filling the space among cellulose, hemicelluloses, and pectin within the cell wall. Its unique characteristics provide natural protection to plant cell walls against microorganisms and is not digestible by animal enzymes.

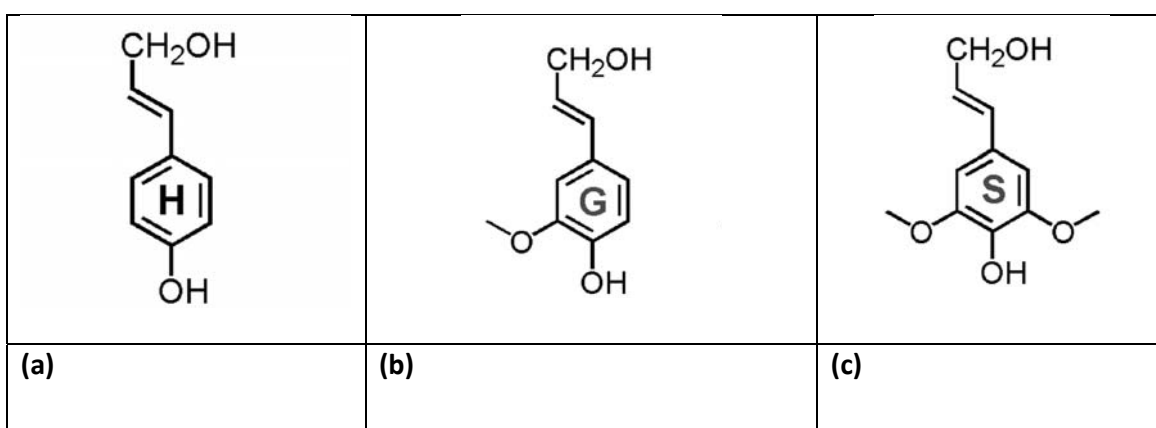


Figure 5. Three different structures of lignin (a) p-hydroxyphenyl, (b) guaiacyl (G), (c) syringyl (S) (Bose *et al.* 2009).

In addition, lignin decreases the permeability of water across the plant cell walls which is important for the role of nutrient transportation within the plant structure (Sarkanen *et al.* 1999). Previous studies have shown, that lignin is more covalently bound to hemicelluloses than cellulose (Lawoko *et al.* 2006). In most plants, cellulose fibers are embedded in a matrix of other structural biopolymers, primarily hemicellulose and lignin. Lignin content varies within and between plant tissues and cell wall layers.

The conversion of cellulose into fermentable sugars can be enhanced by effective lignin removal prior to hydrolysis (Yang and Wyman 2004; Ohgren *et al.* 2007). A study with kraft and sulfite flow-through-pretreatment of corn stover at different temperatures indicated a strong linear correlation between glucan to glucose conversion and lignin removal. Benefits of this desired pretreatment characteristic can be found in removal of barriers to enzymes and increased pore size which promotes susceptibility for hydrolysis (Tarkow 1969; Grethlein and Converse 1985; McMillan 1994; Wyman *et al.* 2005).

The literature suggests that optimum pretreatment methods for biomass can be categorized into several types of studies: 1) Reducing the degree of polymerization of the biomass; 2) minimizing the formation of inhibitors; 3) recovering high purity of value-added by-product streams (lignin and hemicellulose); and 4) pretreatment catalyst recycling, and waste treatment (Banerjee *et al.* 2010).

2.7.2 Cellulose

Research on chemistry of lignocellulosic material began with the isolation of the sugars of cell wall by the French scientist Anselme Payen in 1838 (Ek *et al.* 2009). After treating plants with acids and ammonia, he found cellulose remaining as a resistant and solid fibrous material. Cellulose, the most abundant organic material on earth, is a bio-based organic compound with the formula ($C_6H_{10}O_5$). Cellulose is composed of a linear chain, from several hundred to over ten thousand β (1 $\rightarrow$ 4) linked D-glucose units, with intra-molecular hydrogen bonds between adjacent chains (Gardner 1974). Due to this linkage, cellulose is made of repeat units of monomer cellobiose (Figure 6).

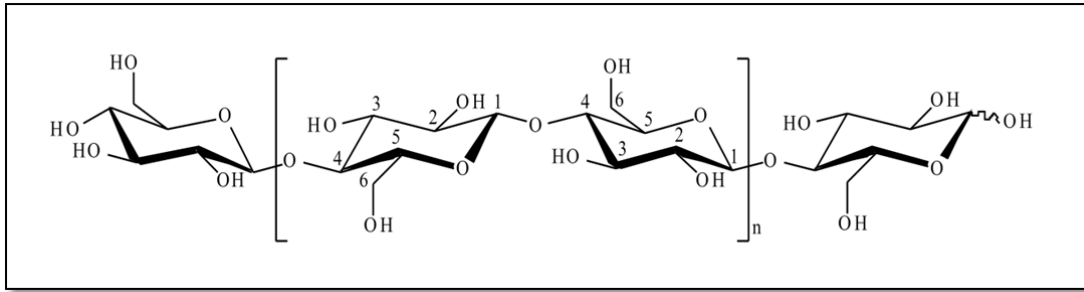


Figure 6. Molecular structure of cellulose (Crawford 1981)

The degree of polymerization (DP) of native cellulose [equation 1] is in the range of 7,000-15,000.

$$DP = \frac{\text{Molecular weight of cellulose}}{\text{Molecular weight of one glucose unit}} \quad [1]$$

Cellulose is a polyglucose, composed of polysaccharide chains which are aligned in parallel direction and form a robust crystalline molecular structure in longitudinal direction. Microfibrils are formed by bundles of linear cellulose chains which are tightly packed and uniformly distributed within the compact structure, so that even small molecules such as water are not able to penetrate the compact structure. Non-crystalline, less ordered regions within the microfibril structure are referred to as amorphous regions (Knauf and Moniruzzaman 2004). Microfibrils impart strength to the plant structure and cell walls. Many studies in the past have shown that amorphous areas can be more easily broken down by hydrolysis compared to crystalline regions (Fan *et al.* 1981; Lee *et al.* 1983; Lynd *et al.* 2002).

2.7.3 Hemicellulose

Hemicellulose is primarily composed of short chains of hetero-1, 4- β -D-xylan with 70-200 DP in a highly branched structure (Poutanen *et al.* 1986). Hemicellulose does not aggregate to form microfibrils, which makes it more vulnerable to thermochemical pretreatment. The major hemicelluloses in softwoods (SW) are galactoglucomannans and arabinoglucuronoxylan, while the predominant hemicellulose in hardwoods (HW) is glucuronoxylan (Pu *et al.* 2006).

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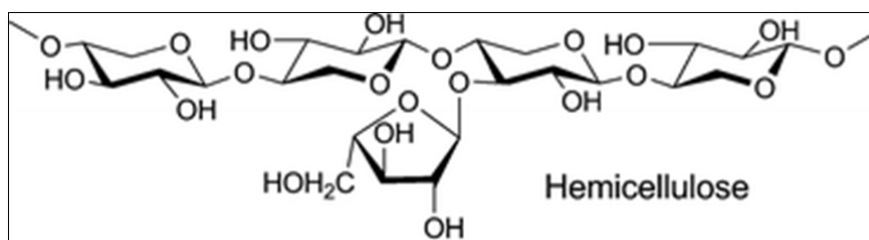


Figure 7. Structure of hemicellulose (Dhepe and Sahu 2010).

2.8 The Pretreatment Principle

Conversion of lignocellulosic materials into ethanol and chemicals from biobased feedstocks requires three major processes: pretreatment, hydrolysis, and fermentation. Efficient pretreatment is required to alter the biomass both in macroscopic and microscopic size and structure as well as the submicroscopic structural and chemical composition which

facilitates rapid and efficient hydrolysis of carbohydrates to fermentable sugars (Brownell *et al.* 1986).

Different pretreatment studies have been explored to evaluate the mechanisms, advantages and disadvantages, and economic feasibility for different pretreatment methods. Different pretreatment options for different feedstock types have been studied such as biological, mechanical, and chemical (McMillan 1994; Wyman *et al.* 2005).

The literature suggests that optimum pretreatment methods for biomass can be categorized into several types. Current practices of the biochemical and biofuel industry in pretreatment technologies are primarily fermentation of sugars derived from starch and sugar crops. These pretreatment technologies are well evolved and have limited opportunities for process improvement.

Among the necessary operations for biological conversion of cellulosic biomass to ethanol, pretreatment accounts for about 18% of the total process cost (Wooley *et al.* 1999). In addition, the initial pretreatment step has a significant impact on the overall subsequent conversion processes. Due to the complex structure of cellulosic biomass, pretreatment is required to alter the structure of lignocellulosic materials in order to make cellulose more accessible to enzymes for the subsequent conversion to sugars (Mosier *et al.* 2005b), see Figure 8.

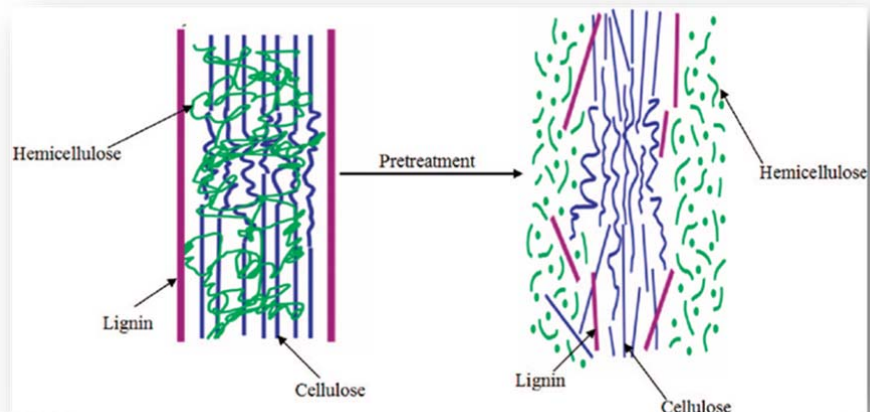


Figure 8. Schematic breakdown of lignocellulosic material (Mosier *et al.* 2005a)

Chemical breakdown and delignification of biomass during pretreatment results in modification of lignin's structure and change in properties such as molecular weight. Many pretreatment methods for lignocellulosic materials have been studied in the past and are related to physical, physico-chemical, chemical, and biological processes (Mosier *et al.* 2005b). The most important pretreatment techniques for a biorefinery are described and discussed in the following section.

2.9 Pretreatment Techniques for a Biorefinery

Feasible pretreatment techniques for biorefineries include organosolv fractionation, hot water, dilute acid, lime, steam explosion, and ammonia fiber expansion (AFEX). They were evaluated as a precursor to this research because of their well-suited ability to remove lignin and hemicelluloses.

By applying different pretreatment parameters to lignocellulosic biomass such as temperature, pH, different chemicals, pretreatment time, and reactor configurations, the aforementioned pretreatment methods exhibit characteristics which differ in pulp quality, delignification, and sugar yields. To identify the proper pretreatment process resulting in

preferred attributes is the key for advancing pretreatment methods. In general, pretreatment at high pH, or with lime, show more ability to remove lignin than hemicellulose. Dilute acid pretreatment at low or near neutral pH shows a minor change in lignin yield but good performance in hydrolysis of most of the hemicelluloses into monomers or oligomers, with little change in the lignin content (Mosier *et al.* 2005b).

Physical pretreatment methods include reduction of biomass by dry and wet milling, vibratory ball milling, steam explosion, and hydrothermolysis. Comminution including dry-milling, wet-milling (Sidiras and Koukios 1989), and compression milling (Tassinari *et al.* 1982) is sometimes applied to make material handling easier within subsequent processing steps. The use of acids or bases promotes hydrolysis and improves the yield of glucose recovery from cellulose by removing hemicellulose or lignin during pretreatment. The most commonly used acid and base are H_2SO_4 and NaOH , respectively.

In this review, potential candidates for pretreatment methods using dilute acid, lime, and ammonia pretreatments are discussed. Key differences are noted. These pretreatments are considered to be cost-effective.

2.9.1 Organosolv Processes

Organosolv fractionation is the primary separation technology examined in this work. These processes use a mixture of organic solvents and water for removal of lignin and hemicellulose in relatively pure streams. Solvents for these processes are mainly ethanol and methanol, but have also been performed using acetone or ethylene glycol at temperatures up to 200°C . However, lower temperatures can be sufficient, depending on the solvent and the type of biomass. Organic and inorganic acids can be used as catalysts for this process (Sun and Cheng 2002). Major benefits of this process are high quality lignin, which may be used for further downstream processing and can serve for platform chemicals, and reduced enzyme

costs from the removal of lignin from the cellulose fraction. In recent years, organosolv pretreatment methodology has been widely studied by applying different solvent-mixtures (Gilarranz 1998; Gonzalez *et al.* 2008 Rodriguez *et al.* 2008; Zhao 2008) from agricultural and forestry residues feedstocks (Oliet *et al.* 2002; Shatalov 2002; Kishimoto, 2002).

2.9.2 Steam explosion

For pretreatment lignocellulosic materials, steam explosion is a widely used method where biomass is treated with high-pressure saturated steam, before the pressure is suddenly vented and exposed to atmospheric pressure. This pretreatment method is usually initiated at temperatures between 160-260°C (corresponding pressure, 0.69-4.83 MPa) and held several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis (Jakobsons *et al.* 1995; Josefsson, 2002).

2.9.3 Ammonia Fiber Explosion (AFEX)

Ammonia fiber explosion (AFEX) is a physical-chemical pretreatment, where biomass is broken down under high pressure using saturated steam at temperature levels between 160°C and 180°C. This is followed by a sudden pressure drop which causes an abrupt decompression of the biomass (McMillan 1994). This technique removes lignin and hemicellulose and improves the following hydrolysis of cellulose. This pretreatment method appears to be most effective for hardwood and agricultural residues (Sun and Cheng 2002). This method delignifies and reduces crystallinity of cellulose but does not effectively remove hemicellulose (Vlasenko *et al.* 1997). The major effect of AFEX can be characterized by the effective improvement of digestibility of pretreated cellulose residue, although this process

suffers due to low hemicellulose sugar yield, which affects the efficient downstream processing of glucose (Wright 1988; Excoffier *et al.* 1991; Heitz *et al.* 1991).

2.9.4 Dilute Acid Pretreatment

Dilute acid pretreatment has been successfully developed for pretreatment of lignocellulosic biomass with various catalysts such as dilute solutions of sulfuric (Zheng *et al.* 2008), nitric, hydrochloric, and phosphoric acid (Zhao *et al.* 2008). Hemicelluloses are completely hydrolyzed into monosaccharides by adjusting pretreatment conditions, although sugar degradation products are generated during hydrolysis. However, significantly increased xylose yield is the major advantage of dilute acid pretreatment compared to steam explosion. Xylose yields approach 80% to 90% of the theoretical value by using a dilute sulfuric acid process in batch mode (Grohmann *et al.* 1987). This pretreatment method has been extensively studied since it is inexpensive. Drawbacks include more expensive reactor materials needed to minimize corrosion resulting from the use of acid, formation of degradation products which may result in release of fermentation inhibitors, a need for initial biomass particle size reduction, and the economic feasibility given sulfuric acid prices.

2.9.5 Alkaline Pretreatment Technology

Alkaline pretreatment technology is one of the major technologies which uses various bases including calcium hydroxide (lime), sodium hydroxide, potassium hydroxide, aqueous ammonia, and ammonia hydroxide (Prior and Day 2008). Alkaline pretreatments are generally considered as delignification processes, although considerable amounts of hemicellulose are also dissolved. These pretreatments are effective on hardwood and herbaceous crops.

2.10 Summary of Pretreatment Methods

Different pretreatment technologies published in the public domain were described above in terms of the mechanisms involved and advantages/disadvantages with economic considerations. A summary is provided in Table 2 of the main pretreatments and their distinct characteristics. The choice of the optimum pretreatment process for a biorefinery depends on the objective of the overall biomass pretreatment given that different methods result in different product streams. The choice of a pretreatment method is not only based only potential yield, but also on other important parameters such as its economic feasibility and environmental impact. Pretreatment methods such as hot water, dilute acid, steam explosion, AFEX, and lime appear to be effective in hemicellulose and lignin removal (Table 2). Most pretreatments are primarily focused on EtOH production.

Table 2. Pretreatment effects of different methods on the biomass (Mosier *et al.* 2005b)

Pretreatment method	Increases accessible surface area	Decrystallizes cellulose	Removes hemicellulose	Removes lignin	Alters lignin structure
Uncatalyzed steam explosion	xx	-	xx	-	x
Liquid hot water	xx	ND	xx	-	x
pH controlled hot water	xx	ND	xx	-	ND
Flow-through liquid hot water	xx	ND	xx	x	x
Dilute acid	xx	-	xx	-	xx
Flow-through acid	xx	-	xx	x	xx
AFEX	xx	xx	x	xx	xx
Lime	xx	ND	x	xx	xx
xx: Major effect x: Minor effect ND: Not determined					

Most of the above mentioned methods indicate acceptable yields when applied to hardwoods and lignocellulosic biomass but most of them are focused on the recovery of cellulose and disregard further utilization of lignin and hemicellulose. In this study, we will describe a novel organosolv fractionation process using a ternary mixture of solvents to perform fractionations of mixed feedstocks. Diluted water, ethanol, and MIBK are the components of this ternary mixture. MIBK improves the overall quality of lignin and hemicellulose streams and offers a promising option for separation of lignocellulosic feedstocks. This process with its beneficial separations is considered to be a potential candidate for a biorefinery.

There is a lack of research on the utilization of hemicellulose and lignin, however with the development of new conversion techniques, the yield of sugars and lignins from lignocellulosic feedstocks can be optimized (Sarath *et al.* 2008). The economics of the overall conversion process from lignocellulosic materials can also be improved by developing value-added by-products derived from lignin and hemicelluloses. Lignin yield is the focus of this research given its potential for value-added materials such as carbon fibers. In this study, Taguchi's engineering methodologies (Taguchi 1993) for designed experimentation (Robust Product Design) were used to maximize lignin yield and are described in the following section.

2.11 Taguchi Robust Product Design

In this study, optimization of the organosolv fractionation process for mixed feedstocks was carried out through application of the Taguchi Robust Product Design methodology, or TRPD (Taguchi 1993). Designing a purposeful experiment starts with a well-defined problem. The response variable and factors (independent variables) with appropriate levels should be well

defined. Sometimes pre-studies are required to determine appropriate or feasible levels of factors. Factors in TRPD are classified as controllable and uncontrollable (Taguchi 1993; Taguchi 1995). Controllable factors (inner array), defined by Taguchi as “*signal*,” are factors that can be easily controlled and manipulated, *e.g., temperature, acid concentration, feedstock weight* (Montgomery 2008). Uncontrollable factors (outer array) are those factors that are classified as being too difficult or expensive to control, and are defined as “*noise*,” *e.g., operators, ambient humidity, feedstock mix, ambient temperature, etc.*

TRPD methodology consists of three elements: “*system design*,” “*parameter design*,” and “*tolerance design*.” “*System design*” is achieved through careful selection of parts, materials, and equipment (Taguchi 1995). “*Parameter design*” which was conducted in this study produces a robust product or process that will remain close to target and will perform well under a range of variation elements in the production environment. “*Tolerance design*” reduces variation around the target value by tightening tolerances on factors that will affect the variation.

TRPD is based on the theory of orthogonal arrays and is related to the theory of fractional factorial designs. Fractional factorials allow for many factors to be examined at typically two levels with a minimum number of runs (Ross 1988). Note that true optimal factor levels are not guaranteed by using Taguchi design (*i.e., local maxima*) because true optimal factor levels may not have been considered as part of the fractional factorial design (Antony *et al.* 2006).

Taguchi proposed a two-step optimization process:

- Find a factor level which reduces performance variability;
- Adjust the factor levels to bring closer to the target.

TRPD defines quality as minimum variation around the targets or set-points. Minimum variation around the targets or set-points will minimize cost or operational losses (Ranjit 2010).

2.11.1 Taguchi Loss Function

In the 1960s Genichi Taguchi was best known for implementing successful quality improvement of Japanese products (Taguchi 1995). Fundamental to economic justification of TRPD was the concept of the “*Loss Function*.” The philosophy behind the loss function is the measurement of financial loss assigned to a product caused by product variation. Taguchi emphasized in the loss function the importance of minimizing variation around the target and manufacturing product to target. Taguchi believes that if variation is minimized around the target, the cost due to variation is minimized, and the products from these processes are more robust, *i.e., product performance has minimal variation and therefore has superior product quality*. Taguchi stressed that any deviation from the target will result in increased cost. In Taguchi’s loss function the financial loss to an organization increases as a quadratic function the farther the product deviates from the target (Figure 9). Taguchi felt that some manufacturers believe they incur a loss only when the product is manufactured outside of specification. Taguchi believed it was too late once a product was manufactured outside of customer specifications, *i.e., the customer may be lost forever*. Taguchi’s philosophy promotes the continuous reduction of variation in a process or service (Taguchi 1993; Hermens 1997; Young and Winistorfer 1999).

Taguchi’s loss function is defined by an objective characteristic y (*e.g., size, yield, etc.*) as it deviates from a target value m . The financial loss from deviations from the target can be

assumed to be a function of y , which is designated $L(y)$. If $y = m$, $L(y) \cong 0$. Taguchi made sustainable contributions to designed experimentation in manufacturing.

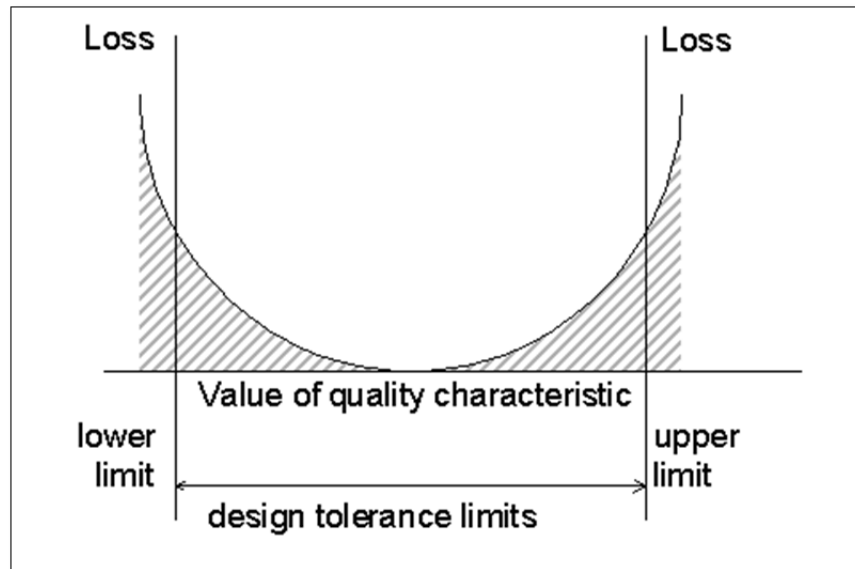


Figure 9. Illustration of the Taguchi Loss Function (Taguchi 1995).

2.11.2 Signal-to-Noise Ratio

In this study, lignin yield from woody and herbaceous biomass using organosolv fractionation was maximized by the experimental methodology of TRPD. As previously stated, TRPD is not a statistical method, it is an engineering methodology. TRPD relies on the metric known as “*signal-to-noise ratio*” or S/N. The S/N of the response variable is nominal, maximized, or minimized. In this study, lignin yield was maximized. The equations for S/N are given in equations [2], [3], and [4]:

$$\textbf{Nominal:} \quad SN_T = 10 \log_{10} \left(\frac{\bar{Y}^2}{s^2} \right) \quad [2]$$

$$\textbf{Maximization:} \quad SN_L = -10 \log_{10} \left(\frac{1}{n} \sum_{i=1}^n \frac{1}{y_i^2} \right) \quad [3]$$

$$\textbf{Minimization:} \quad SN_S = -10 \log_{10} \left(\frac{1}{n} \sum_{i=1}^n y_i^2 \right) \quad [4]$$

The theory of manufacturing a robust product, *i.e.*, *one that is affected minimally by process noise*, is illustrated in Figure 10. Developing products along the S/N function produces products that have less variation regardless of the level of input variation in the process. Products with less variation are considered products of higher quality. Figure 10 illustrates a S/N function with lignin yield as the response (y axis) and switchgrass percentage as the input (x-axis).

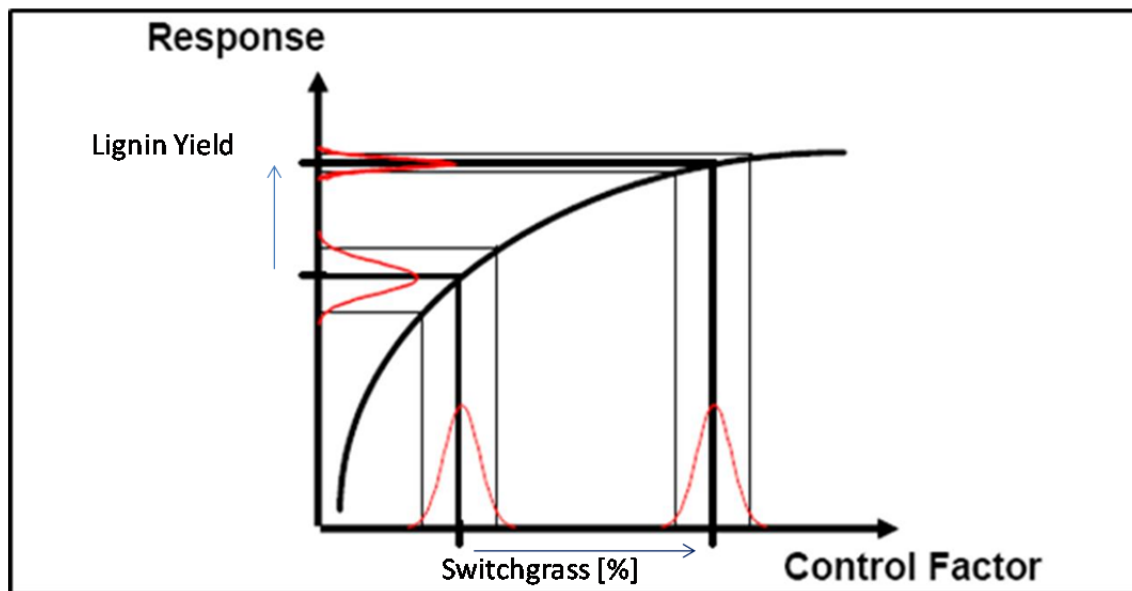


Figure 10. Illustration of TRPD (Taguchi *et al.* 1998)

CHAPTER 3 which follows describes the specific materials and methods used in this study.

CHAPTER 3 MATERIAL AND METHODS

3.1 Feedstock Type 1 – Switchgrass (*Panicum virgatum*)

Alamo switchgrass, harvested in East Tennessee, was comminuted in a 25.4 mm (1 inch) knife mill to give material with an average length of 50.8 mm (2 inches), see Figure 11a. Moisture content was determined on average to be 8% by weighing the biomass before and after drying in an oven at 105°C for 12 h. Results from compositional analysis of the biomass are published in a preliminary study (Bozell *et al.* 2011b).

3.2 Feedstock Type 2 – Tulip Poplar (*Liriodendron tulipifera*)

Tulip poplar chips used for this study with dimensions of approximately 4 cm² and thicknesses of 0.5-1cm were purchased from Oak Ridge Hardwoods, Oak Ridge, Tennessee. The wood chips (Figure 11b) were air dried at 20°C to a moisture content of 8% dry weight basis and stored in cardboard boxes. Compositional analysis results of the poplar chips were performed according to protocol NREL/TP-510-4268.

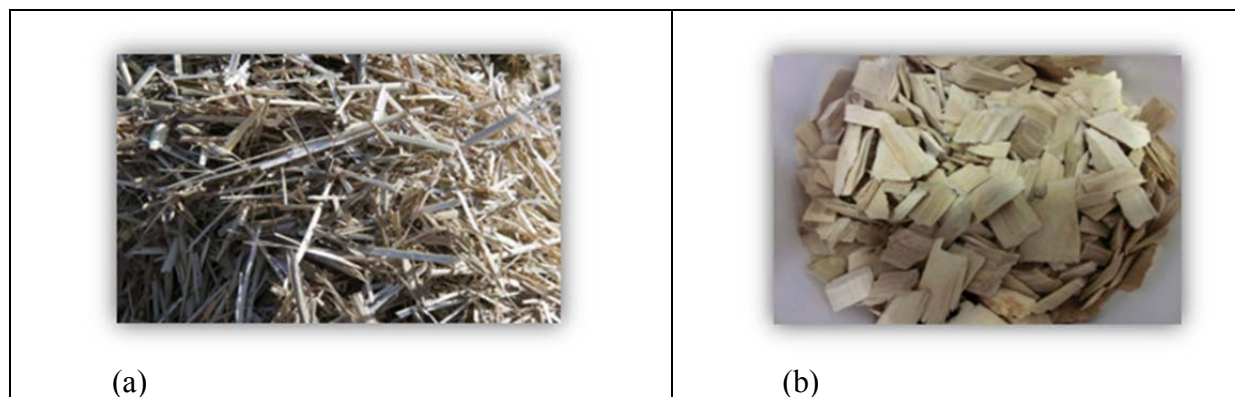


Figure 11. Feedstock used for organosolv fractionation (a) switchgrass and (b) tulip polar chips.

3.3 Organosolv Fractionation Process

In this study, maximization of lignin yield of switchgrass and tulip poplar feedstocks was performed using organosolv fractionation. The fractionation of woody and herbaceous feedstocks is described in further detail in Bozell *et al.* (2011a) and Bozell *et al.* (2011b). The pretreatment fractionation experiments were carried out in a flow-through Hastelloy C276 pressure reactor using a single phase 16:34:50 (by mass) ternary mixture of methyl isobutyl ketone (MIBK), ethanol, and water in the presence of a sulfuric acid catalyst. The fractionation system works in batch flow-through mode and is designed to operate with pressures up to 70×10^5 Pa (1000 psi) and temperatures up to 200°C. The reactor is electrically heated and flow is controlled by an air driven solvent pump combined with an automated valve. System operations are controlled and monitored using Lab-VIEW 8.6 software in combination with a pressure transducer, thermocouples, and an analog to digital converter (Figures 12 and 13).

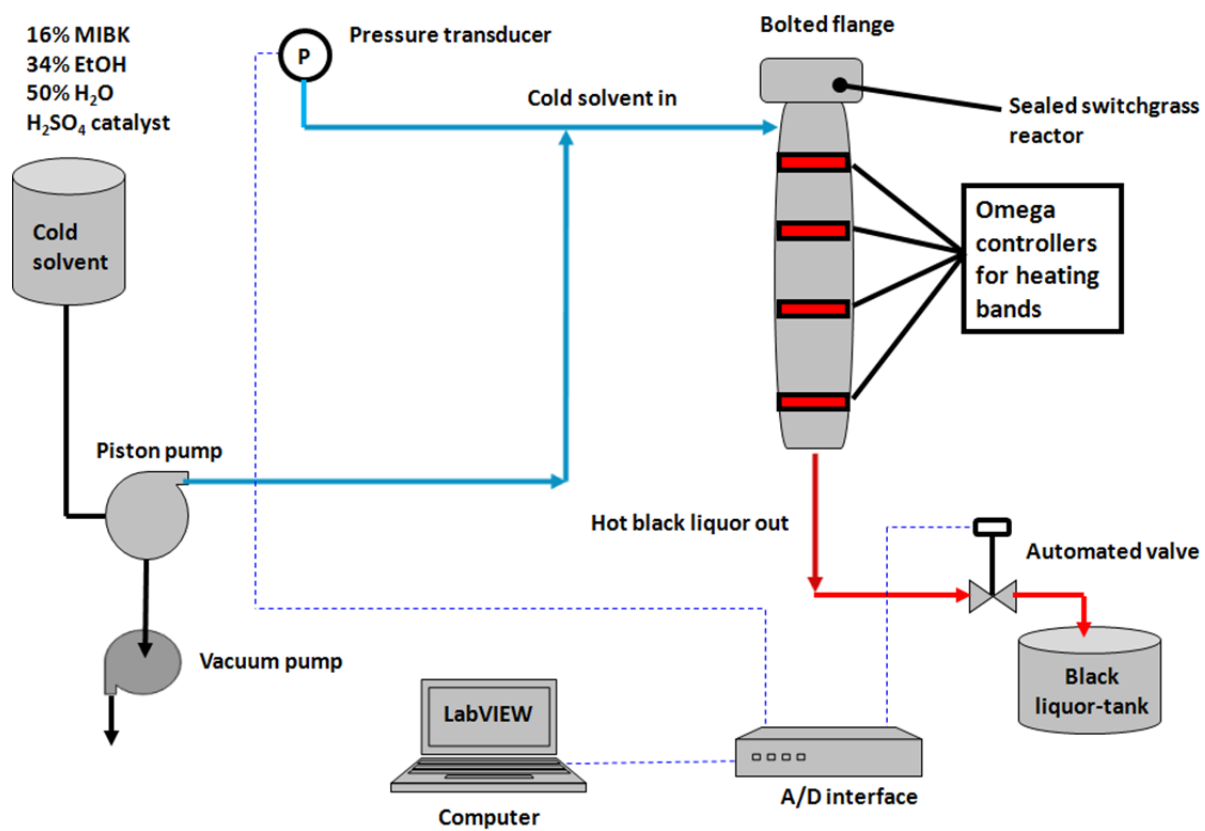


Figure 12. Reactor layout and flow diagram.



Figure 13. Organosolv fractionation reactor with computer interface.

3.3.1 Solvent Composition

For this study the solvent mixture was a single phase solution of MIBK, EtOH, and H₂O, and was prepared based on the ternary phase diagram shown in Figure 14. Ethanol (190 proof), methyl isobutyl ketone (MIBK) and sulfuric acid (catalyst) were purchased from Thermo Fisher Scientific Inc. NJ. In a preliminary study, the specific solvent ratio, termed as “*minus 1-mixture*” (Figure 15), was found to be the most efficient composition for the solvent fractionation process (Bozell *et al.* 2011a). The phase transition line of Figure 14 was determined by preparing EtOH/H₂O mixtures of different compositions, and adding MIBK until a phase separation occurred at room temperature.

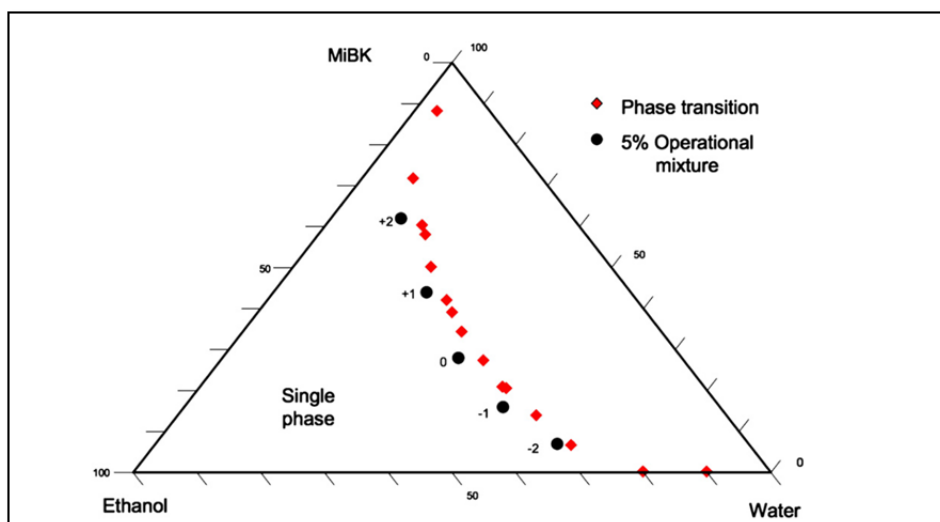


Figure 14. Ternary phase diagram of solvent (Bozell 2011a).

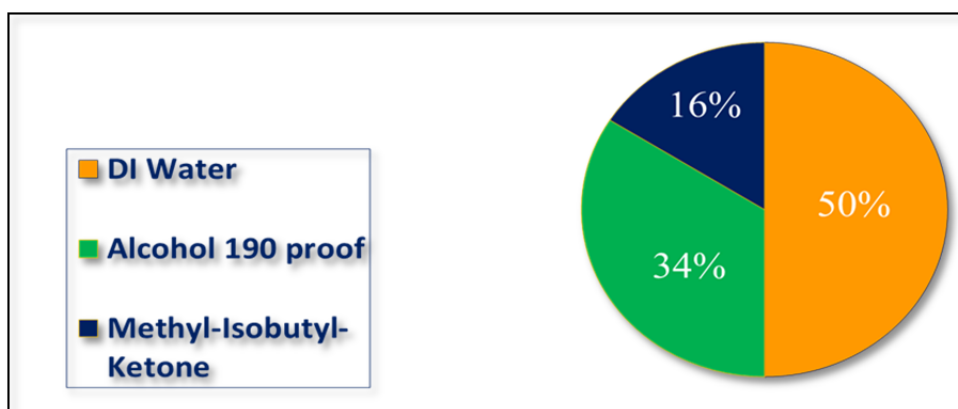


Figure 15. Composition of solvent for organosolv fractionation (Bozell 2011a).

3.3.2 Fractionation with Mixed Feedstocks

The organosolv fractionations were performed with different switchgrass/tulip poplar ratios based on either constant mass or constant volume. Different feedstock compositions (Table 3) were selected, mixed, and loaded into a perforated Teflon basket and placed in the reactor.

The reactor was then sealed and evacuated via a vacuum pump for 20 minutes to remove air and enhance the solvent penetration into the biomass.

Table 3. Feedstock ratios for mass and volume based experimental runs.

Ratio in % [SG/Pop]	Constant mass 400 [g]		Constant volume 3.5 [L]	
	SG [g]	Poplar [g]	SG [g] 100%=270 g	Poplar [g] 100%=500 g
[90/10]	360	40	243	450
[50/50]	200	200	135	200
[10/90]	40	360	27	40

After this stage a solvent tank was opened and the solvent mixture was pulled into the reactor by the vacuum at a level of -0.76×10^5 Pa (-11.5 psi). After filling, the system was electrically heated to the desired fractionation temperature over a duration that varied from 35 to 50 minutes giving a reactor pressure of $2.76\text{--}9.8 \times 10^5$ Pa (40-145 psi) and depends on the temperature set-point. After the fractionation temperature was reached the solvent mixture was pumped through the system into a collection tank for two different runtimes: 56 minutes or 90 minutes. After completion of the run, the remaining solvent in the reactor was carefully drained into a collection tank for subsequent phase separation and product recovery. After cooling the reactor for approximately one hour, the reactor was opened and the un-dissolved cellulose within the Teflon basket was removed.

3.3.3 Cellulose Recovery

The contents of the reactor basket were emptied into a container, mixed with three liters of deionized water and allowed to soak overnight to remove residual ethanol and MIBK. After

soaking, the sample was fiberized in a blender. The fiber suspension was filtered through a polypropylene filter cloth in a Büchner funnel using a water aspirator. The cellulose was continually washed with deionized water for one to two hours until a clear filtrate was observed. To remove excess water, the remaining fiber cake was then vacuum-pressed in a Büchner funnel under a latex sheet for an additional one to two hours. The cellulose sample was weighed and frozen for storage to prevent decomposition.

3.3.4 Lignin Recovery

The black liquor received from the reactor was placed in a separatory funnel, mixed with solid NaCl (15g per 100 ml deionized water contained in solvent mixture) shaken, and allowed to stand for 30 minutes to generate aqueous and organic phases. The layers were separated and the remaining organic layer was washed twice with ~30% v/v deionized water to remove residual EtOH and sugars from the solvent, until a dark organic layer resulted (Figure 16). Lignin was isolated from the organic fraction by solvent removal on the rotary evaporator at a water bath temperature of 50°C. The resulting lignin residue was triturated with diethyl ether in order to remove impurities from the lignin. After decanting the ether, the organic fraction was placed under vacuum. The trituration step was repeated (up to five times) as necessary to give a free flowing brown lignin-powder.



Figure 16. Phase separation between organic (top) and aqueous layer (bottom).

From the aqueous fraction, ethanol and MIBK were removed on the rotary evaporator at 50°C to precipitate a second lignin fraction that was isolated by filtration through a double layer of filter paper in a Büchner funnel and dried under vacuum for 12 h to give a free flowing brown powder. From the aqueous filtrate, 500 ml were collected and stored in a freezer to provide samples for HPLC determination of hemicellulose sugars. The aforementioned processes of lignin and cellulose recoveries are illustrated in Figure 17.

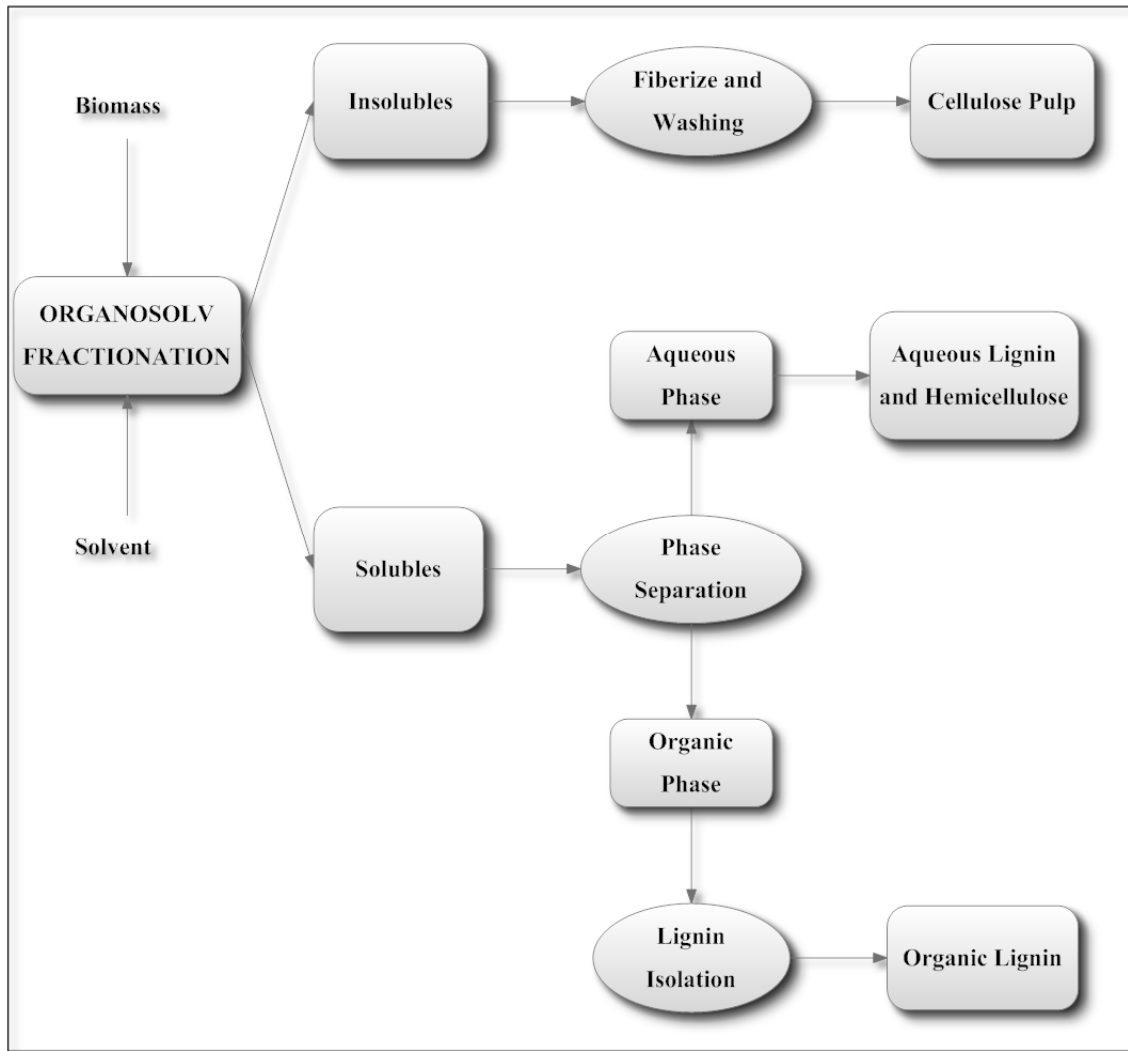


Figure 17. Isolation principle of the organosolv fractionation process.

The lignin yield used for this study was calculated from the combined mass of the lignins isolated from the aqueous and organic fractions (equation [5]).

$$\text{Lignin yield [\%]} = \frac{\text{Lignin after pretreatment [g]}}{\text{Klason lignin in biomass [g]}} \times 100 \quad [5]$$

Moisture content results of organic and aqueous lignins were determined under standard conditions at 105°C and for 24 hours (NREL/TP-510-4268). Analysis for acid soluble lignin and acid insoluble lignin was performed using protocol NREL/TP-510-4268. Results of both analyses methods were implemented in the final lignin yield calculation.

3.4 Taguchi Robust Product Design (TRPD)

3.4.1 Signal Factors for the Inner Array

For this study, lignin yield maximization was performed by applying the Taguchi Robust Product Design (TRPD) methodology (Taguchi 1993; Taguchi 1995). Consistent with the TRPD methodology for maximization problems, the signal-to-noise (S/N) ratio was maximized in this study. Taguchi considers design factors parameters of a process that can be controlled and manipulated. These design factors in the TRPD methodology are also defined as the “*inner array*” of the design matrix. Based on the outcomes from preliminary studies, four significant factors were used as design factors in the TRPD. The design factors in this experiment were: temperature, acid level, feedstock ratio, and runtime.

3.4.2 Noise Factor for the Outer Array

In TRPD, noise factors are considered factors that are expensive or too difficult to control and represent potential factors that induce variation in the process. Taguchi (1993) defined these noise factors as the “*outer array*” of the TRPD design matrix. These noise factors are also used for replication in the fractional factorial designs of the TRPD methodology.

Defining the noise factors and associated levels requires a clear understanding of the process. Different densities associated with the varying mass/volume ratios of the feedstocks were considered difficult factors to control in the organosolv fractionation process and were used as “noise” in the experiment (Table 4)⁴ The first set of runs were chosen based on a constant feedstock mass of 400 g and the second set was based on a constant feedstock volume of 3.5 L and were calculated with three different feedstock ratios (Table 5).

Table 4. Factors and levels in the TRPD experiment.

Design factor	Role	Levels		
Temperature [°C]	Signal (Inner Array)	120	140	160
Acid concentration [%]	Signal (Inner Array)	0.1	0.05	0.025
Feedstock ratio [SG/Pop %]	Signal (Inner Array)	90/10	50/50	10/90
Runtime [min]	Signal (Inner Array)	56		90
Feedstock [kg/dm ³]	Noise (Outer Array)	Constant Mass		Constant Volume

⁴ The solvent flow may affect the dissolution and efficiency of lignin removal within runs.

Table 5. Assignment of feedstock ratios for the noise factors

Feedstock-Ratio in % [SG/Pop]	Constant mass 400 [g]		Constant volume 3.5 [L]	
	SG [g]	Poplar [g]	100 [%] SG = 270 [g]	100 [%] Poplar = 500 [g]
[90/10]	360	40	243	50
[50/50]	200	200	135	250
[10/90]	40	360	27	450

The experiments were arranged using the L18 fractional factorial design associated with a TRPD experiment. A total of 36 reactor runs were performed as part of the L18 design. The noise factor required 18 runs based on mass feedstock composition and 18 runs based on volume based feedstock composition. The levels for each of the factors of the controllable TRPD design factors (inner array) are given in Table 6. The levels for the factors were determined from preliminary studies. The (-) indicates a low-level of the factor such as 120°C. The (+) indicates a high-level of the factor. The (0) is between the low and high levels, and this (0) is not always equidistant between (-) and (+), e.g., H_2SO_4 .⁵

⁵ The largest S/N ratio is considered the most robust product design in the Taguchi design methodology.

Table 6. Assignment of the factors and levels by using the L18 design matrix.

Run Number with replication	Temperature [°C]	H ₂ SO ₄ (mol/L)	Feedstock Ratio [SG/Pop]	Reaction Time [min]	Pattern
1	120	0.025	[10/90]	56	-----
2	120	0.05	[50/50]	56	-00-
3	120	0.1	[90/10]	56	-++-
4	140	0.025	[50/50]	56	0-0-
5	140	0.05	[90/10]	56	00+-
6	140	0.1	[10/90]	56	0+--
7	160	0.025	[90/10]	56	+--+
8	160	0.05	[10/90]	56	+0--
9	160	0.1	[50/50]	56	++0-
10	120	0.025	[90/10]	90	---++
11	120	0.05	[10/90]	90	-0-++
12	120	0.1	[50/50]	90	-+0++
13	140	0.025	[10/90]	90	0--++
14	140	0.05	[50/50]	90	000++
15	140	0.1	[90/10]	90	0+++
16	160	0.025	[50/50]	90	+--0+
17	160	0.05	[90/10]	90	+0+++
18	160	0.1	[10/90]	90	+++0+

To illustrate the levels of controllable factors as a experimental design cube, the inner array factors were assigned to three perpendicular directions and are on a continuous axis varying between (-), (0), and (+). Each dot represents a possible level within the factor-combination of the experimental design cube. The optimal location of the TRPD is easily illustrated in the design space using this three dimensional cube (Figure 18). Note that the cube illustrates all points associated with a full factorial design. If a full factorial design would have

been conducted for the five factors associated with this study, a total of 108 runs using a minimum of two replications would have had to be tested. In the fractional factorial L18 TRPD design a total of 36 runs were conducted, thus making it an efficient design methodology. The points tested using a TRPD are always orthogonal to each other.

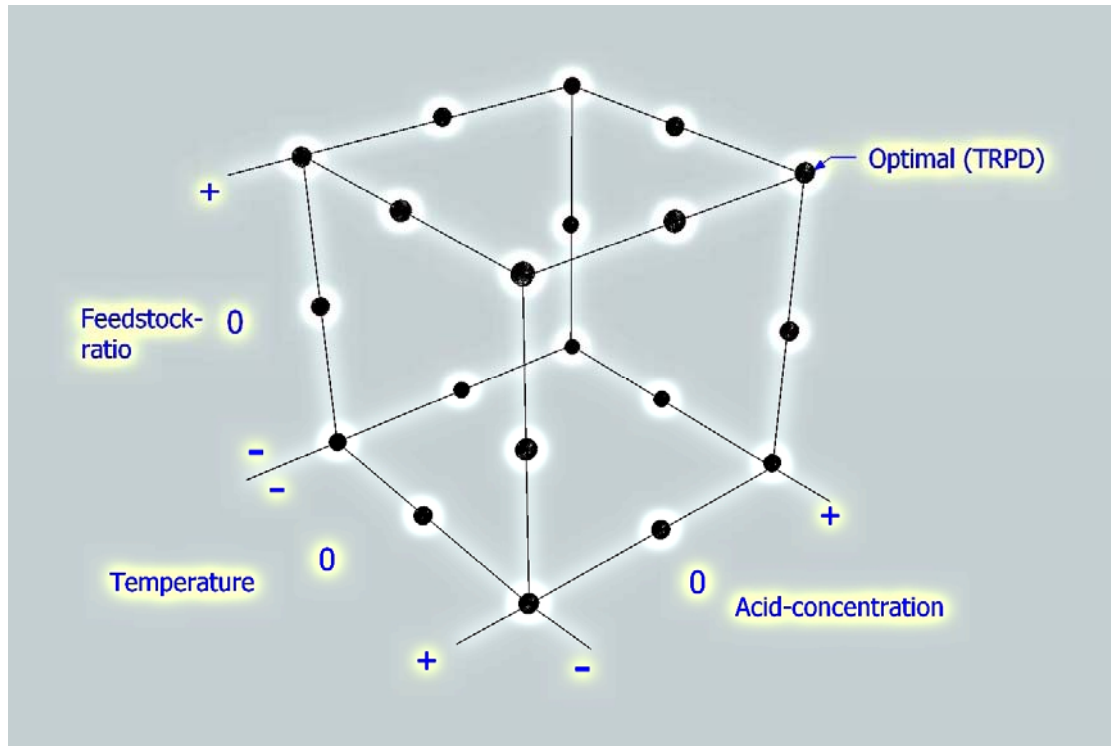


Figure 18. Three dimensional illustration of the TRPD used in this study.

JMP Pro 10.0 software (<http://www.jmp.com/>) was used for creating the L18 design and for the statistical analysis of the TRPD experimental results. In addition to the TRPD S/N ratios, statistical assessments included summary statistics, tests of normality, analysis of variance (ANOVA), and simulation of statistical studies (prediction profiler) were also performed.

CHAPTER 4 RESULTS AND DISCUSSION

According to Klason lignin analysis, the solvent fractionation process successfully provides a lignin fraction with high purity and relatively little cross contamination. The average Klason lignin values for different temperature levels are given in Table 7 and are included in the calculation of the lignin yields.

Table 7. Average values of Klason lignin determination at three different temperature levels.

Temperature [°C]	Klason lignin [wt%]
120	84.63
140	89.79
160	92.08

4.1 Lignin Yield Distribution

In parametric statistics, a general practice is the assumption of an underlying distribution of the observed data which includes independent observations, *i.e., usually assumed to be normal or Gaussian*. In order to validate this assumption, normality tests are used to determine, if the data follow an underlying normal distribution in order to apply the appropriate test-statistics.

The first step of the statistical analyses was a graphical examination of lignin yield for the constant mass and constant volume groups in order to verify that the data are normally

distributed. Recall that mass-based and volume-based feedstock combinations were the noise factors selected for the TRPD experiment. The graphical assessment was done to test for potential outliers and normality of the data. The red “*bell-shaped curve*” exhibits the superimposed normal distribution of the dataset. Histograms of lignin yield for both mass and volume exhibit approximate normality with a slight skewness to the left (Figures 19a and 19b). No outliers in lignin yield were observed. Lignin yields were slightly.

Normal probability plots indicate that the data are approximately normal (Figures 20a and 20b) where the dotted red lines represent the 95% confidence interval and the solid red line represents a “*theoretical*” normal distribution. The more the data fall on the straight line, the more consistency to a normal distribution is given. Slight deviations from normality were observed in the left tails of each distribution where the solid red line exhibits a perfect normal distribution. The Shapiro-Wilk test for normality also confirms the normality assumption. The Shapiro-Wilk statistic for lignin yield for mass-based runs was $W = 0.9321$ (p-value = 0.2110) and for volume-based runs was $W = 0.9070$ (p-value = 0.0793), respectively. Lignin yield for mass was more normally distributed than volume. There is no evidence to suggest that the lignin yield data have severe departures from normality.

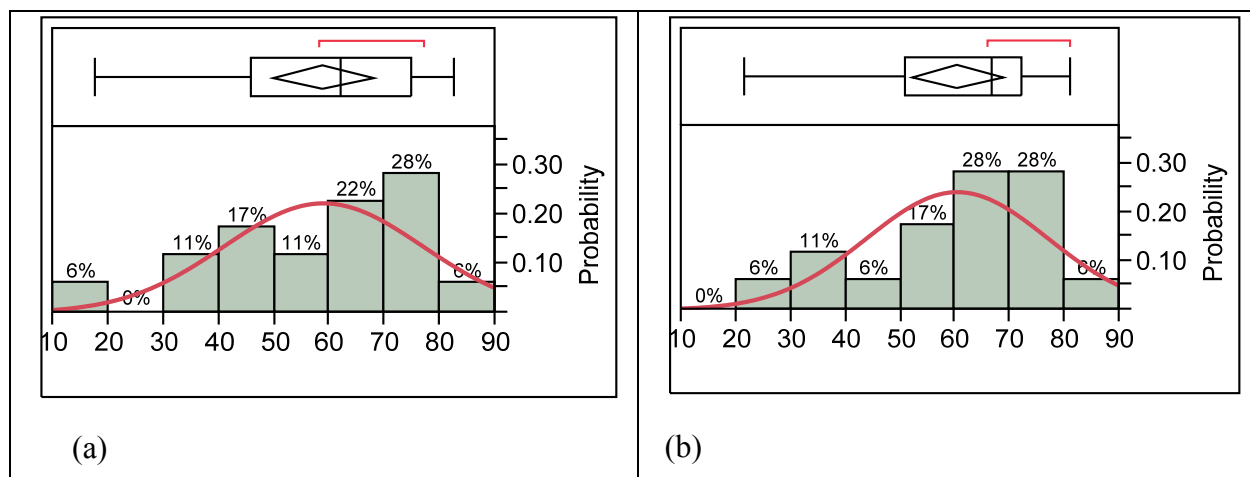


Figure 19. Box plots and histograms of lignin yield for (a) constant mass and (b) constant volume.

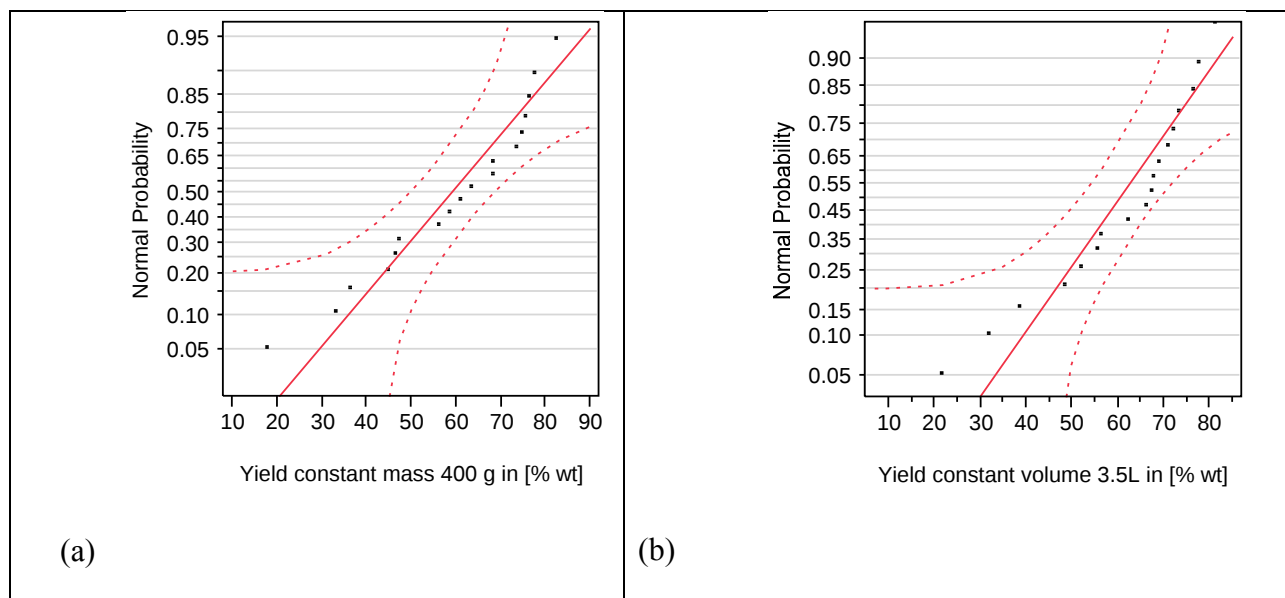


Figure 20. Normal probability plots of lignin yield for (a) constant mass and (b) constant volume.

Another check whether the equal spread of the distribution assumption is reasonable (*i.e., whether the t -test and ANOVA inferences that assume equal variance will be valid*), was to use the rule of thumb that the ratio of the largest sample standard deviation to the smallest sample standard deviation (Table 8) is less than two. Here the ratio was $17.98 \text{ wt\%}/16.56 \text{ wt\%} = 1.09$ which is less than two. Thus, ANOVA analyses assuming equal variances are valid for comparisons of the mass and volume groups.

4.2 Descriptive Statistics of Lignin Yield

Overall mean lignin yields for mass and volume taken over all runs show similar results. The mean lignin yield based on mass ($\bar{x}_M$) was 58.88 wt%. The mean lignin yield based on volume ($\bar{x}_V$) was 60.33 wt%, refer to Table 8. The standard deviation in lignin yield based on mass (s_M) was 17.98 wt% and the standard deviation in lignin yield based on volume (s_V) was 16.56 wt%. The coefficient of variation which is a measure of the dispersion of the data, for mass-based runs (CV_M) was 30.54% and $CV_V = 27.45\%$, indicating that the mass-based and volume-based approaches had similar dispersion within their respective datasets. There was no significant difference between the mean lignin yields based on mass or volume (p-value = 0.5364). There was no significant difference in the variance based on mass or volume (p-value = 0.6769).

Table 8. Summary statistics of lignin yield for constant-mass and constant-volume.

Descriptive Statistics	Constant mass [wt%]	Constant volume [wt%]
Mean of lignin yield	58.88	60.33
Standard Deviation	17.98	16.56
Coefficient of Variation	30.54%	27.45%
Std Error of the Mean	4.24	3.90
Upper 95% Mean	67.82	68.57
Lower 95% Mean	49.94	52.10
N	18	18

4.3 Correlation Analysis for Lignin Yield Between Mass and Volume

Since the experimental conditions of the runs were the same for both mass-based and volume-based runs, a correlation analysis was performed to determine if differences existed. The correlation coefficient $r = 0.71$ indicated that lignin yield from mass and volume are correlated (Figure 21). This might be due to the fact that the dispersion of data-points at low temperatures such as 120°C is higher compared to higher temperatures. The increased temperature resulted in positive effects on lignin yield during the separation process. Lower temperatures (below 120°C) generated significantly lower lignin yield. This correlation also suggests the assumed “noise” factor in the TRPD was not large. Since noise in the TRPD was also used as replication in the experiment, the implications of small noise on overall experimental results is not profound, *i.e.*, *similar results for lignin yield would be expected based on either mass or volume.*

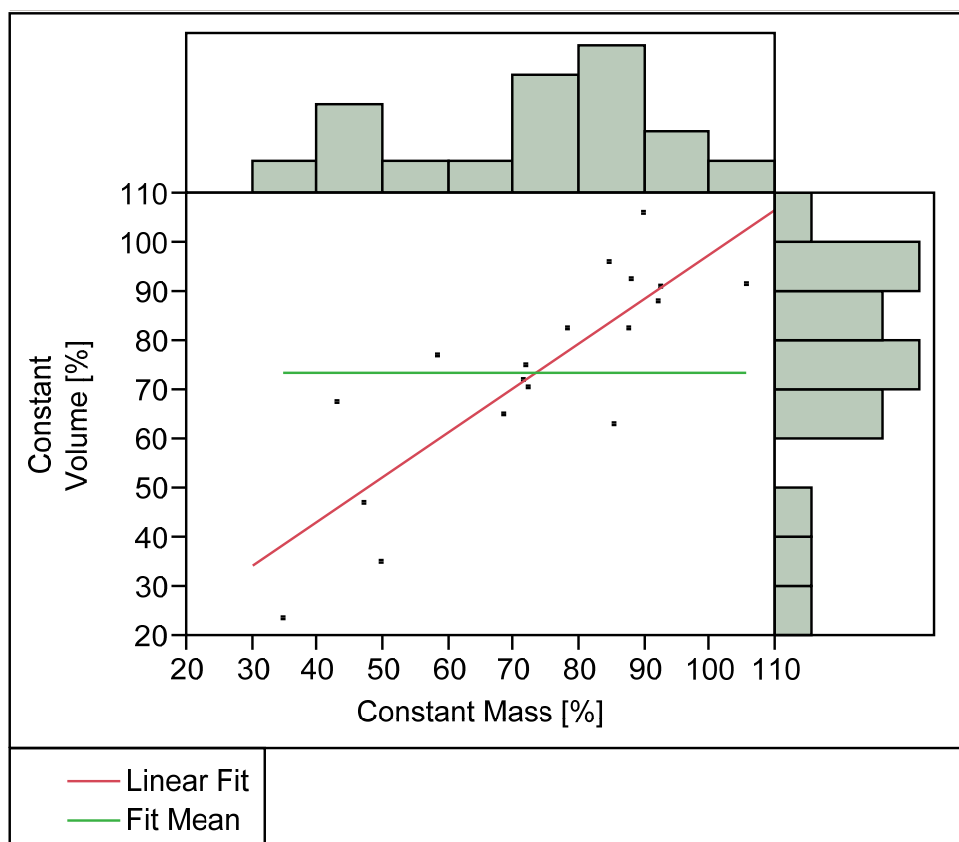


Figure 21. Goodness of fit test between constant mass-based and -volume-based data-set.

4.4 Taguchi Robust Product Design Experimental Results

Optimization was focused on maximizing lignin extraction and maintaining the predictability of the processing conditions using a TRPD to define the 18 runs shown in Table 9. Lignin yield and the Taguchi signal-to-noise ratios (S/N) were maximized at a processing temperature of 160°C, acid level of 0.1M, a volume-based feedstock ratio of 10% switchgrass and 90% tulip poplar, and a 90-minute runtime during processing (run #18, Table 9). Under these conditions, the lignin yield was 81.12 wt% and the Taguchi S/N ratio was 37.9. These operating

conditions were found at the highest levels of the factors during the TRPD experiment and are designated as “+, +, +, +” in the design.

The minimum lignin yield of 17.60 wt% and S/N = 25.68 was found in run #1 for a temperature of 120°C, acid level of 0.025M, a mass-based feedstock ratio of 10% switchgrass and 90% tulip poplar, and a 56-minute runtime. These conditions were found at the lowest levels of the factors examined and are designated as “-, -, -, -” in the experiment (Table 9). One possible reason for this low lignin yield might be the low amount of black liquor (BL) produced (5300 ml) which was the lowest for all 36 runs of the TRPD experiment. If the amount of BL was small, the resulting fraction of organic phase was usually smaller, thus also the solid fraction of lignin.

Table 9. Lignin yields and S/N ratios for volume-based and mass-based feedstock compositions.

Run #	Temperature [°C]	Acid Level [M]	Feedstock Ratio [SG/Pop]	Runtime [min]	Yield of constant mass [%]	Yield of constant volume [%]	Mean [%]	S/N Ratio
1	120	0.025	[10/90]	56	17.60	21.43	19.52	25.68
2	120	0.05	[50/50]	56	36.41	38.56	37.48	31.47
3	120	0.1	[90/10]	56	56.23	51.87	54.05	34.63
4	140	0.025	[50/50]	56	46.99	56.28	51.63	34.15
5	140	0.05	[90/10]	56	60.82	66.01	63.41	36.02
6	140	0.1	[10/90]	56	68.30	67.78	68.04	36.66
7	160	0.025	[90/10]	56	63.53	77.43	70.48	36.83
8	160	0.05	[10/90]	56	82.48	62.22	72.35	36.93
9	160	0.1	[50/50]	56	73.28	72.97	73.12	37.28
10	120	0.025	[90/10]	90	46.52	48.42	47.47	33.52
11	120	0.05	[10/90]	90	44.56	31.58	38.07	31.23
12	120	0.1	[50/50]	90	33.18	55.26	44.22	32.09
13	140	0.025	[10/90]	90	68.16	72.01	70.08	36.90
14	140	0.05	[50/50]	90	75.44	76.25	75.84	37.60
15	140	0.1	[90/10]	90	58.40	68.66	63.53	35.97
16	160	0.025	[50/50]	90	77.28	70.88	74.08	37.37
17	160	0.05	[90/10]	90	74.55	67.25	70.90	36.98
18	160	0.1	[10/90]	90	76.14	81.12	78.63	37.90

Additional statistical analyses reported in the next section were performed on the TRPD experimental data as a function of three key operating parameters: processing temperature, acid concentration, feedstock ratio and runtime. Further discussion on the TRPD results using a “*prediction profiler*” are reported at the end of the chapter.

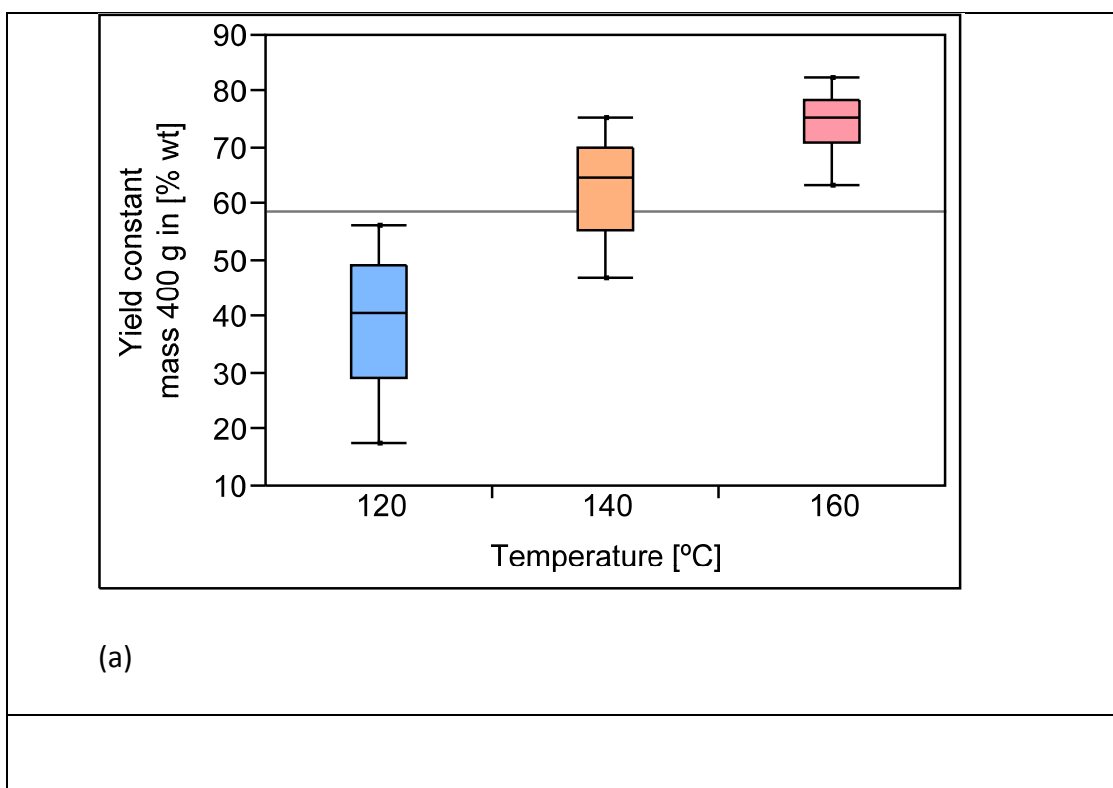
4.4.1 One-Way ANOVA of Lignin Yield by Processing Temperature

A one-way ANOVA was performed to detect any statistical differences in mean lignin yield at different processing temperatures. The blue colored boxplot of lignin yield at 120°C based on mass (Figure 22a) had a larger dispersion in lignin yield compared to volume-based feedstocks (Figure 22b). A reason could be the higher variation in feedstock density and thus a broader band of variation in the lignin yield compared to mass-based feedstock. Note the box plots are grouped by temperature and each box plot contains all other data associated with acid level, runtime, and feedstock ratios.

The one-way ANOVA of mean lignin yields at 140°C and 160°C were not significantly different ($\alpha = 0.05$). However, the lignin yield for 120°C was significantly different ($\alpha = 0.05$) compared to yields at 140°C and 160°C (Table 10). The assumption of equal variances for the temperature level groups was validated (p-value = 0.25 for mass and p-value = 0.08 for volume) using the Levene test. This leads to the conclusion, that if all other factors are constant, the runs could be carried out at 140°C without significant loss of mean yield, offering an opportunity for separation under milder conditions. The letter A is designated to the temperature levels, which are not statistically significant different. If there is a difference between means of the groups, the letter will be different from A, which in this case it is for 120°C (Table 10). Study results support previous research by Pan *et al.* (2006) for organosolv fractionations with single feedstocks. Xu (2005) and Pan *et al.* (2006) found that that lignin yield from switchgrass and poplar, increased at higher temperatures.

Table 10. Lignin yields by temperature levels for mass-based and volume-based feedstocks.

Temperature [°C]			Mass based [wt%]	Volume based [wt%]
160	A ⁶		74.542	71.977
140	A		63.015	67.832
120		B	39.085	41.185



⁶ Temperature levels with the same letter were not statistically different at an $\alpha = 0.05$.

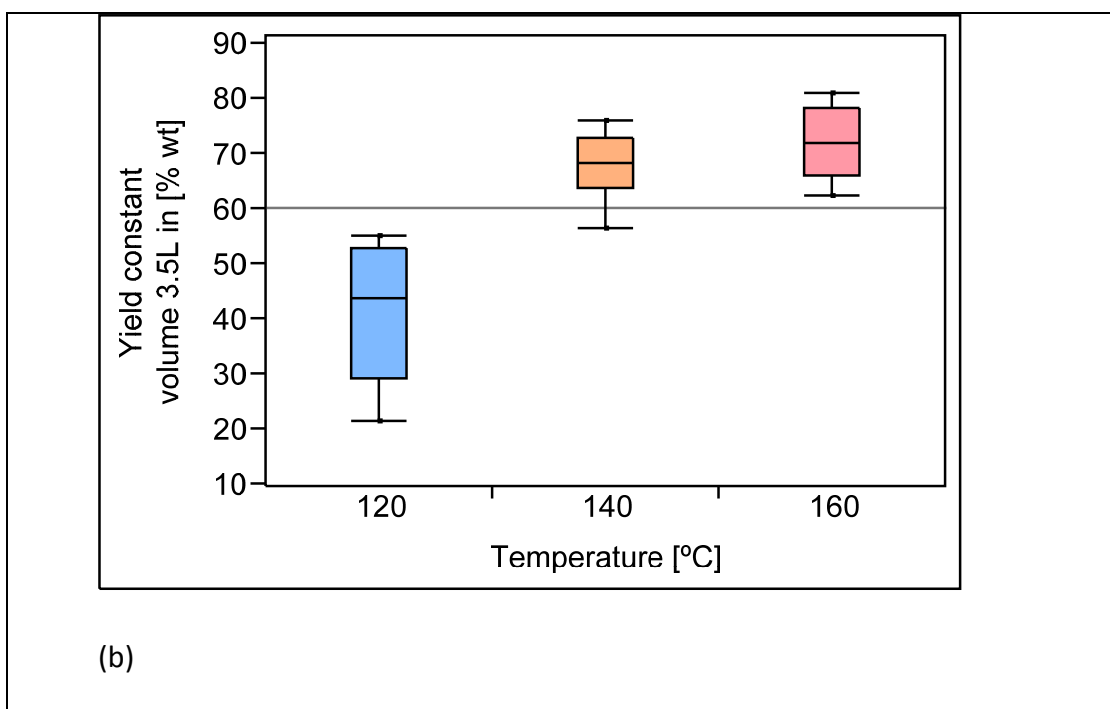


Figure 22. Boxplots of lignin yield for the (a) mass-based and (b) volume-based feedstocks by processing temperature.

4.4.2 One-Way ANOVA of Lignin Yield by Acid Level

A one-way ANOVA analysis was conducted to detect any statistical differences in mean lignin yields as a result of changing the solvent acid levels of groups from mass-based and volume-based feedstock loadings.

In general, a larger variation of the data can be observed within the low levels of 0.025- and 0.05 [M] than in the results of the highest acid levels between mass-based and volume-based feedstocks (Figures 23a and 23b). This leads to the conclusion, that higher solvent concentrations

will not be significantly increased by choosing higher acid levels. Note the maximum lignin yield was found at 82.479 wt% at an acid level of 0.05 [M].

The Levene's test for unequal variances was conducted and supported the assumption that the variances were not significantly different by acid concentration (p-value = 0.6987 for mass and p-value = 0.3335 for volume) from which we can conclude that lignin yield of mass and volume based feedstocks was not significantly influenced by the selection of the acid level.

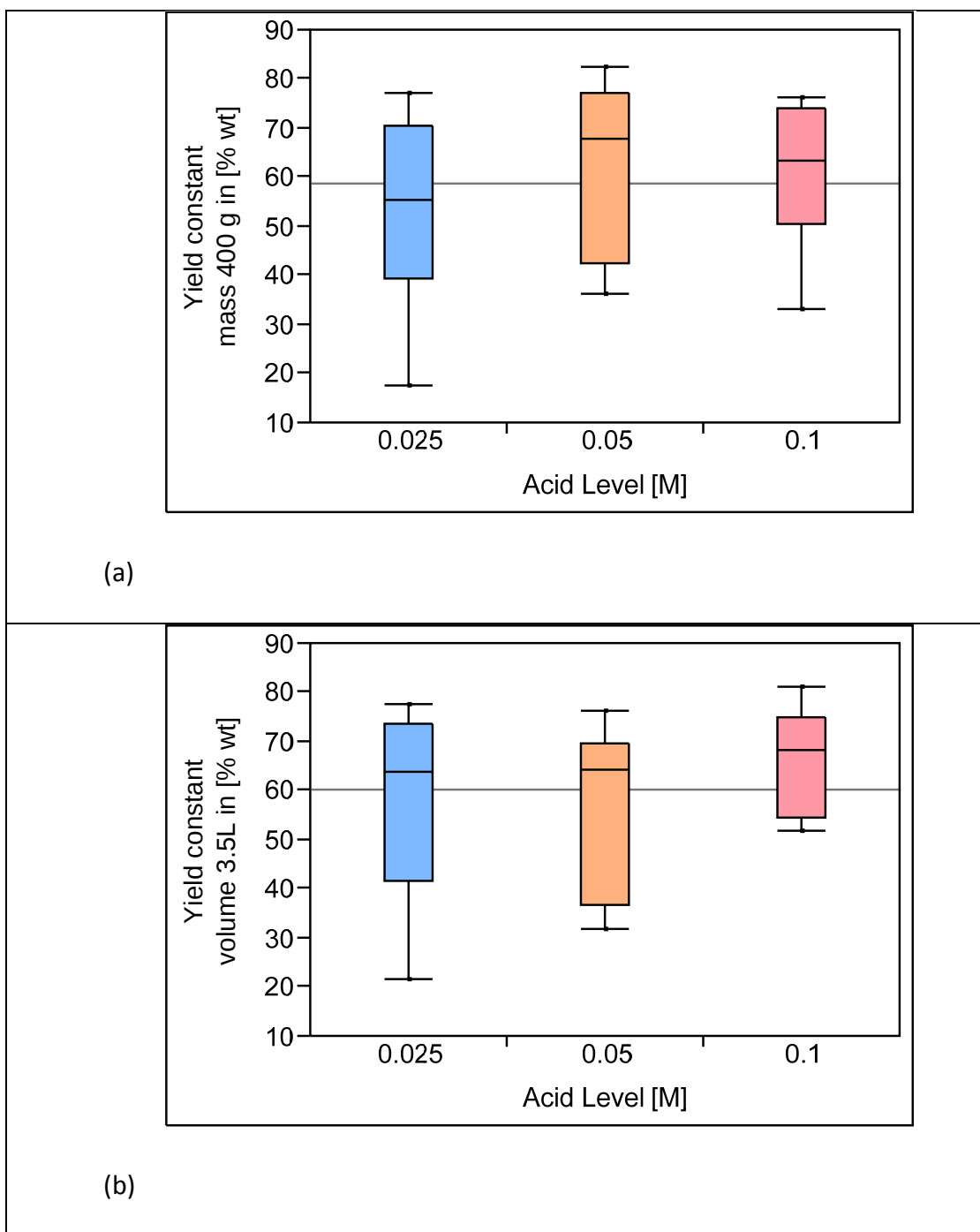


Figure 23. Boxplots of lignin yield for the (a) mass-based and (b) volume-based feedstocks by solvent acid level.

4.4.3 One-Way ANOVA of Lignin Yield by Feedstock Ratio

A one-way ANOVA was performed to detect any statistical differences in mean lignin yield as a result of changing the feedstock ratio. The boxplots of lignin yield by feedstock ratios revealed a smaller variance as the switchgrass proportion increased for both mass-based and volume-based proportions (Figures 24a and 24b).

The Levene's test for unequal variances was applied and supported the conclusion that the variances were not equal by feedstock ratio (p-value = 0.0346 for mass and p-value = 0.0678 for volume). This is a significant result of the study in that it suggests that lignin yield variability is reduced as the proportion of switchgrass increases. Reduced variability implies improved predictability and improved product quality from a manufacturing perspective. The one-way ANOVA of mean lignin yields at different feedstock ratios was not statistically different for mass and volume-based runs ($\alpha = 0.05$) as shown in Table 11. However, the Welch-ANOVA test was performed given the finding of unequal variances. The Welch ANOVA also indicated no significant difference in mean lignin yields by feedstock ratio at an $\alpha = 0.05$ (Table 11) and supported the conclusion that although the variances are different, feedstock ratio does not affect average lignin yield for the mass-based and volume-based runs.

Table 11. Lignin yields by feedstock ratio for mass-based and volume-based feedstocks.

Level			Mass based [wt%]	Volume based [wt%]
160	A		60.000	63.273
140	A		59.540	61.698
120	A		57.096	56.024

Table 12. Welch ANOVA of mean lignin yield across feedstock ratios.

	F Ratio	DFNum	DFDen	Prob > F
Mass-based	0.0465	2	8.404	0.9548
Volume-based	0.2135	2	9.3265	0.8116

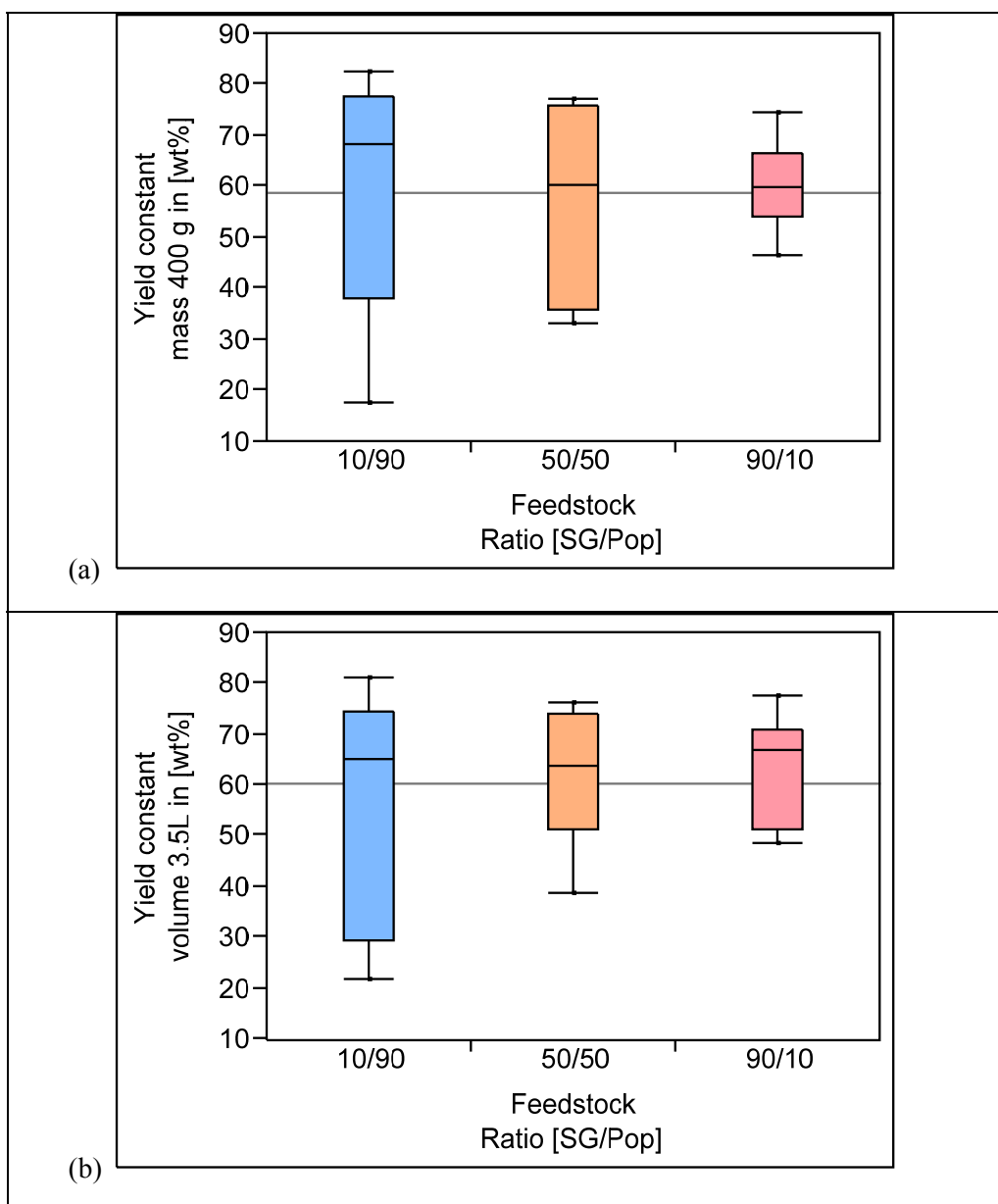


Figure 24. Boxplots of lignin yield for the (a) mass-based and (b) volume-based feedstocks by switchgrass and tulip poplar feedstock ratio.

4.4.4 One-Way ANOVA of Lignin Yield by Runtime

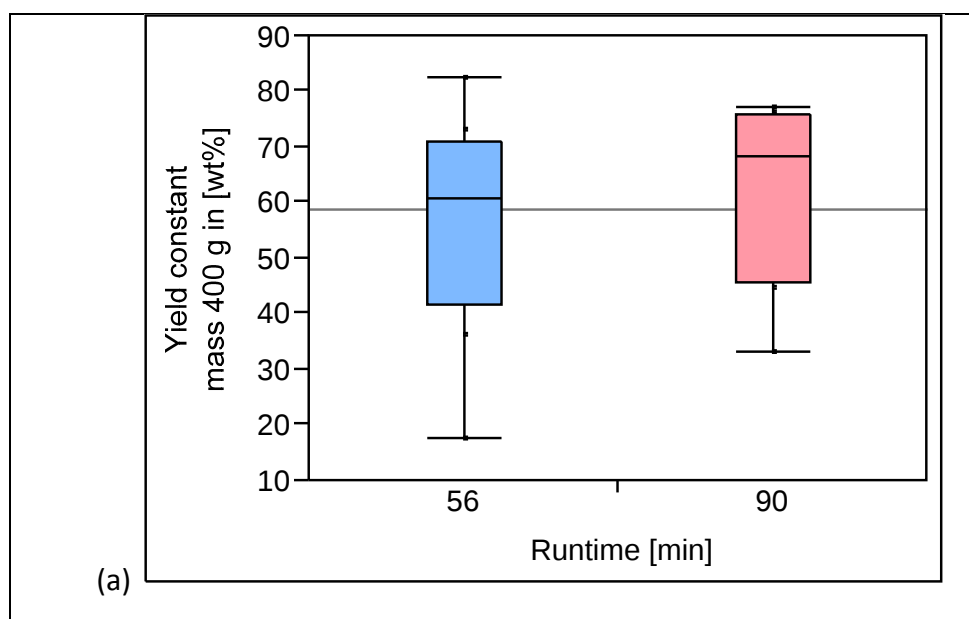
A one-way ANOVA was performed to detect any statistical differences in mean lignin yield as a function of fractionation runtime. The boxplots of lignin yields by runtime indicated no apparent differences in mean lignin yields or dispersions around the means for the 56-minute and 90-minute runtimes for either mass-based or volume-based feedstocks (Figures 25a and 25b). The statistical test of significant differences for means and variances supported this conclusion at $\alpha = 0.05$ (Table 13). The Levene's test for unequal variances was applied to verify if there is a difference between variances and supported the conclusion that the variances were also not equal by processing runtime (p-value = 0.8540 for mass and p-value = 0.7983 for volume).

The results of the one-way ANOVA by runtime may be significant from a practical perspective. No significant difference in either mean lignin yield or variances of lignin yield when going from a 56-minute runtime to a 90-minute runtime may imply that lower runtimes may be feasible. The practical implication is that lower runtimes suggest increased throughput, lower cycle time, and lower costs.

Pressure curves for the 36 experimental runs for the organosolv fractionation process are provided in the Appendix. These pressure curves may be insightful for operational variations in the organosolv fractionation process. They may also improve standard operating procedures for future fractionations.

Table 13. Fisher's least significance difference (LSD) test for mean lignin yields at the 56-minute and 90-minute processing runtimes.⁷

Abs (Dif)-LSD	56 minutes	90 minutes
56	-10.405	-16.729
90	-16.729	-10.405



⁷ Runtimes with positive Fisher LSD values are significantly different at an $\alpha = 0.05$.

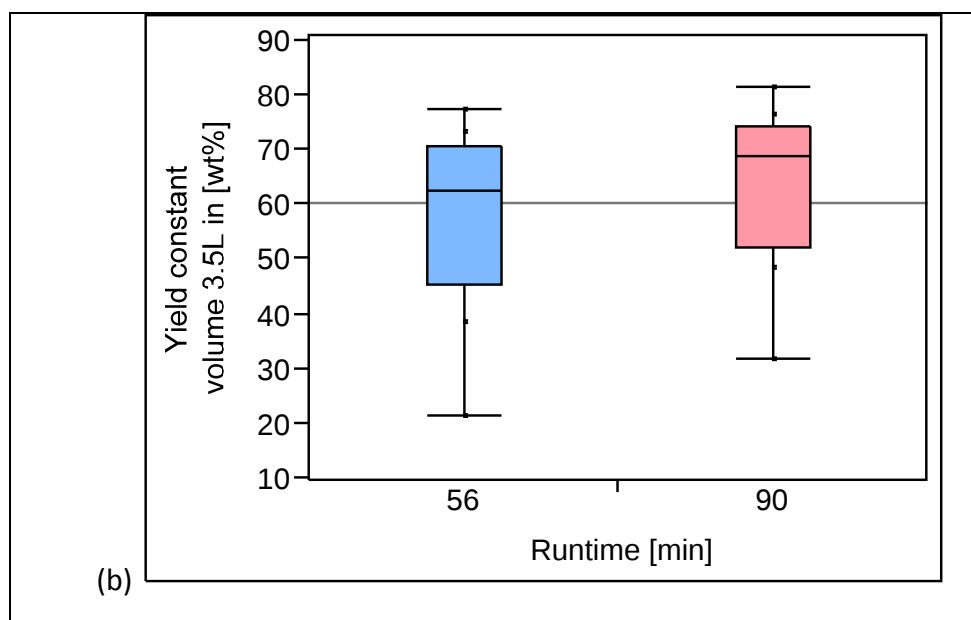


Figure 25. Boxplots of lignin yield for the (a) mass-based and (b) volume-based feedstocks by processing runtimes.

4.5 Predictions from the TRPD

In accordance with the principles of the TRPD, the present study assumes that the maximum lignin yield is indicated by the highest signal-to-noise (S/N) ratio (Equation [4], Chapter 3). Recall from Table 9 that the optimal conditions for L18 TRPD (18 experimental runs) were found at a processing temperature of 160°C, an acid level of 0.1M, a volume-based feedstock ratio of **10% switchgrass and 90% tulip poplar**, and a 90-minute runtime (lignin yield = 81.12 wt% and S/N = 37.9). The prediction profiler provided by JMP 10.0 software expands lignin yield and S/N predictions to the other orthogonal arrays not analyzed in the L18 TRPD experiment. A new global optimal was found using this prediction profiler at a processing temperature of 160°C, an acid level of 0.1M, a feedstock ratio of **90% switchgrass and 10% tulip poplar**, and a 90-minute runtime (predicted lignin yield of 82.22 wt% and a S/N of 39.3), see Figure 26. The key difference was the change in switchgrass/tulip poplar ratio percent from a local optimal of 10/90 to 90/10. Recall from Figures 24a and 24b that the variance in lignin yield significantly declined as switchgrass percent increased and tulip poplar percent decreased.

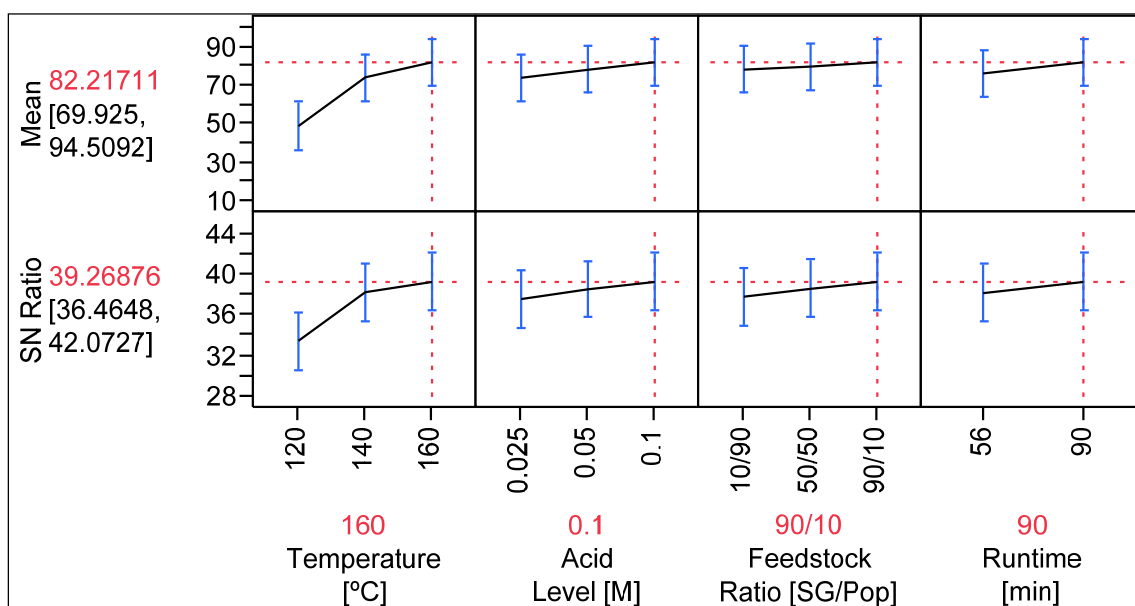


Figure 26. Prediction profiler of the L18 TRPD experiment.

4.6 Simulated Predictions from the TRPD

Using the results from the prediction profiler, it was possible to carry out a much larger simulation of fractionation performance using JMP 10.0 software. Based on the outcome of the TRPD a simulation of 5000 runs was conducted using JMP 10.0 software. Random noise of temperature, acid level, feedstock ratio, and runtime were induced in the simulation. The prediction profiler from JMP 10.0 software associated with this simulation is shown in Figure 27. The mean lignin yield at 140°C from this experiment was 74.41 wt% with a mean S/N of 34.95 (Table 14). A run temperature of 140°C was chosen to allow comparison of the TRPD results with a preliminary study described below. Even though the mean lignin yield and S/N ratio were larger for the simulated experiment of 5000 runs than the TRPD or preliminary study, it does

give a plausible expectation of lignin yields at the optimal conditions based on the results of the TRPD experiment.

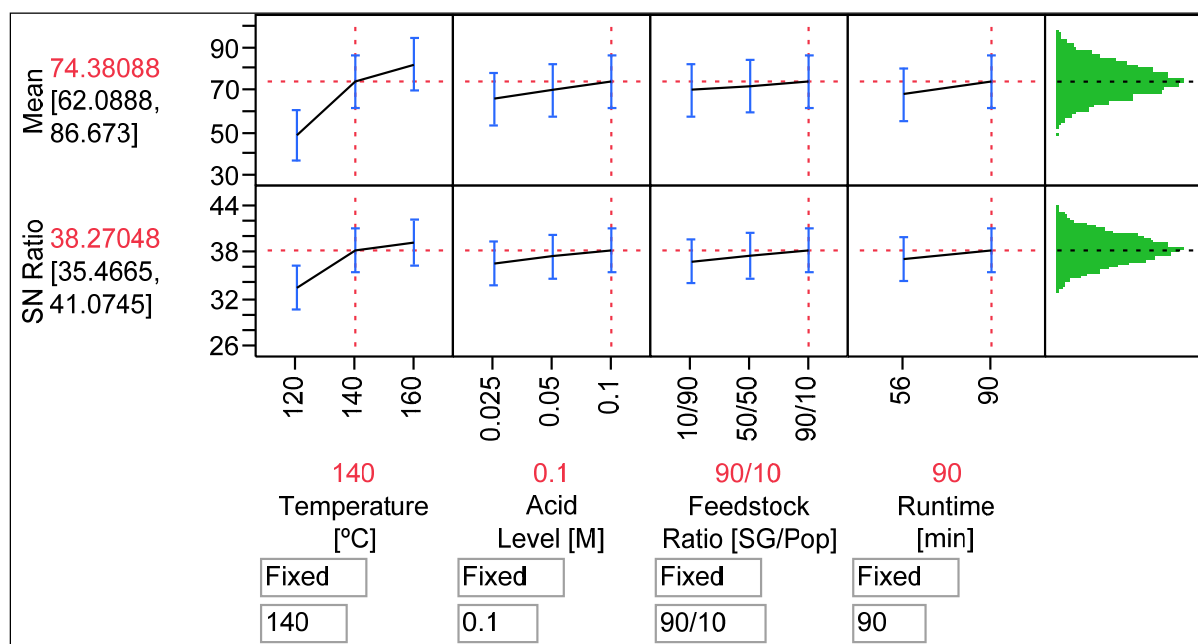


Figure 27. 5000 simulated runs based on the L18 TRPD experimental results.

Table 14. Mean lignin yields of simulation study, TRPD, and preliminary study.

Temp [°C]	Minimum [wt%]	Maximum [wt%]	Simulated Mean [wt%] from 5000 runs	Predicted Mean [wt%]	Preliminary study Mean [wt%]
120	36.80	61.38	49.07	49.09	--
140	62.09	86.67	74.41	74.38	63.58
160	69.93	94.51	82.28	82.21	--

4.7 Comparison of TRPD with Preliminary Study Results

In order to further test the credibility of the TRPD, the results of the experimental design were compared to results carried out on a series of preliminary separations of switchgrass/tulip poplar mixtures. In preliminary research conducted to determine the appropriate factors and levels for the TRPD experiment, solvent fractionation pretreatment was carried out by heating switchgrass and poplar in a ternary, one-phase solvent mixture with sulfuric acid catalyst in a 3.5 L flowthrough reactor. The black liquor product was then phase-separated and evaporated to recover lignin and hemicellulose. A mixed feedstock campaign was chosen to examine the quality of lignin, hemicelluloses and cellulose. This preliminary study was the first attempt to adjust the fractionation process using mixed feedstocks to get reproducible results compared to prior experiments which used only single feedstocks. In order to compare the lignin yields with preliminary runs, an evaluation of the midpoints was conducted. The calculated mean lignin yield of these preliminary runs was found to be 63.58 wt%.

4.7.1 Lignin Yield from Preliminary Mixed Feedstock Runs

During the preliminary studies different feedstock ratios, weights for the different feedstock ratios, and feedstock-masses were analyzed, *e.g.*, 100% switchgrass, poplar at 270 g and 500 g, etc. Lignin was isolated from both the organic phase and the aqueous phase of the black liquor using the salting-out method previously discussed in methodology of Chapter 3. Combined lignin yields were presented in terms of two contributions: organic lignin yield and aqueous lignin yield. Organic yield is the obtained lignin yield from the separated solvent phase of the black liquor. Aqueous yield is the lignin yield obtained from the separated aqueous phase

of the black liquor. The combined lignin yield is the sum of the organic and aqueous phase lignins.

Preliminary study results are summarized in Table 15. A maximum combined lignin yield of 71.11 wt% was determined at 90/10 switchgrass/tulip poplar ratio. The lignin yield of the preliminary runs ranged from 36.66 wt% to 71.11 wt%. The highest yield was achieved when switchgrass was the predominant component of the mixture. This result was similar to the larger TRPD experiment.

Descriptive statistics of the lignin yield data from the preliminary mixed feedstock runs are summarized in Table 16. The mean and median lignin yields were 63.58 wt% and 50.37 wt%, respectively. The sample standard deviation and coefficient of variation (Sorger *et al.* 2003) were 9.22 wt% and 17.51%, respectively. The skewness statistic indicated a right skewed distribution (skewness = 0.4679).

Table 15. Preliminary study results varying the switchgrass/poplar proportion.

Run #	Temp. [°C]	Acid level [M]	Ratio [SG/Pop]	Duration [min]	Black liquor recovered [ml]	Lignin recovered [g]	Combined lignin yield [%wt]
1	140	0.05	90/10	56	5400	46.21	71.11
2	140	0.05	75/25	56	4940	46.25	58.39
3	140	0.05	50/50	56	5040	50.93	49.35
4	140	0.05	25/75	56	5020	59.75	47.05
5	140	0.05	10/90	56	5260	69.20	48.93
6	140	0.05	90/10	56	5520	39.47	60.73
7	140	0.05	75/25	56	4860	29.04	36.66
8	140	0.05	50/50	56	5525	50.81	49.23
9	140	0.05	25/75	56	5480	65.25	51.39
10	140	0.05	10/90	56	5580	75.73	53.55

Table 16. Descriptive statistics of preliminary runs with mixed feedstocks.

Statistics	Lignin yield [wt%]
Mean	63.58
Median	50.37
Standard Deviation	9.22
Coefficient of Variation	17.51%
Interquartile Range (IQR)	10.52
Min	36.66
Max	71.11
Skewness	0.4679
Kurtosis	1.3538
N	10

CHAPTER 5 CONCLUSIONS

A study was performed on the organosolv fractionation process to maximize lignin yield from feedstock mixtures of switchgrass (*Panicum virgatum*) and tulip poplar (*Liriodendron tulipifera*) using a dilute acid pretreatment. A Taguchi Robust Product Design (TRPD) designed experiment was used in this study to identify process parameters and sources of variation for organosolv fractionation that would result in the highest yield of lignin and minimize variance from the feedstock mixtures.

The L18 TRPD with four controllable design factors (inner array) was used in the study to maximize the response defined as lignin yield. The four inner array factors were: process temperature (120°C, 140°C, 160°C); fractionation time (56 minutes, 90 minutes); sulfuric acid concentration (0.025 mol/L, 0.05 mol/L, 0.1 mol/L); and feedstock ratio (switchgrass/tulip poplar ratios of 10%/90%, 50%/50%, 90%/10%). One factor of process noise, the outer array was joined to the inner array and represented the feedstock charges of switchgrass and tulip poplar at two levels (mass-based or volume-based). The TRPD for the aforementioned factors and levels resulted in 36 experimental runs.

A mean maximum lignin yield of 78.63 wt% was found for the run #18 over the other 36 runs of the L18 campaign. Optimum conditions for maximum lignin yield were found at a 90 minute runtime, 160°C process temperature, 0.1 mol/L sulfuric acid concentration, and a feedstock composition of 90 percent switchgrass, and 10 percent tulip poplar. Study results indicated that the 140°C processing temperature during fractionation resulted in a statistically significant increase in lignin yield when compared to the 120°C process temperature (p-value <

0.0001). Study results also indicated that higher proportions of switchgrass (90%) relative to tulip poplar (10%) in the feedstock mixture resulted in reduced variability of lignin yield ($\alpha = 0.05$). The significance of the higher switchgrass percentage inducing higher lignin yields may be due to different particle sizes and densities when compared to tulip poplar. Tulip poplar has a higher density, thus hindering the penetration of the solvent during the fractionation process. As a consequence, the reduction of particle sizes of tulip poplar chips should be considered in future research as an experimental factor.

Simulation results of 5000 runs supported the conclusions of the preliminary and TRPD study results. The mean for lignin yield of 74.41 wt% from the simulations was comparable to the lignin yield mean of 78.63 wt% at the maximum found in the TRPD study. Both simulated and TRPD study lignin yield means were higher than the preliminary study mean of 63.58 wt%.

The organosolv fractionation technique for lignocellulosic biomass is promising in its ease of post-processing steps, using simple chemical mixtures, low material costs, and trainability. The research with this system in combination with mixed feedstocks such as switchgrass and tulip poplar will assist in the refinement of the procedure to maximize product purities. A process scale up unit could be simulated using the large reaction chamber that is already built into the solvent fractionation reactor system. Run times based upon reaction rates on lignin purity could be calculated which would assist in the determination of optimum flow rates through the scaled-up reactor. In addition, a recycle line would reduce dependence on fresh solvent, as would refinement of the black liquor separation method in the workup of the process stream. The salting-out method indicates good solvent recovery.

According to Klason lignin analysis, the solvent fractionation process successfully separates switchgrass into its individual components with relatively little cross contamination. The highest solvent fractionation yield of lignin from switchgrass was 81.12 wt%. The yield of the lignin fraction indicates that most of the material contained in the starting feedstock can be recovered for use in the biorefinery. The purity of lignin suggests that it will be an excellent substrate for conversion into prospective materials such as chemicals and polymers.

5.1 Future Research

To integrate the organosolv fractionation process into a biorefinery, future biomass feedstocks will be expanded to include lignocellulosics from many sources including agricultural wastes and residues or fast-growing trees.

Novel processes will be needed to fractionate biomass (regardless of the source) into its individual components. Based on the findings of this study, reduction of particle size and optimization of feedstock geometry will be an essential factor in order to enhance the purity of individual streams of solvent fractionation. According to the biorefinery concept, a main goal should be a full recovery of the feedstocks through optimized utilization of all lignocellulosic components including sugars and non-sugar compounds as marketable products.

The next steps will be to pursue the compositional analysis of the lignins and hemicelluloses in order to get quantitative proof across different runs, especially around the 140°C and 160°C levels of processing temperature. It is also recommended to apply high acid levels and keep the switchgrass portion on a high level in order to verify these study results and potentially find a new global maximum for lignin yield. Further response surface experimental

designs (*e.g.*, *Box-Behnken methodology*) may be helpful in locating the global maximum for lignin yield and may result in a mathematical model of the organosolv fractionation process.

The results of NMR analysis on the lignin showed no significant difference compared to single feedstock analysis. Future research will explore the integration of the compositional results from lignins, hemicelluloses, and celluloses in order to address the efficiency of quantitative yields in correlation with the qualitative evaluation of the individual streams. Several other experiments might be conducted to understand the effect of pretreatment technologies on mixed feedstocks as well as optimization of the whole pretreatment process. For future research, some recommendations are given.

Response surface design (RSD) is a promising approach for further investigation using experimental design. Based on the results from the TRPD, RSD can be applied using the TRPD maximum result as the starting point. RSD is usually very efficient in terms of number of required runs, and they are either rotatable or nearly rotatable. The Box-Behnken design is known as a “*spherical design*” where all points are located on a sphere of a radius $\sqrt{2}$ (Montgomery 2008). Process factors such as temperature or acid concentration in combination with feedstock ratio may be globally optimized from this RSD.

A suggested Box-Behnken design requiring 30 runs would use the following factors and levels: process temperature (140°C, 150°C, 160°C); acid concentration (0.025 mol/L, 0.05 mol/L); and switchgrass/tulip poplar feedstock ratio (75%/25%, 90%/10%, and 100%/0%). The suggested runtime for this Box-Behnken design would be 56 minutes. This approach may provide a tool for design, scale up, and predictive process control. Variation in the amount of solvent flow for individual runs can also be considered for further investigation.

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APPENDIX

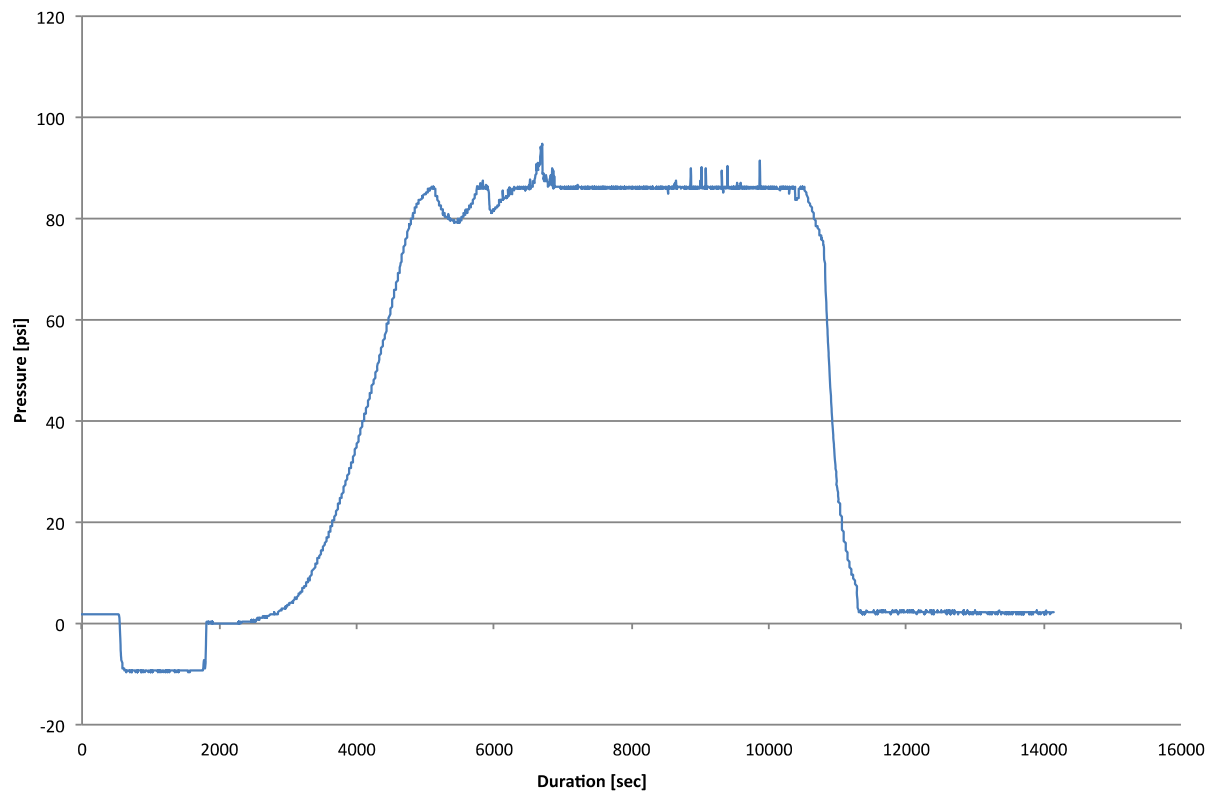


Figure 28. Pressure diagram for Run #1.

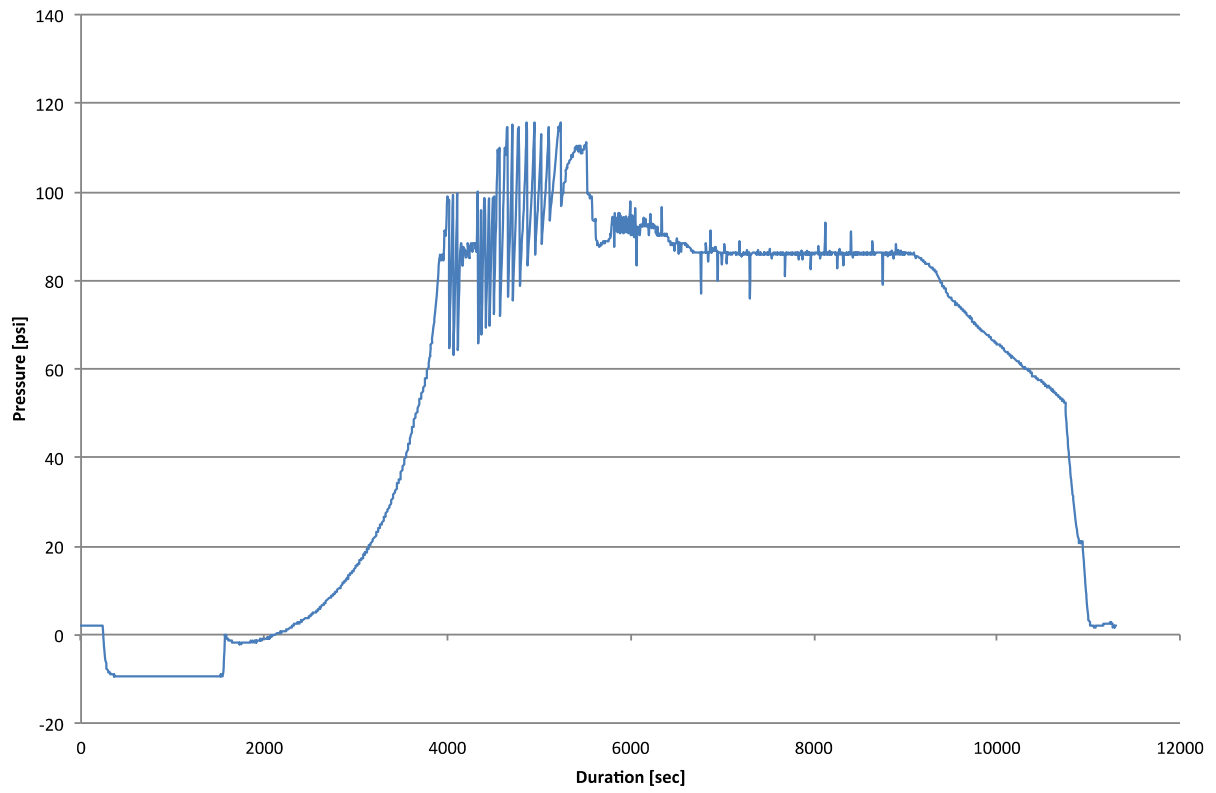


Figure 29. Pressure diagram for Run #2.

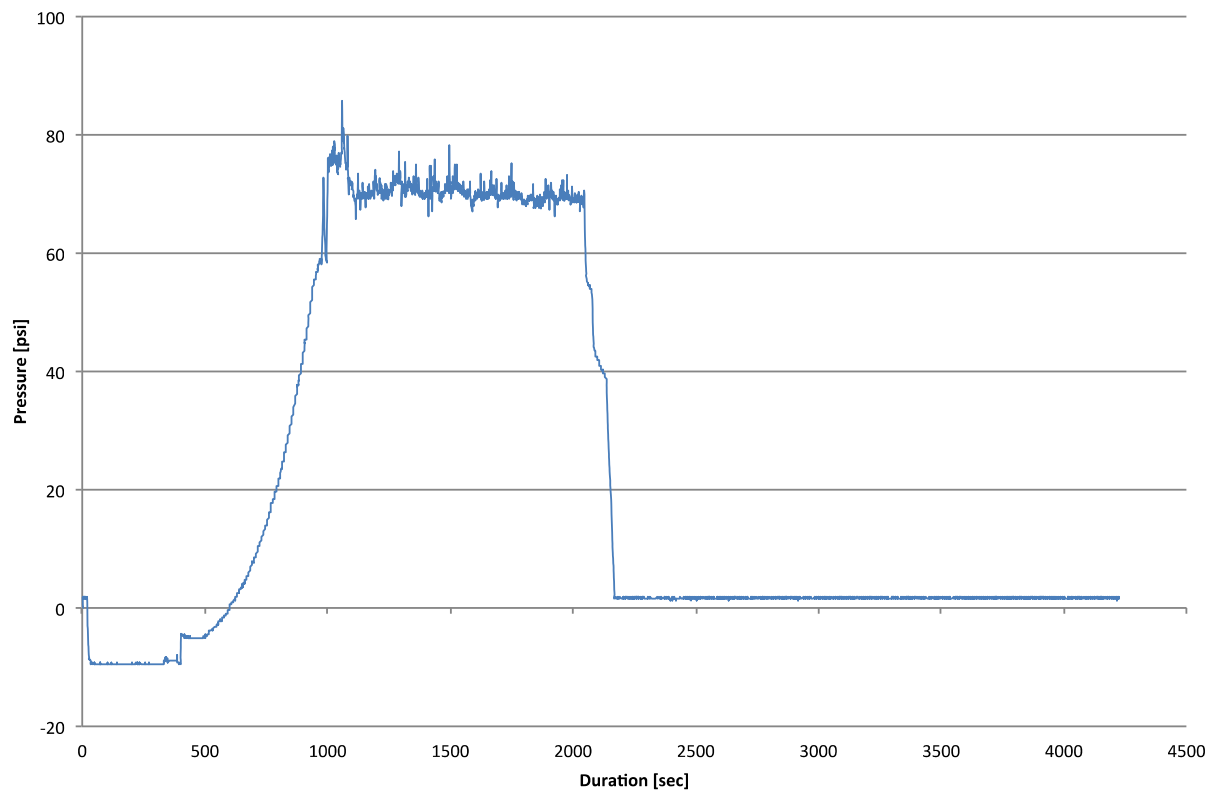


Figure 30. Pressure diagram for Run #3.

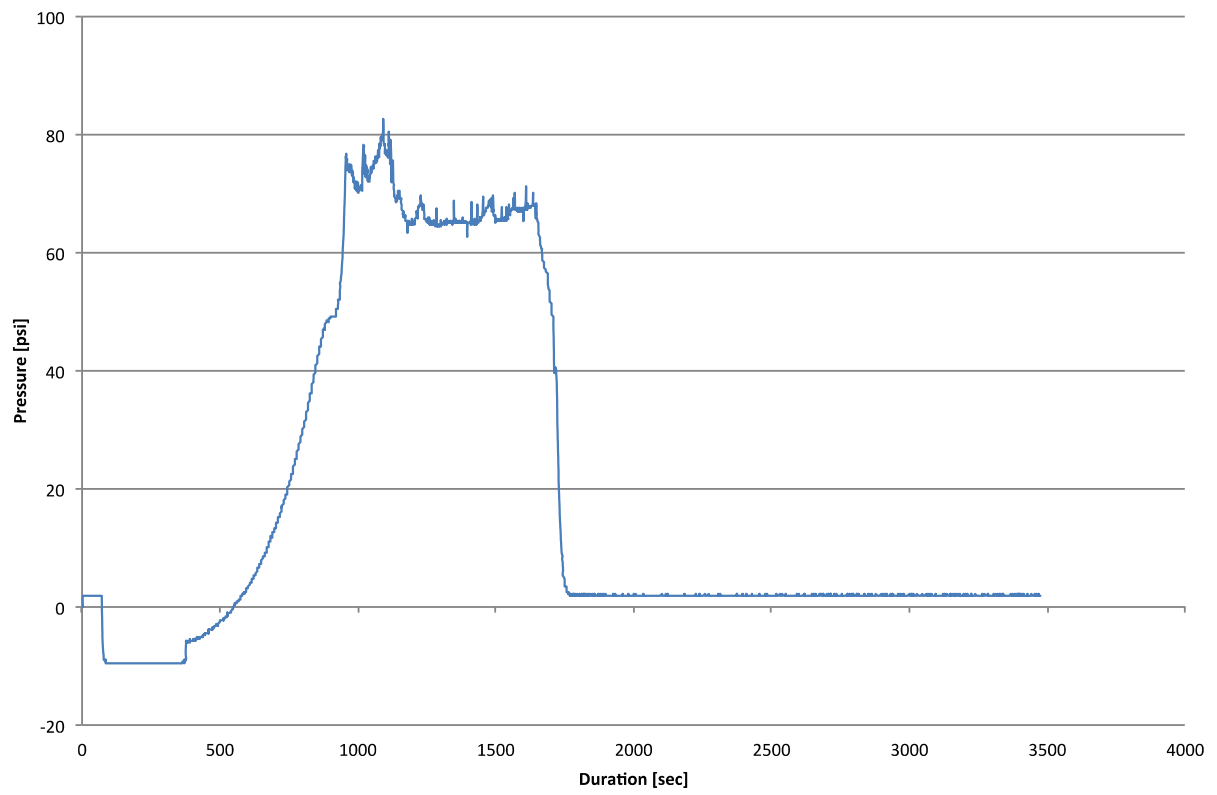


Figure 31. Pressure diagram for Run #4.

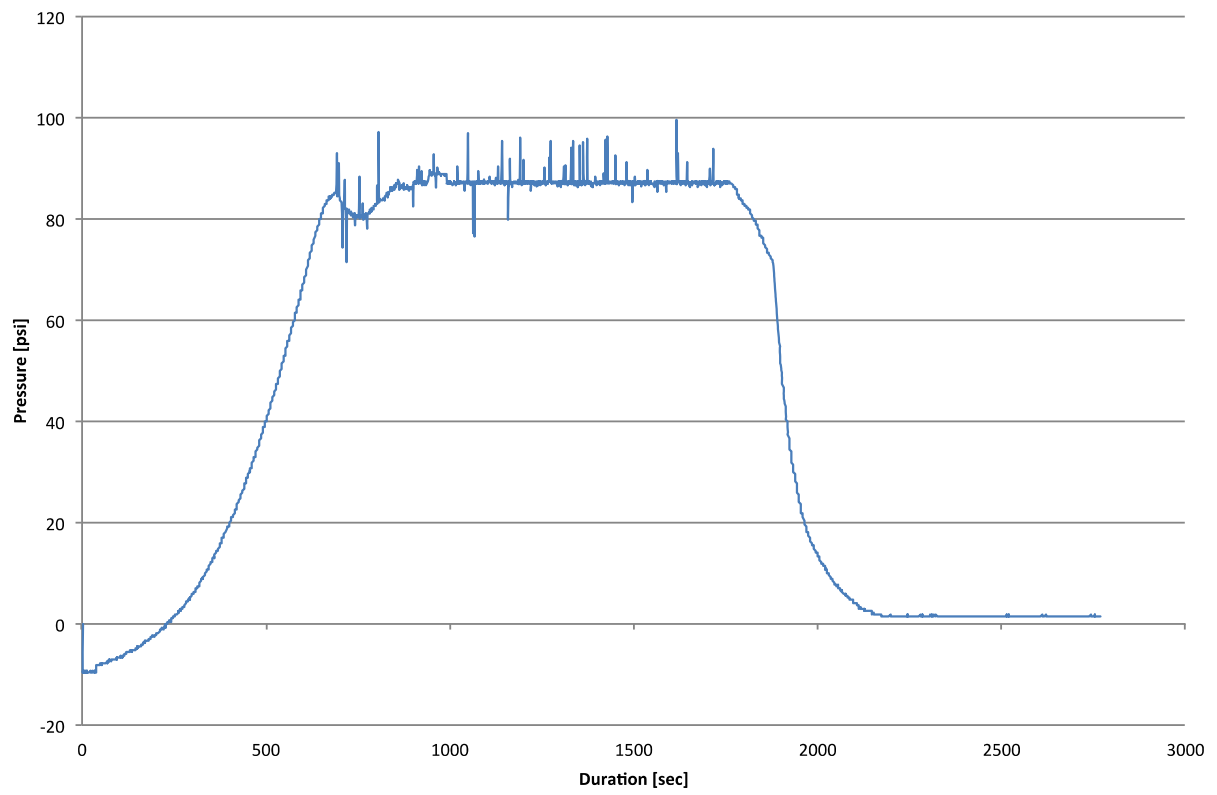


Figure 32. Pressure diagram for Run #5.

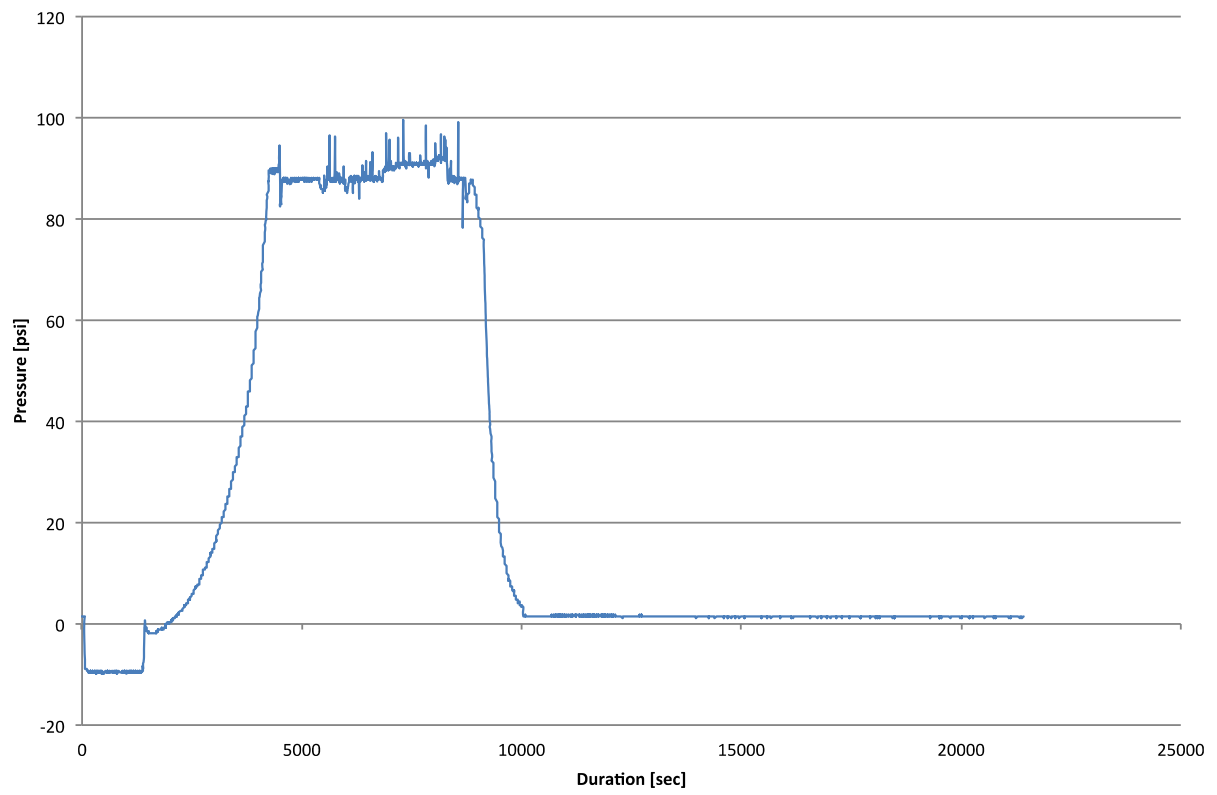


Figure 33. Pressure diagram for Run #6.

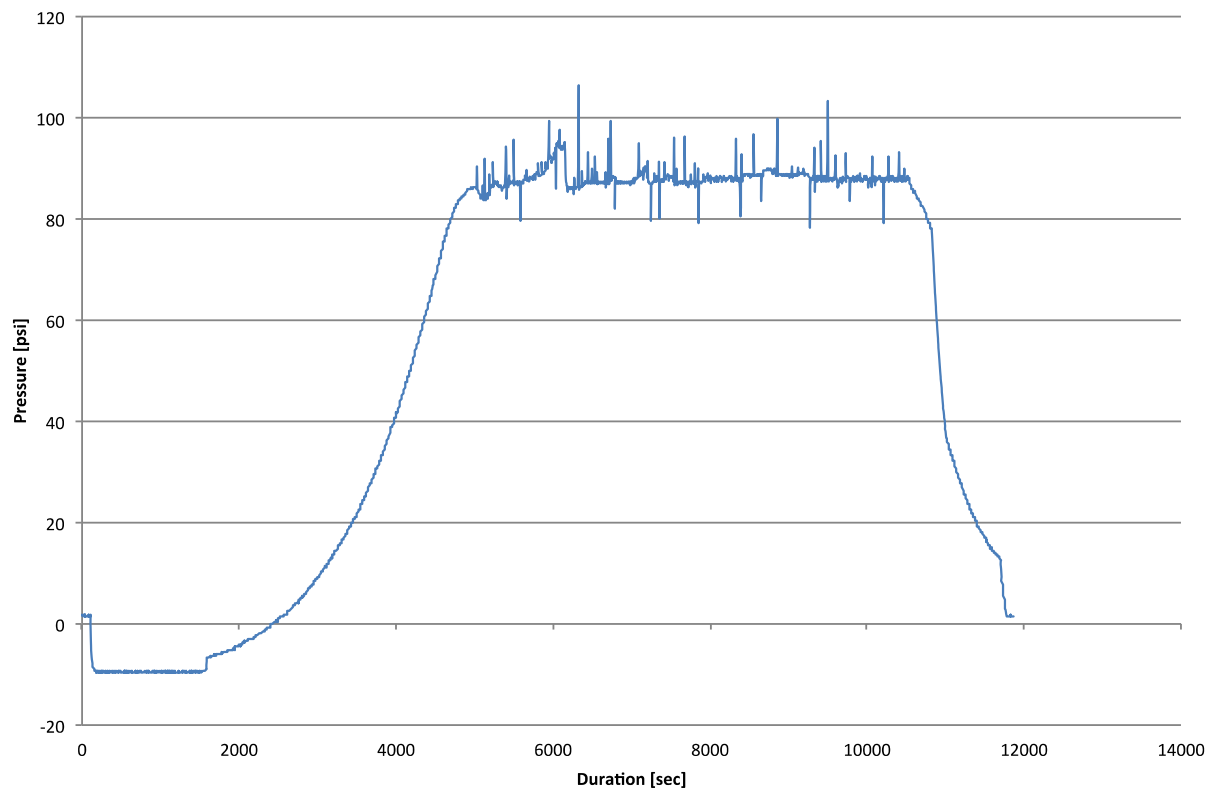


Figure 34. Pressure diagram for Run #7.

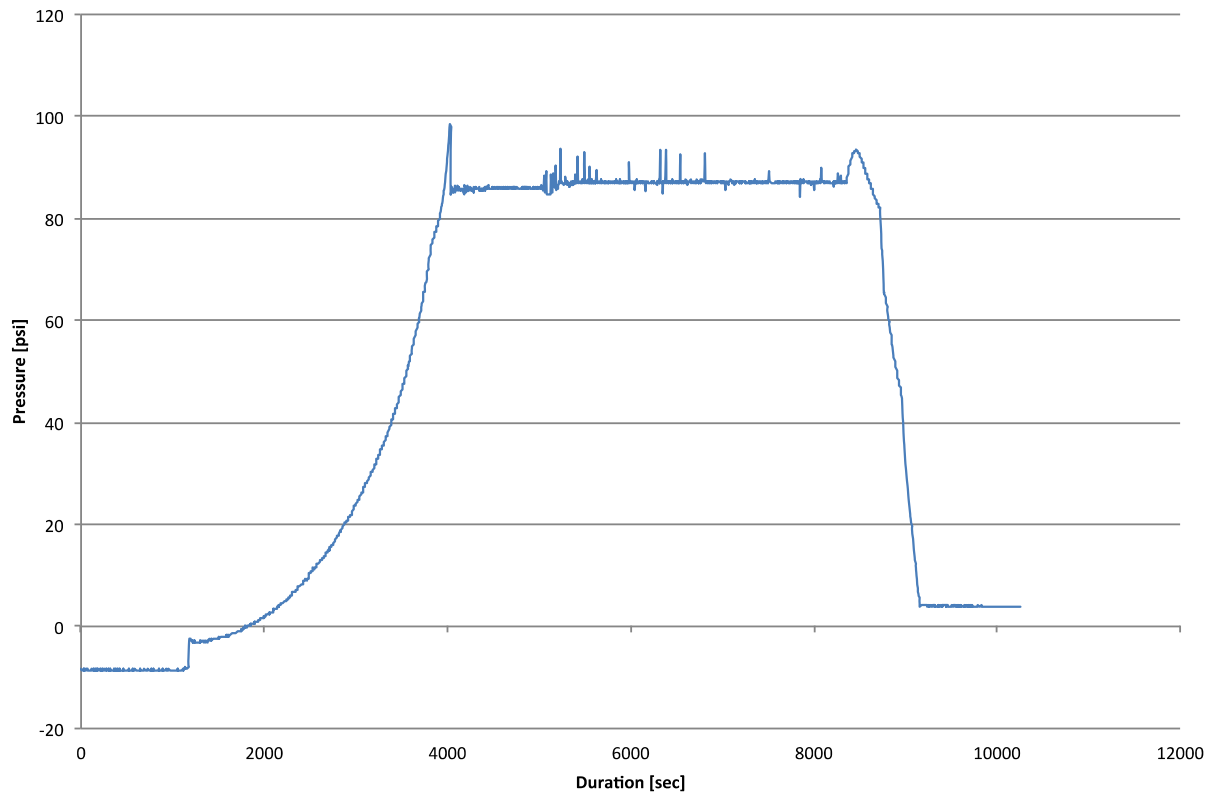


Figure 35. Pressure diagram for Run #8.

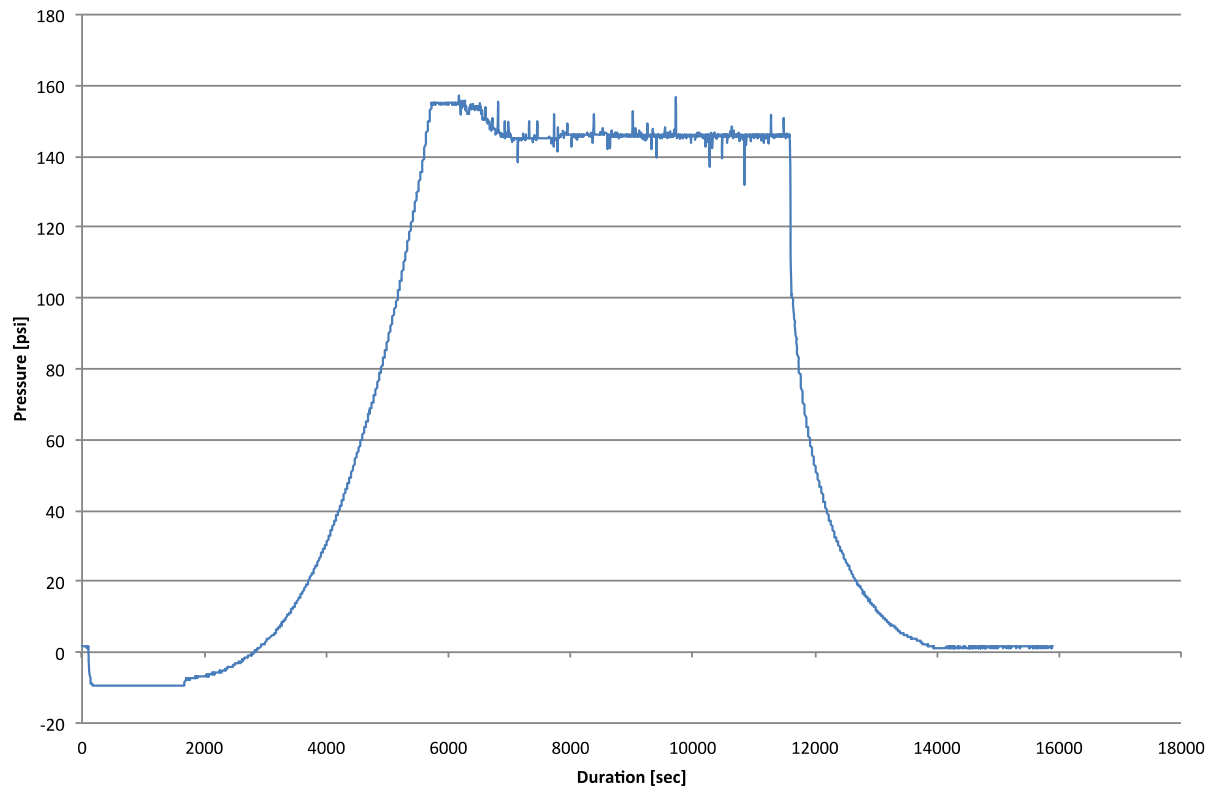


Figure 36. Pressure diagram for Run #9.

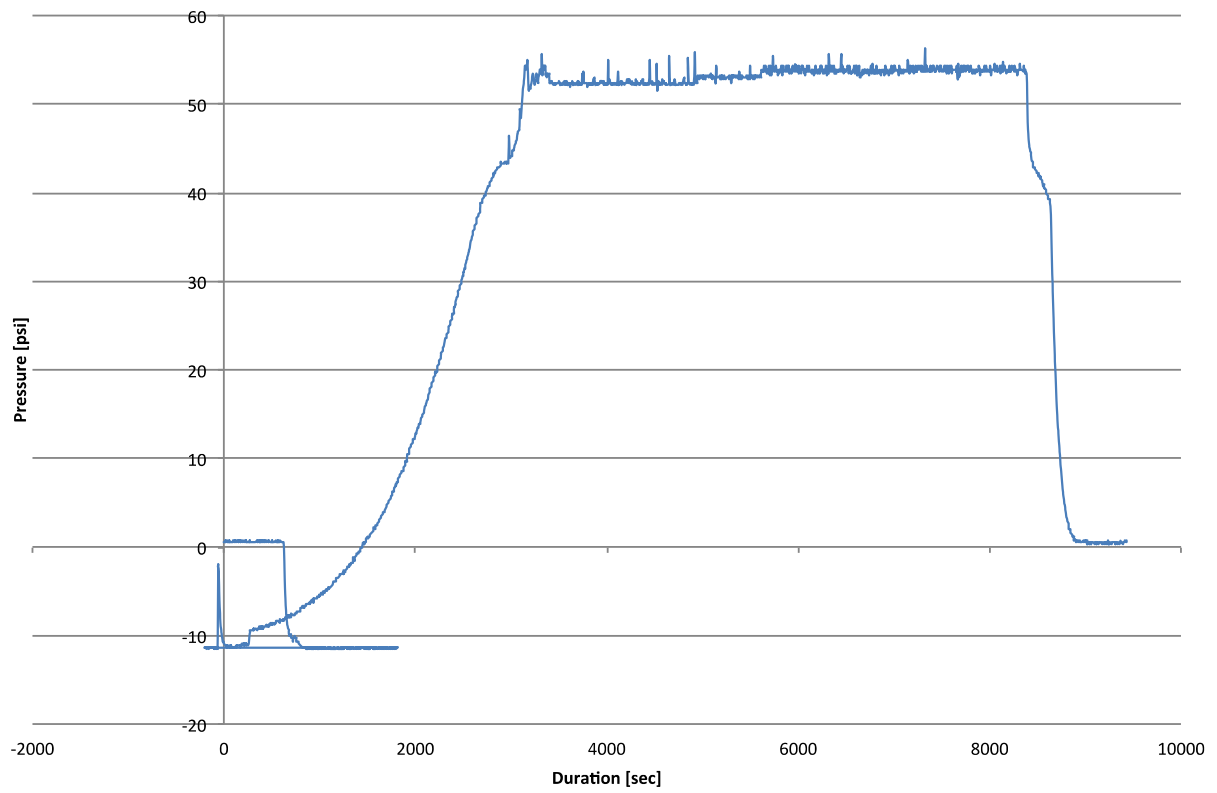


Figure 37. Pressure diagram for Run #10.

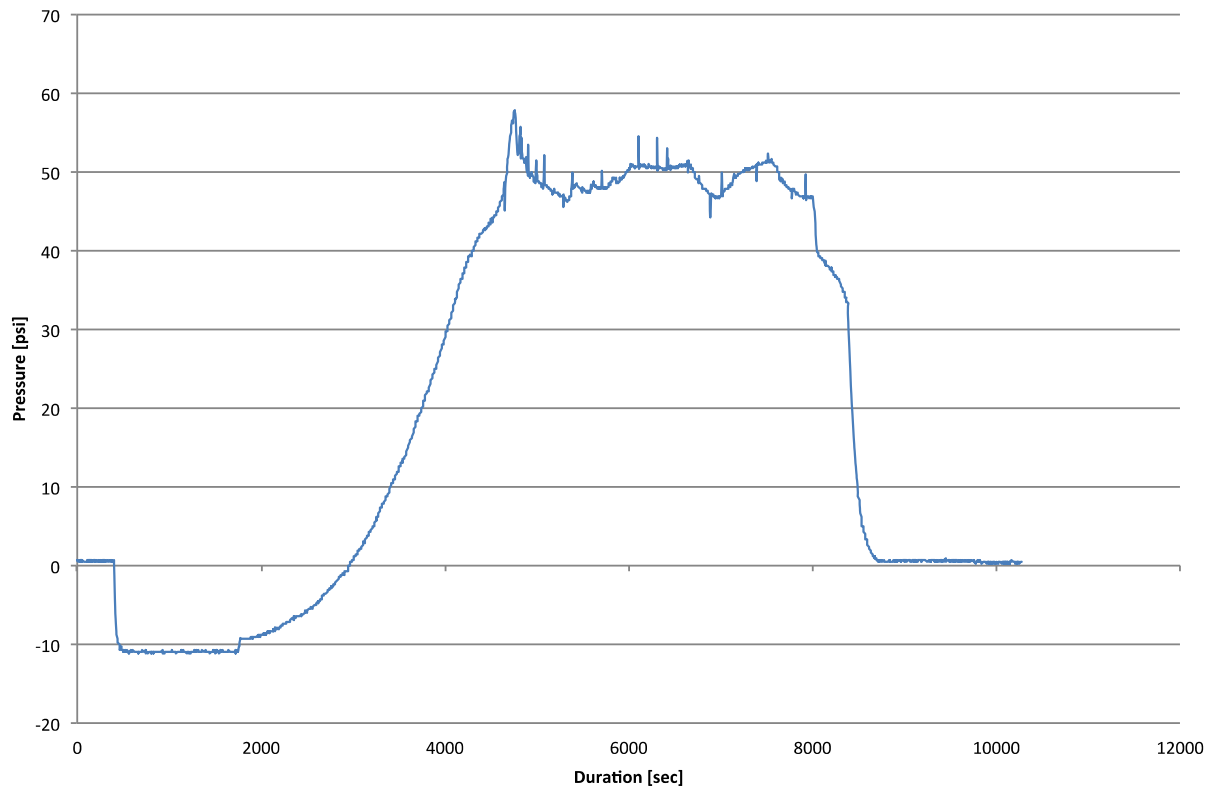


Figure 38. Pressure diagram for Run #11.

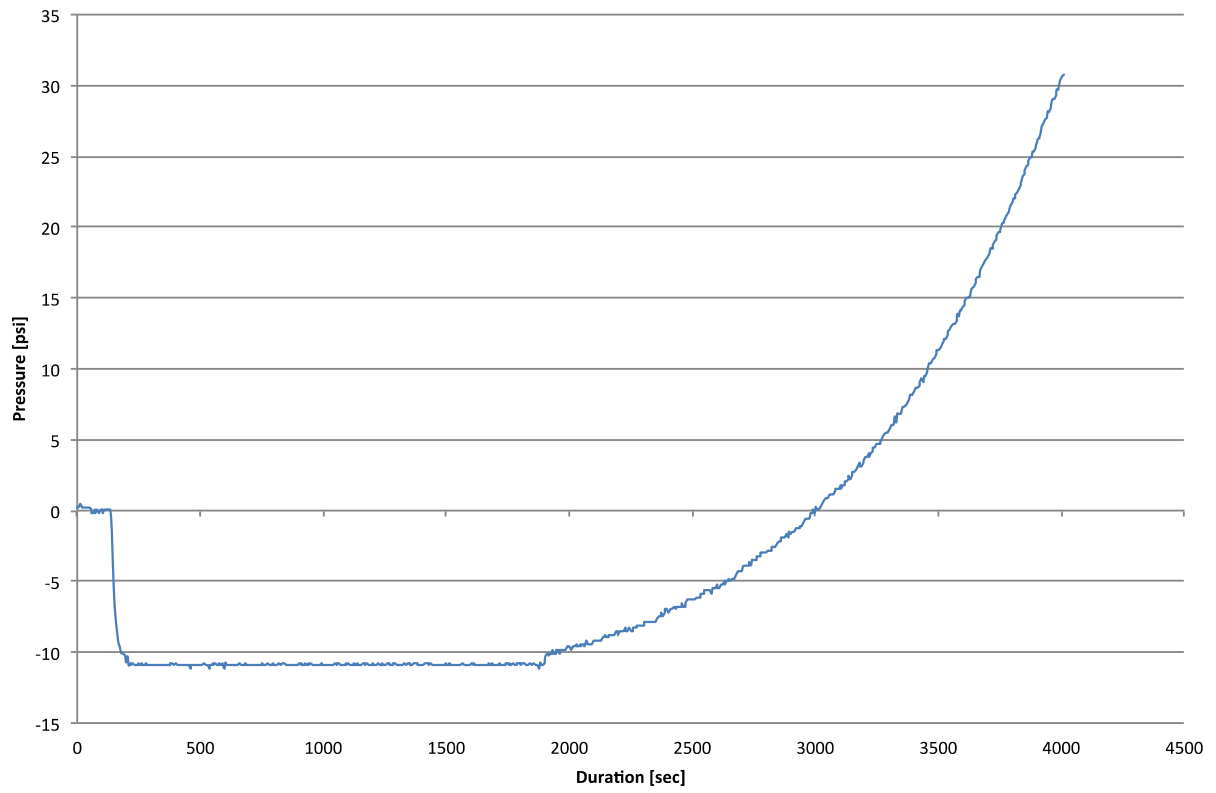


Figure 39. Pressure diagram for Run #12.

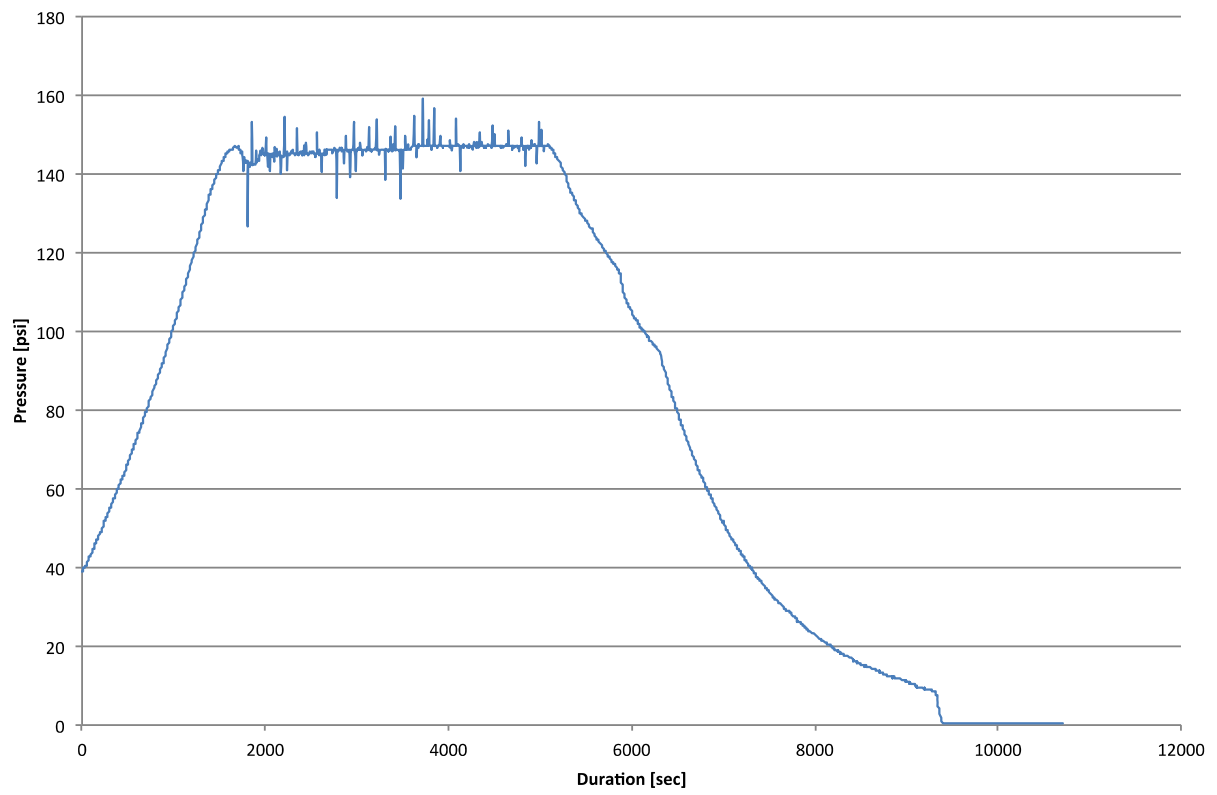


Figure 40. Pressure diagram for Run #13.

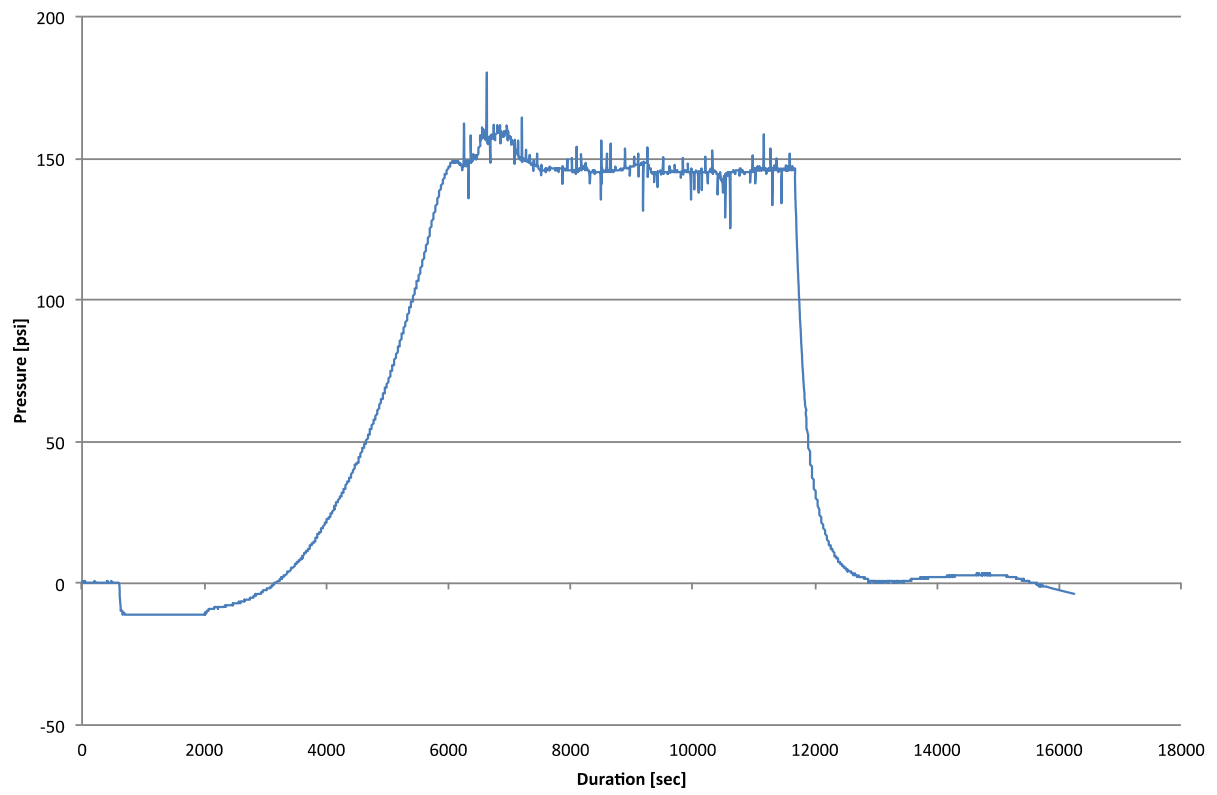


Figure 41. Pressure diagram for Run #14.

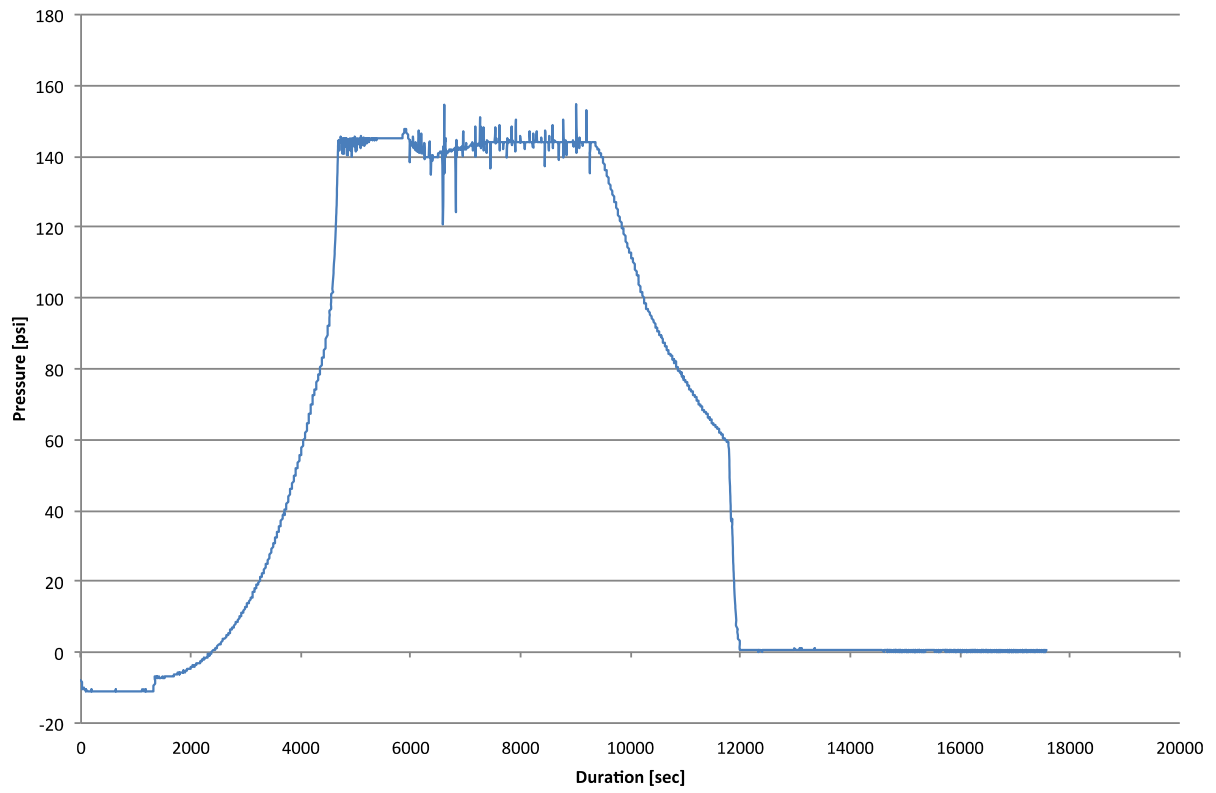


Figure 42. Pressure diagram for Run #15.

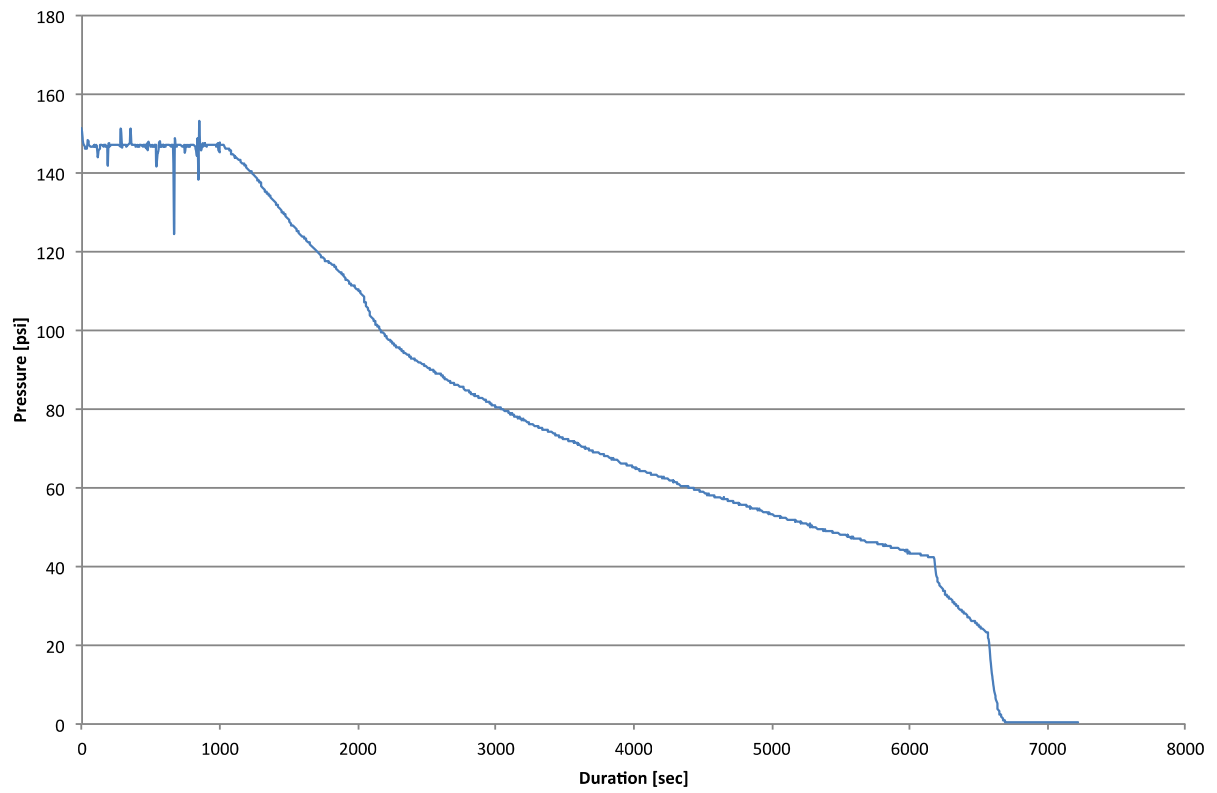


Figure 43. Pressure diagram for Run #16.

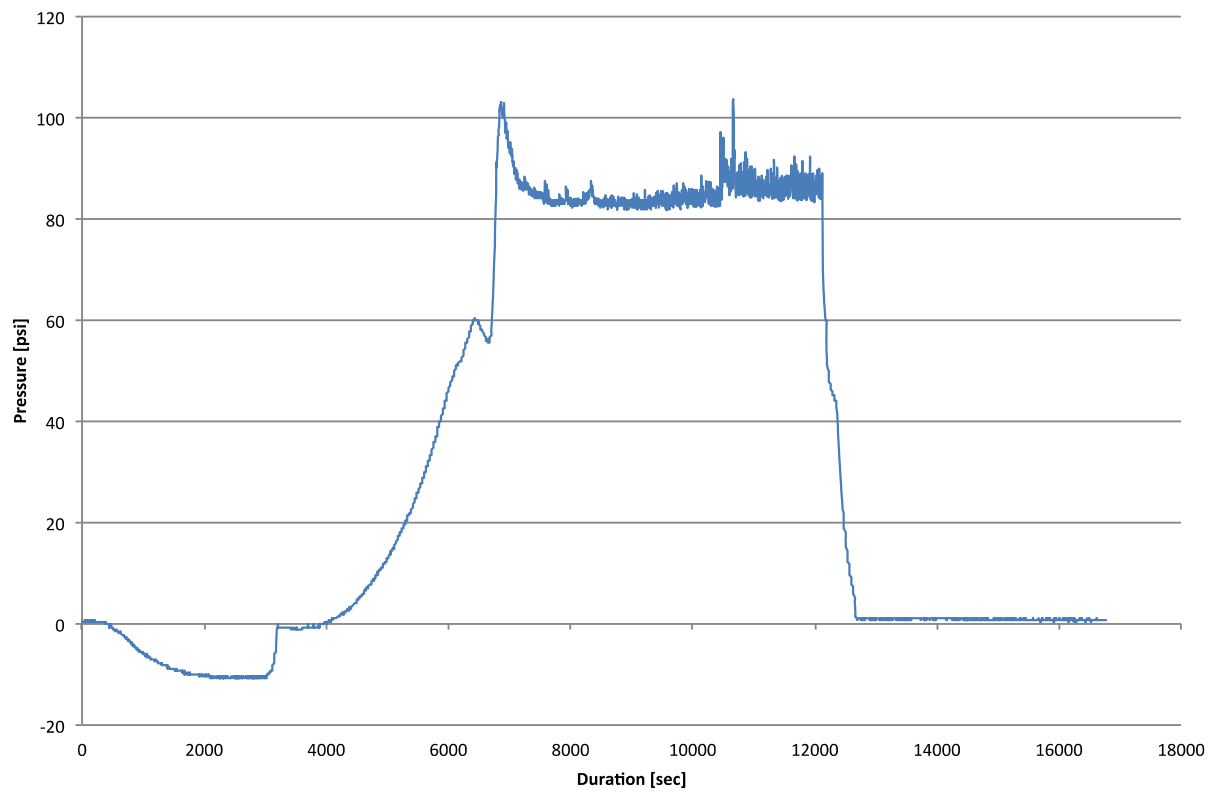


Figure 44. Pressure diagram for Run #17.

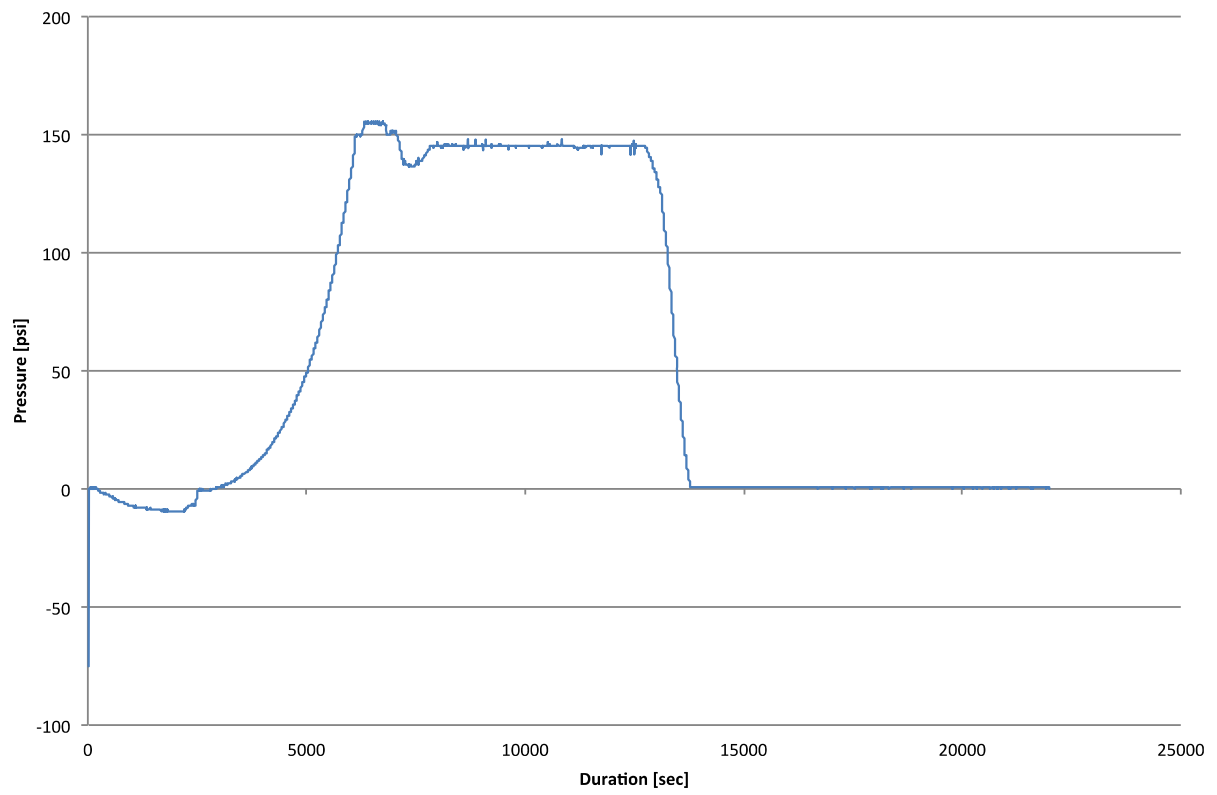


Figure 45. Pressure diagram for Run #18.

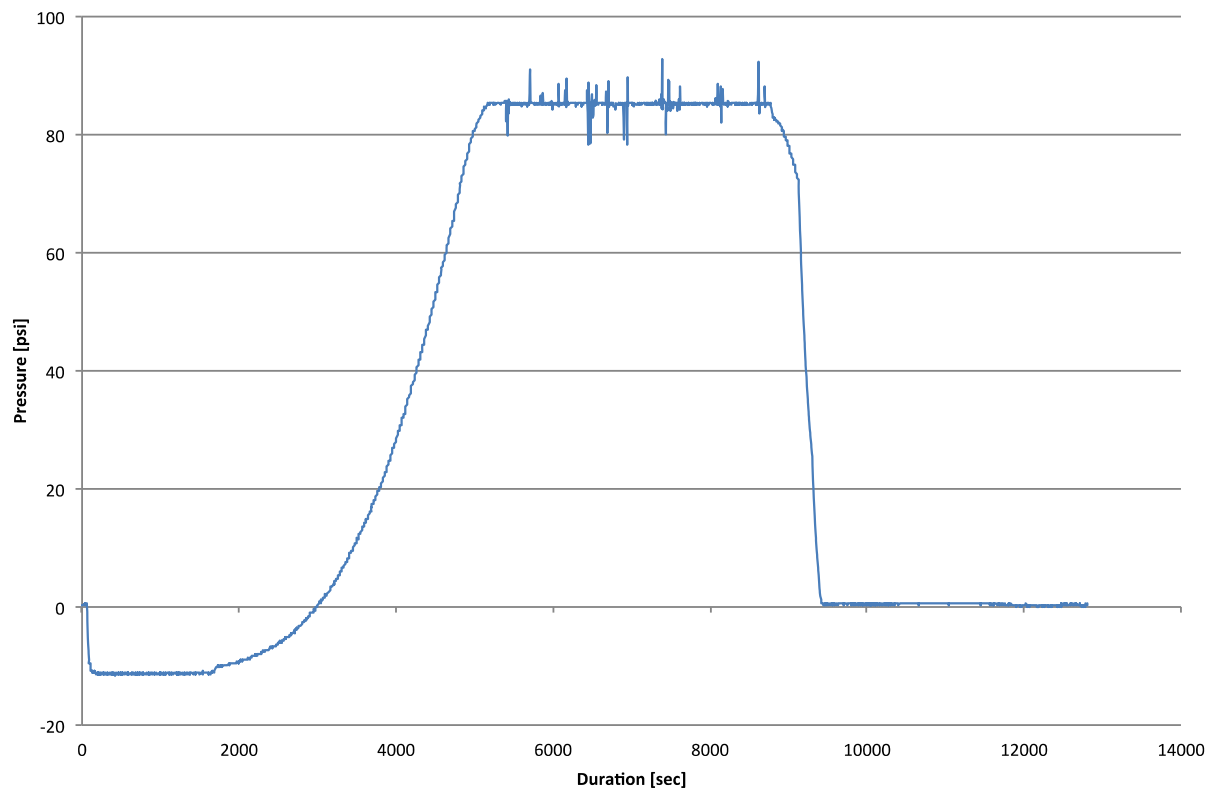


Figure 46. Pressure diagram for Run #19.

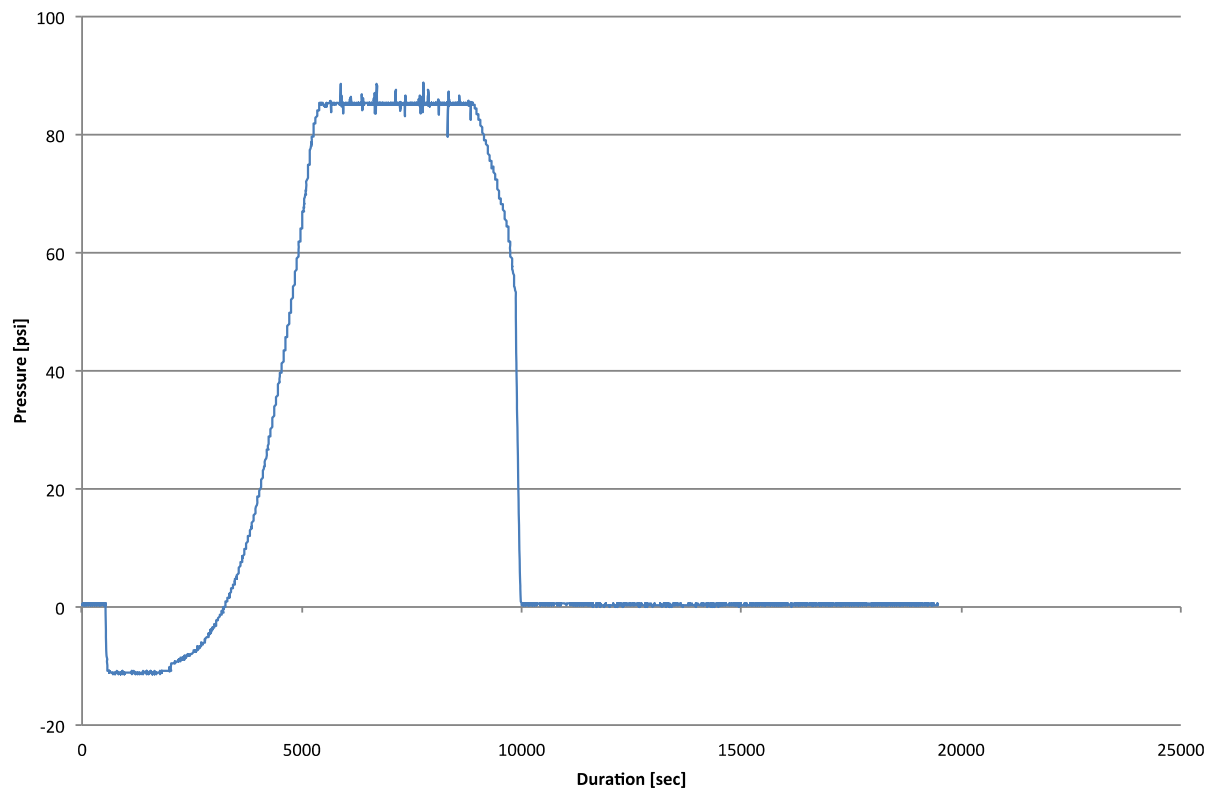


Figure 47. Pressure diagram for Run #20.

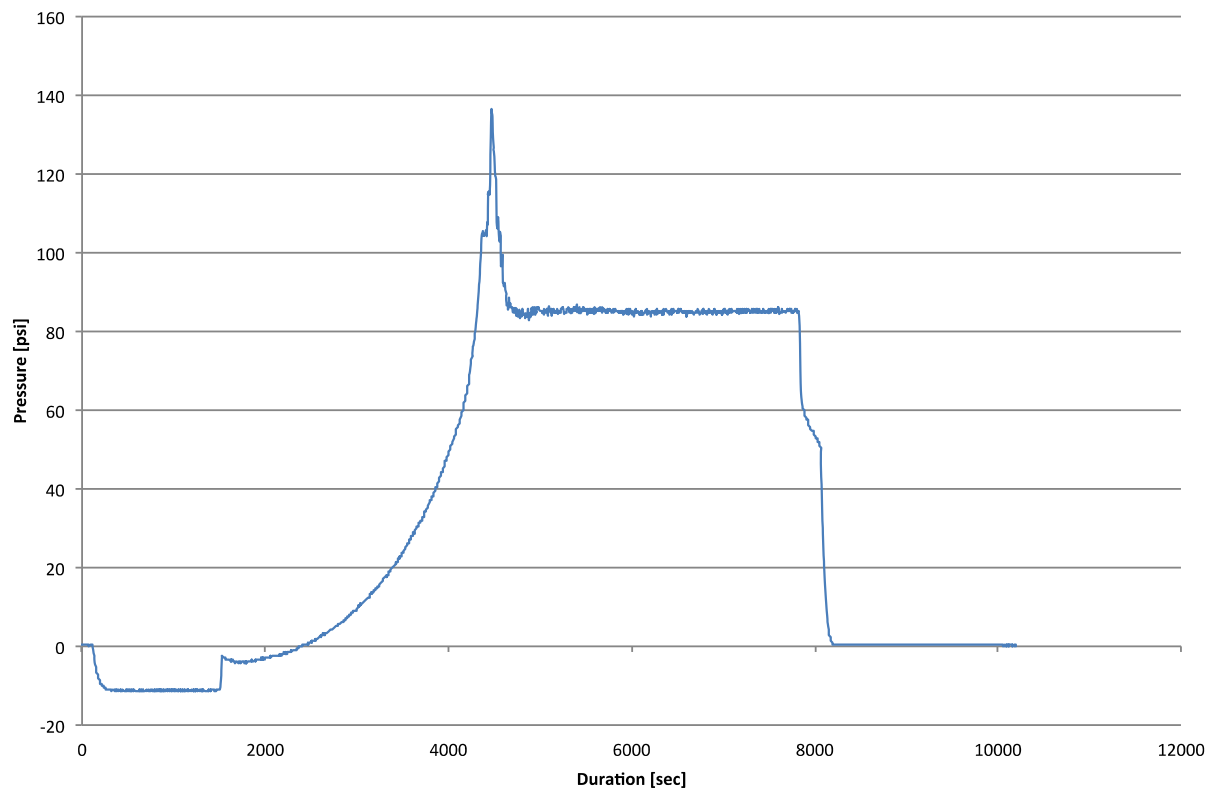


Figure 48. Pressure diagram for Run #21.

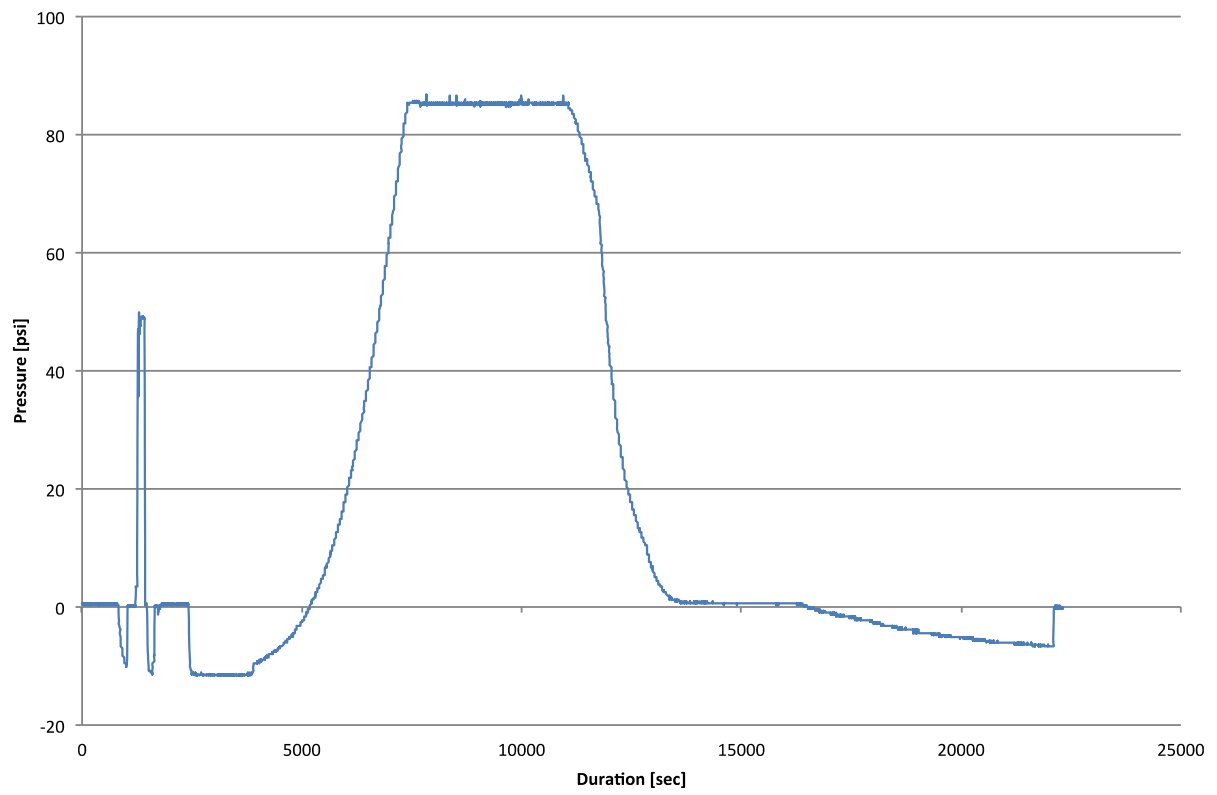


Figure 49. Pressure diagram for Run #22.

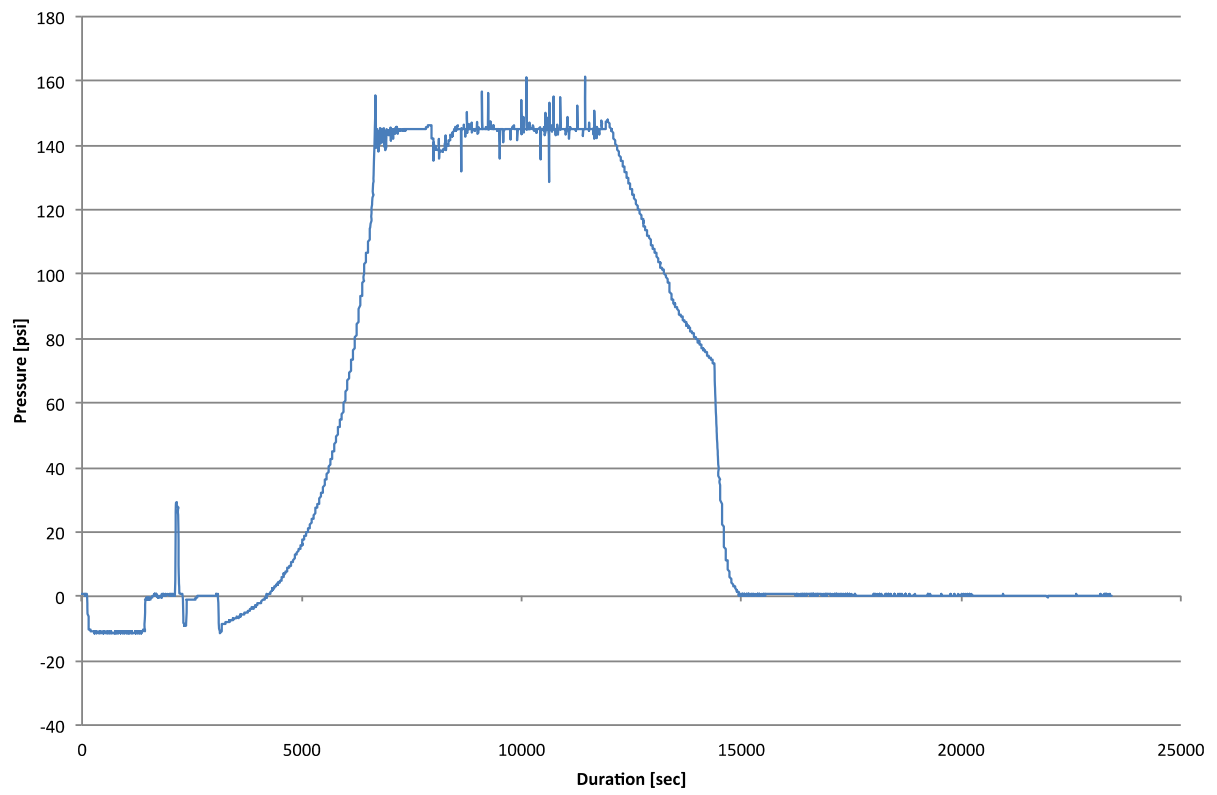


Figure 50. Pressure diagram for Run #23.

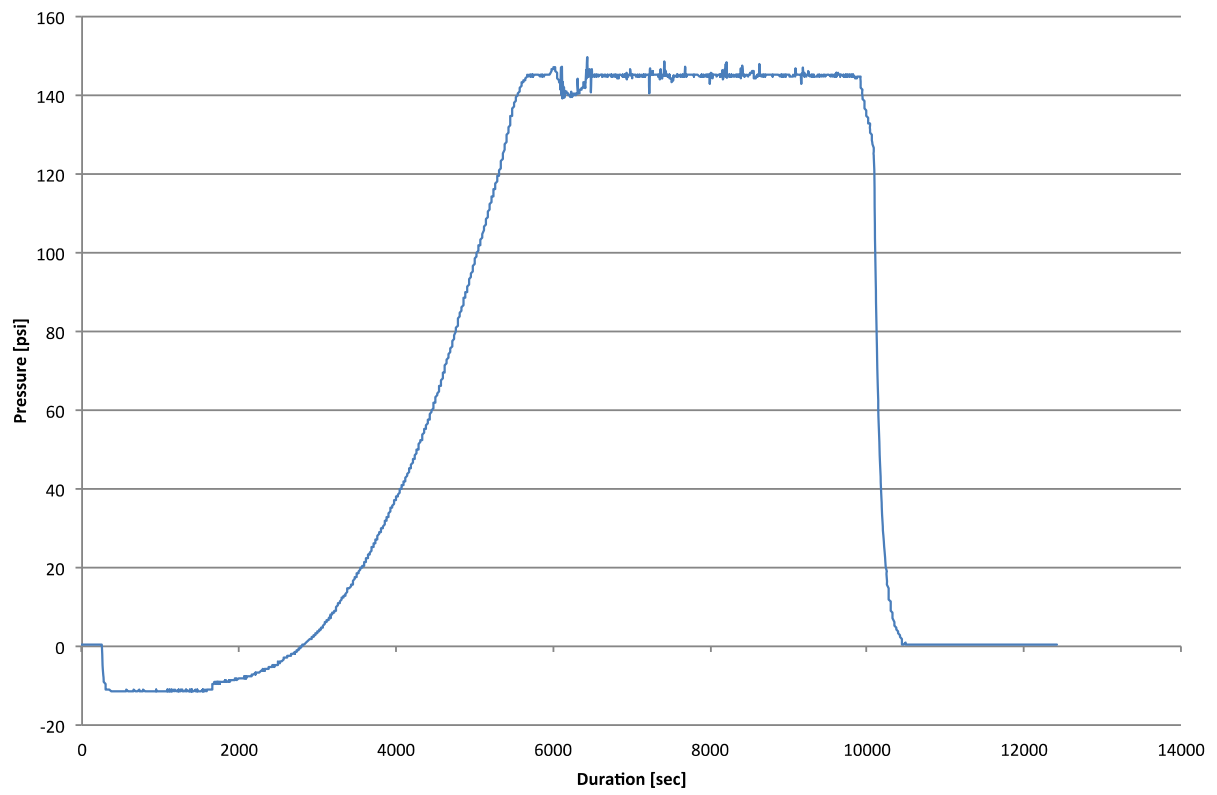


Figure 51. Pressure diagram for Run #24.

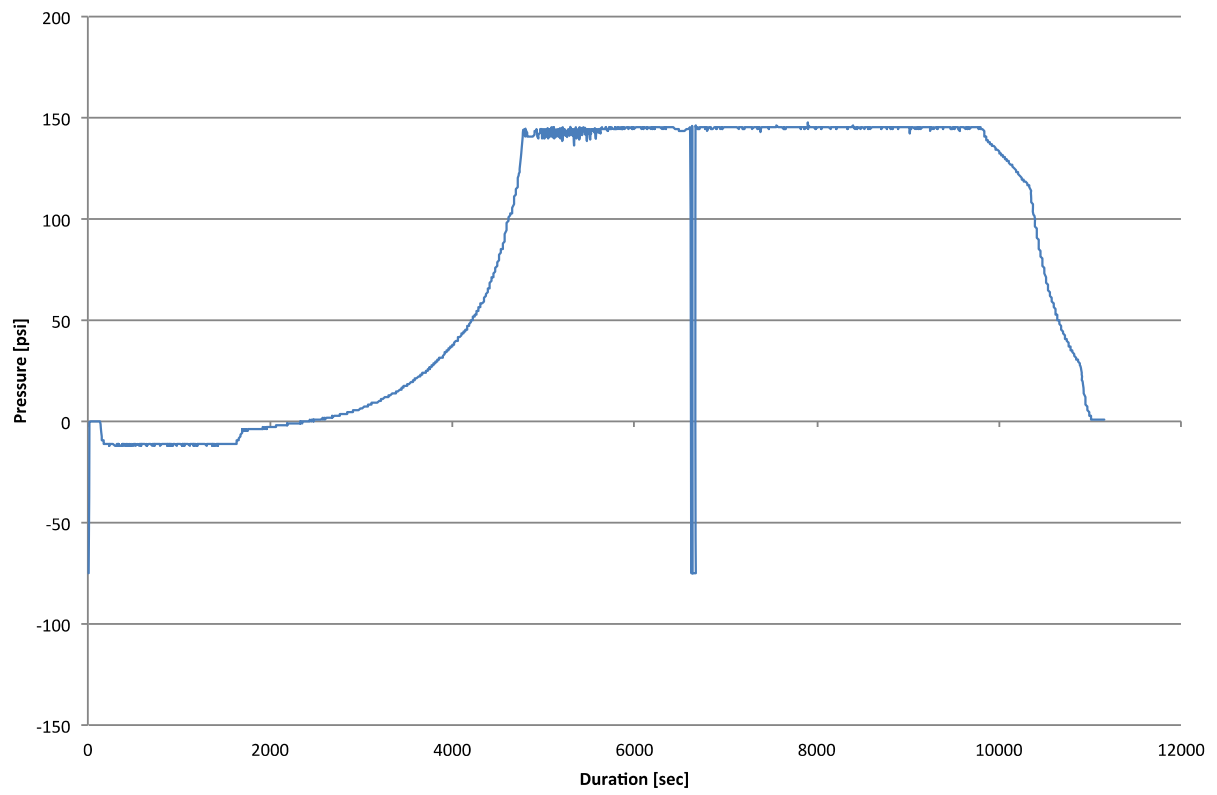


Figure 52. Pressure diagram for Run #25.

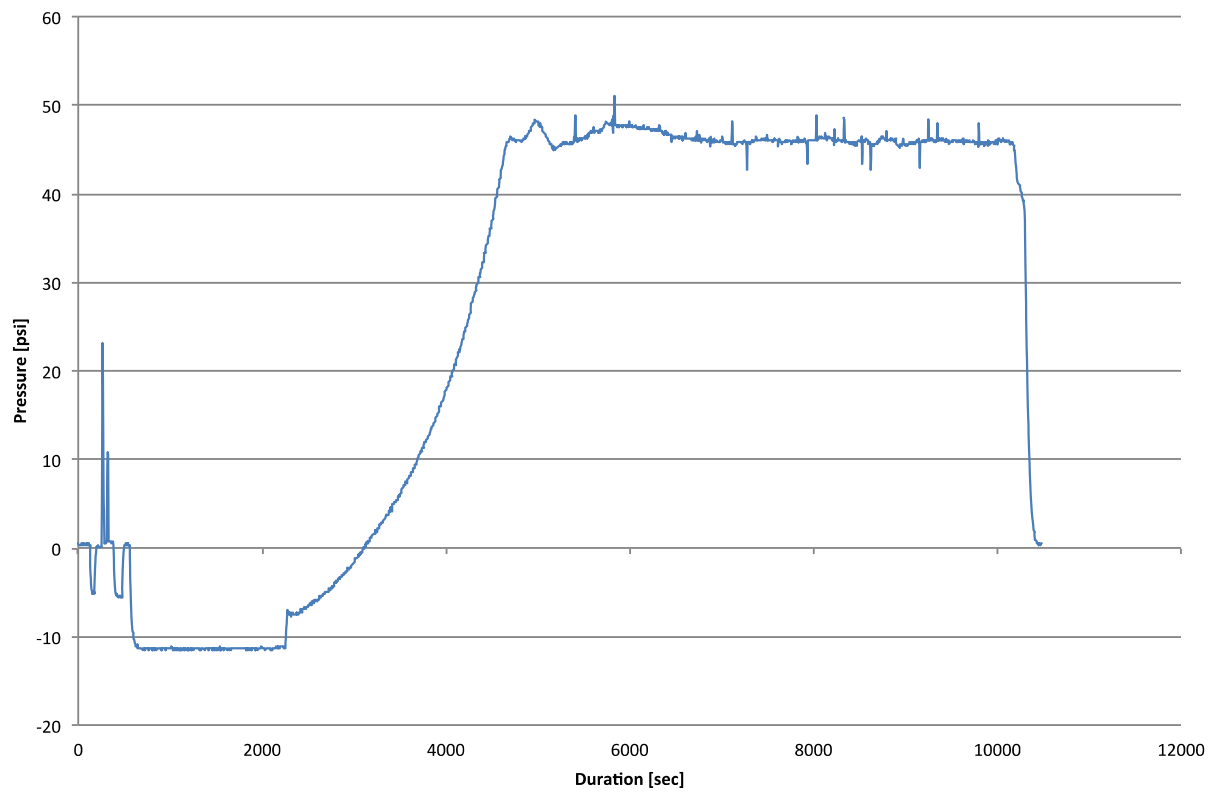


Figure 53. Pressure diagram for Run #26.

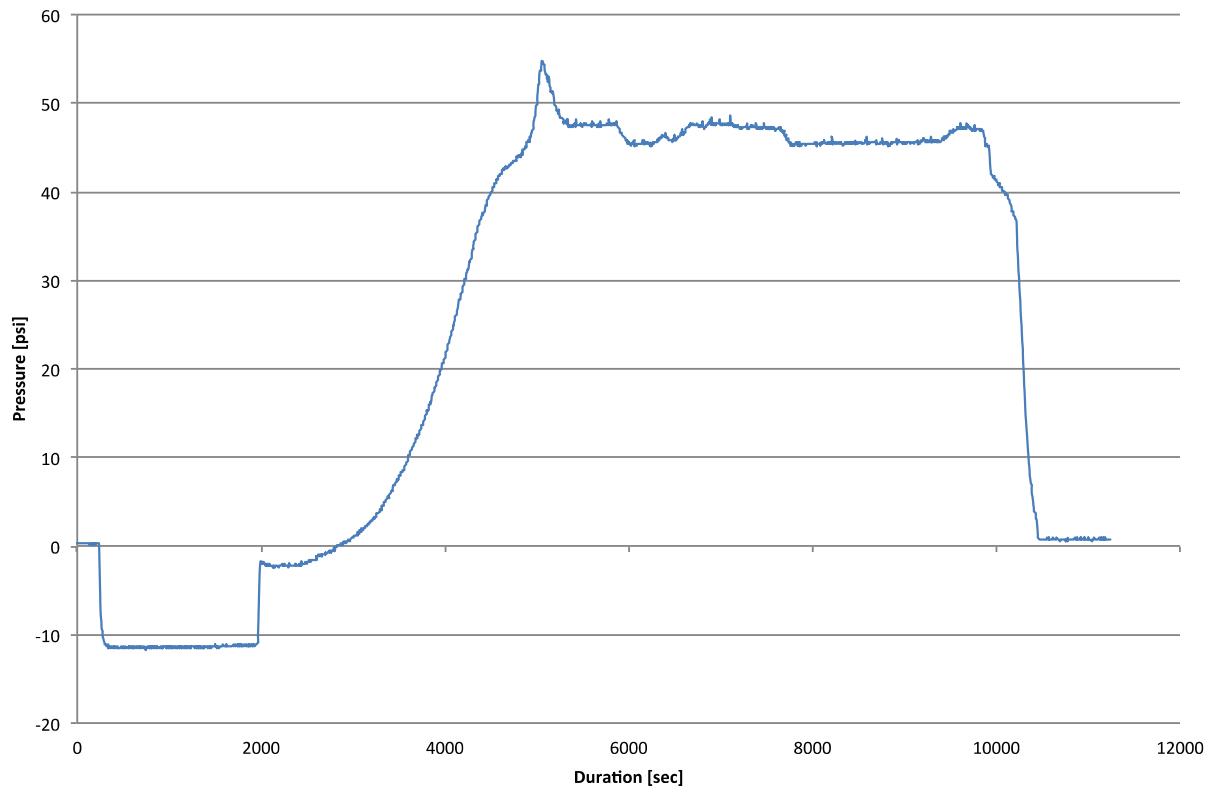


Figure 54. Pressure diagram for Run #27.

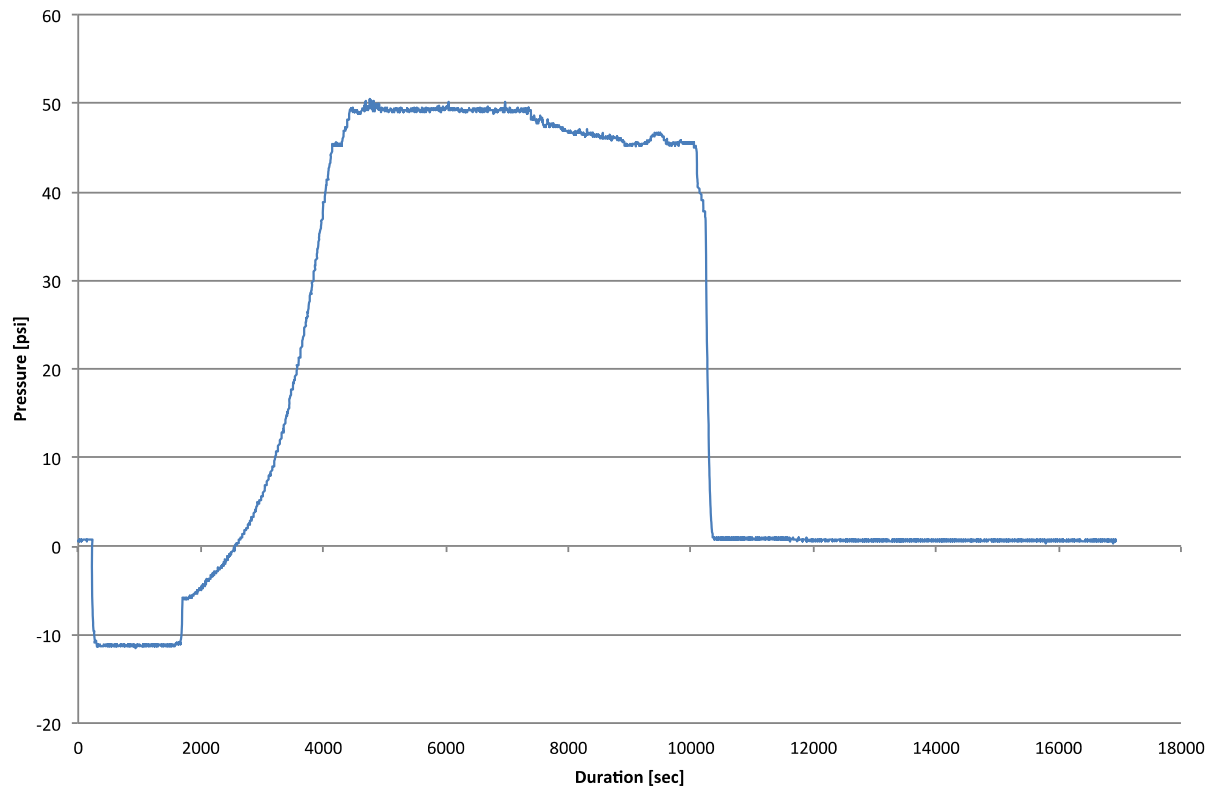


Figure 55. Pressure diagram for Run #28.

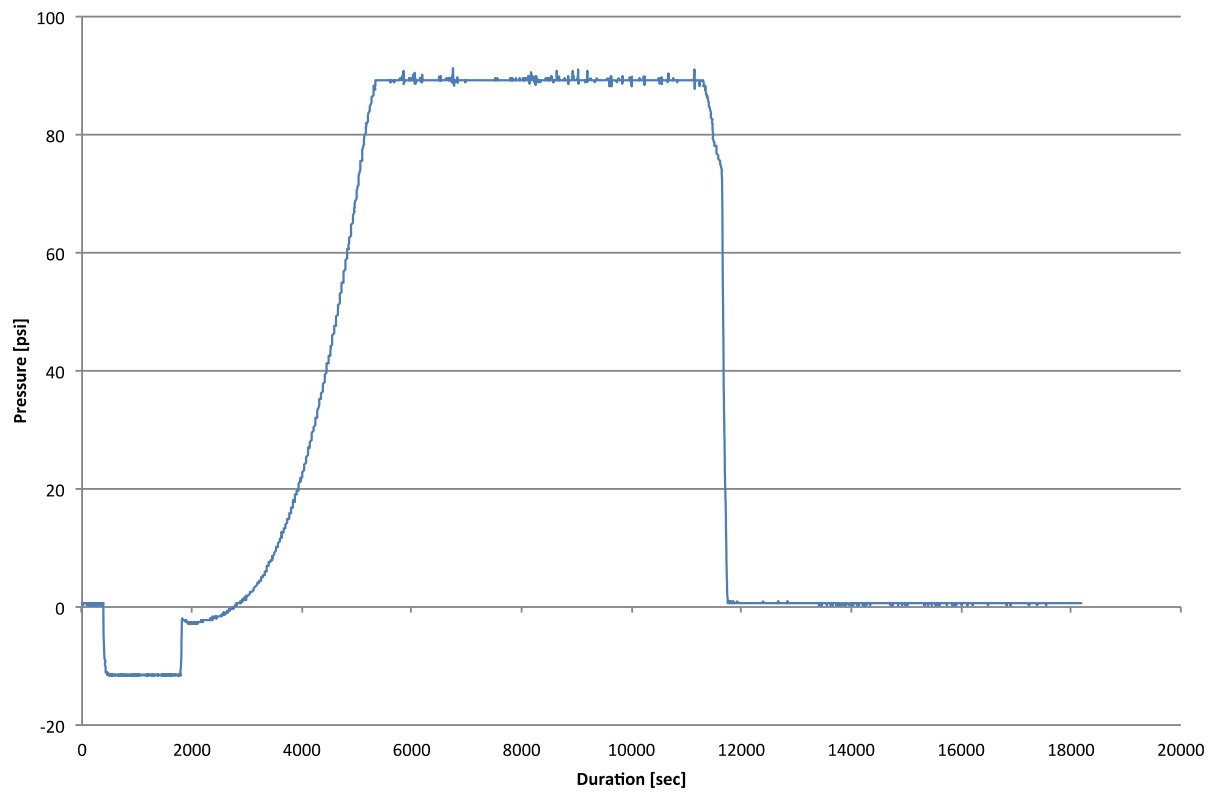


Figure 496. Pressure diagram for Run #29.

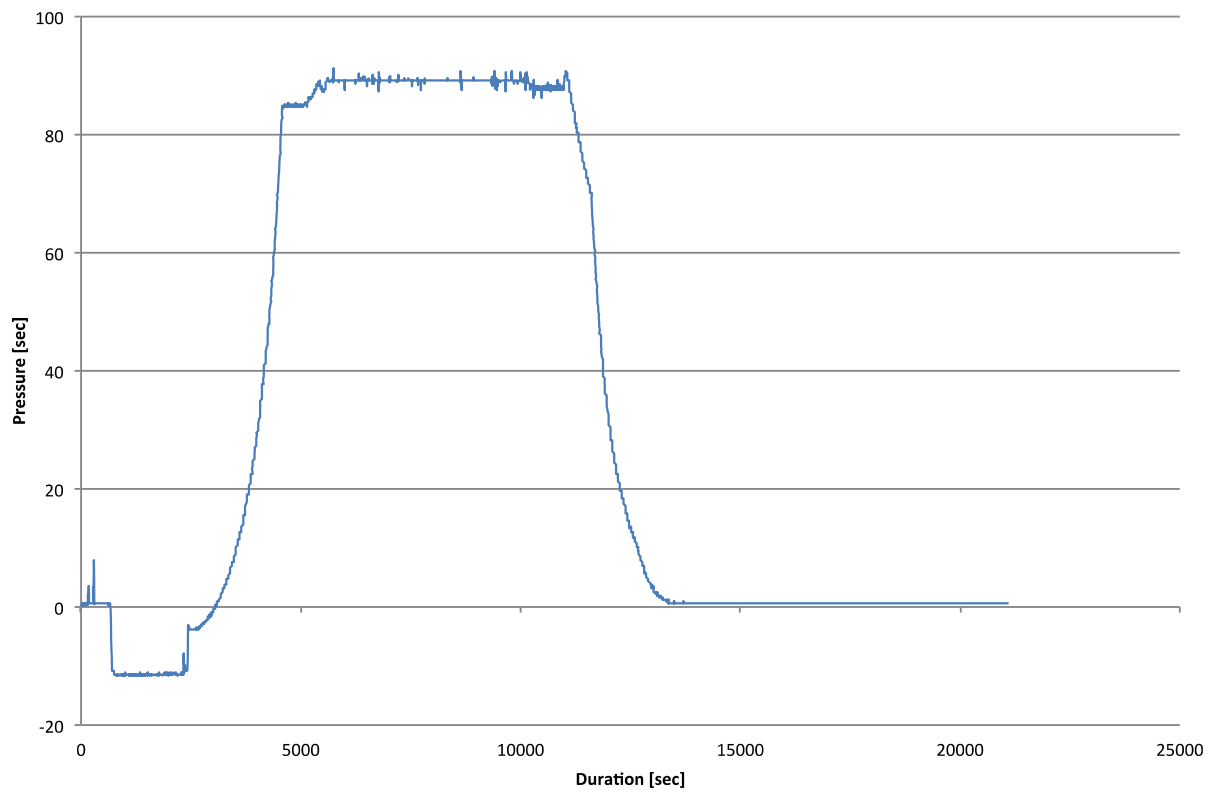


Figure 57. Pressure diagram for Run #30.

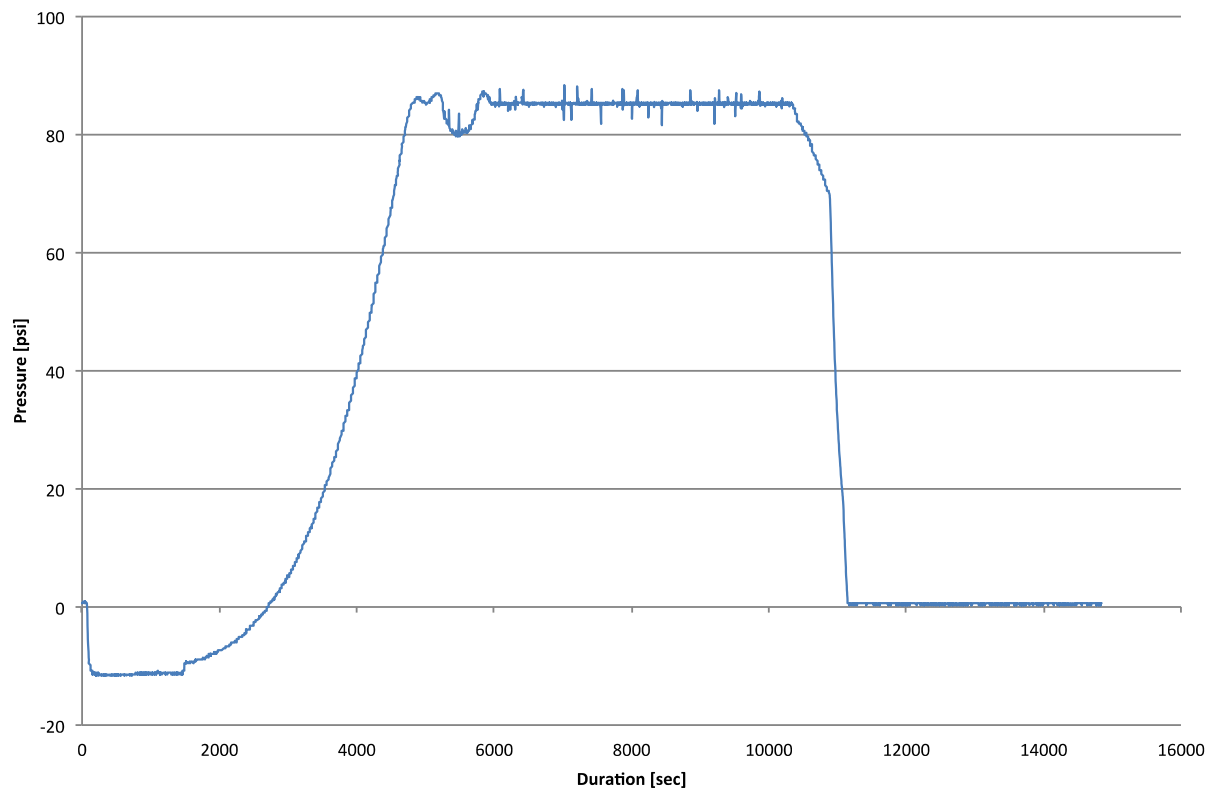


Figure 58. Pressure diagram for Run #31.

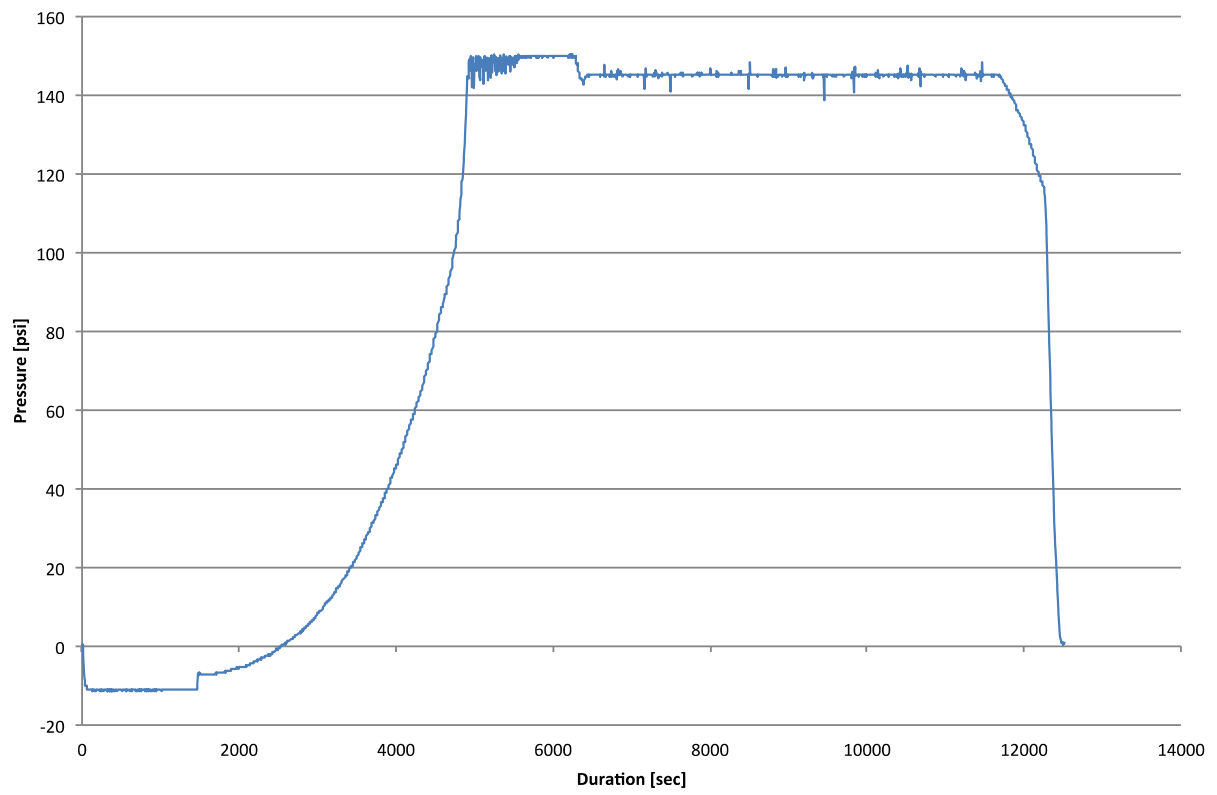


Figure 59. Pressure diagram for Run #32.

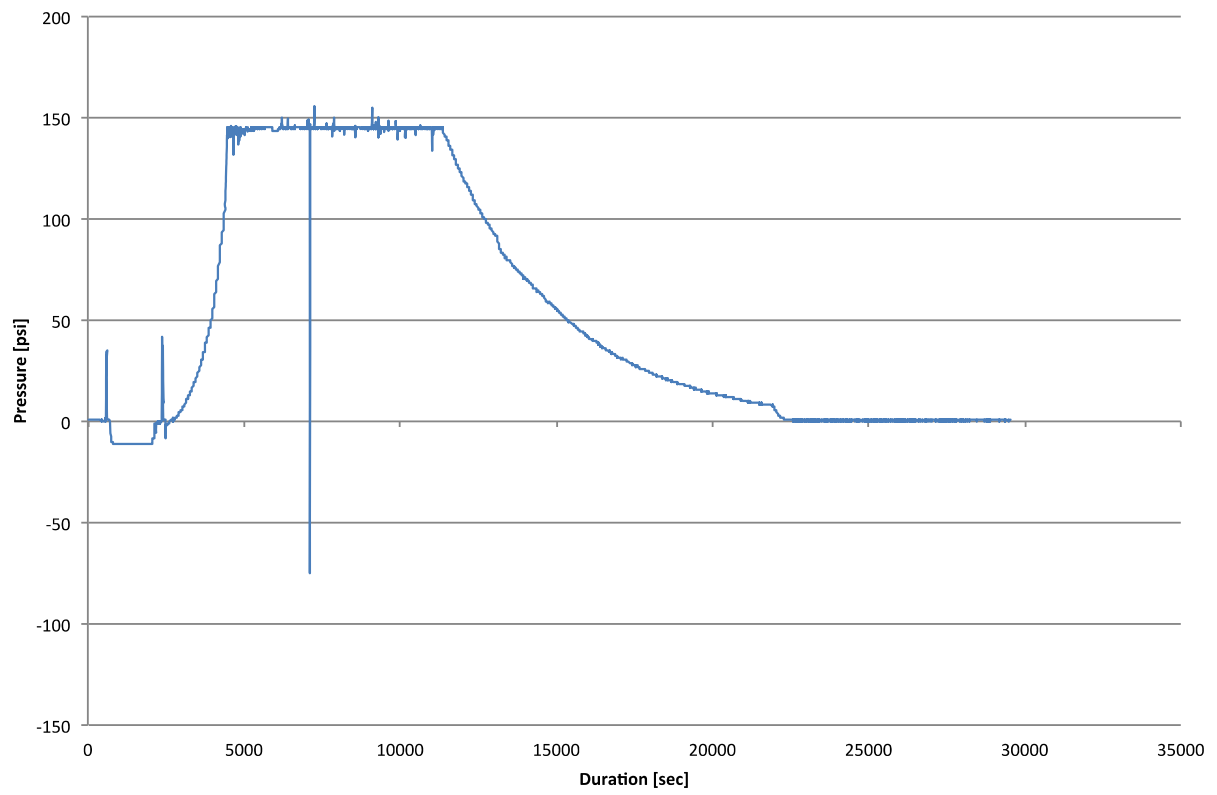


Figure 60. Pressure diagram for Run #33.

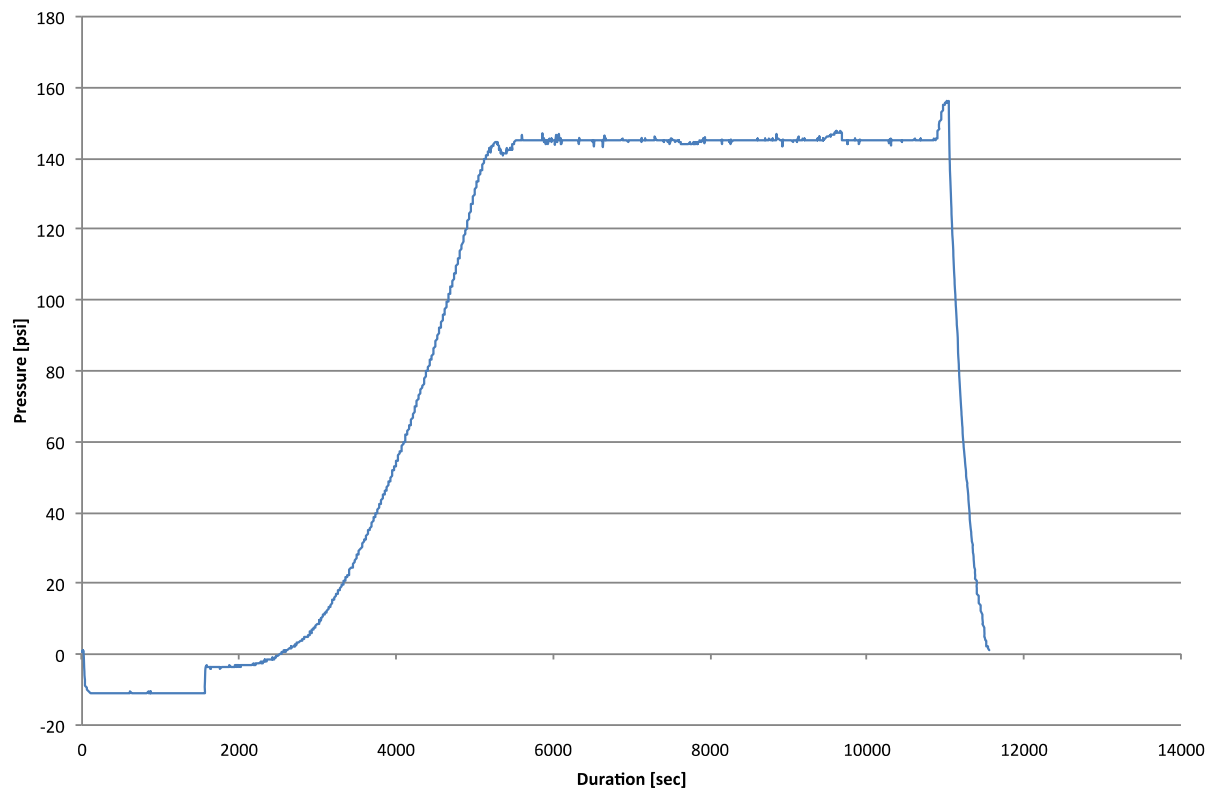


Figure 61. Pressure diagram for Run #34.

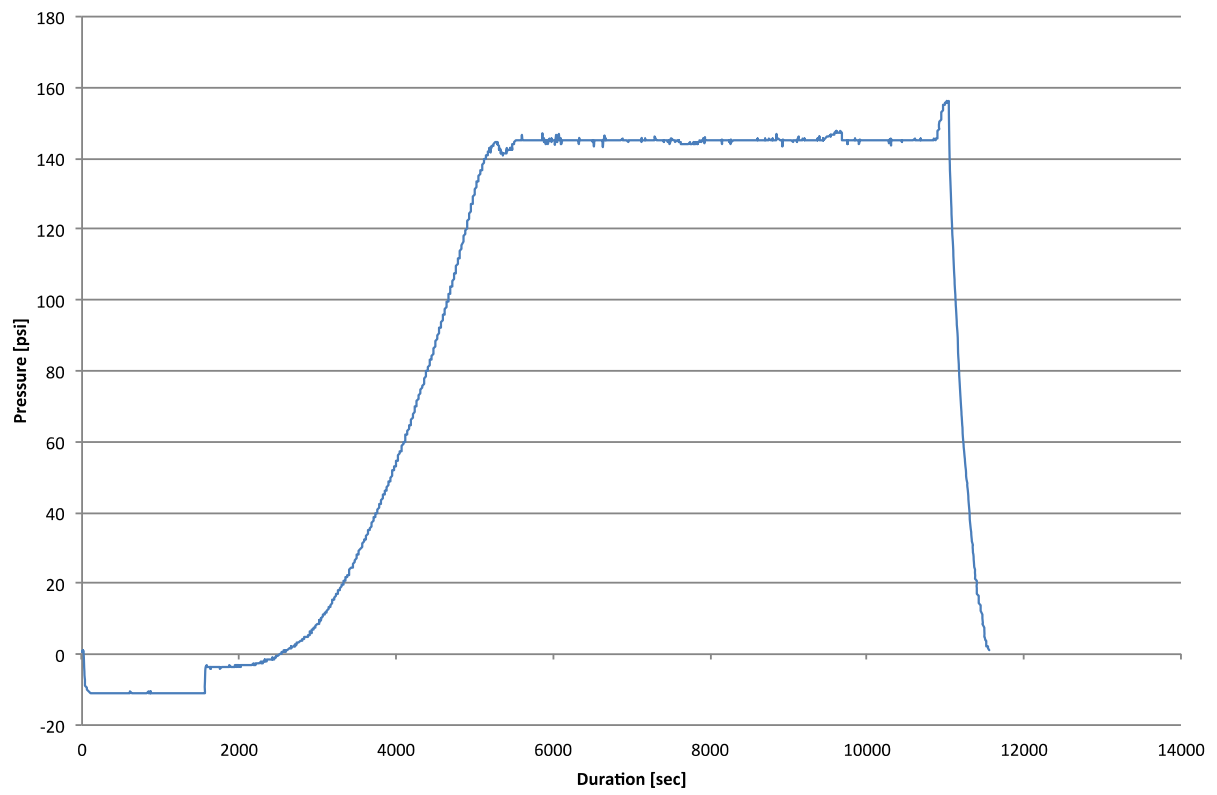


Figure 62. Pressure diagram for Run #35.

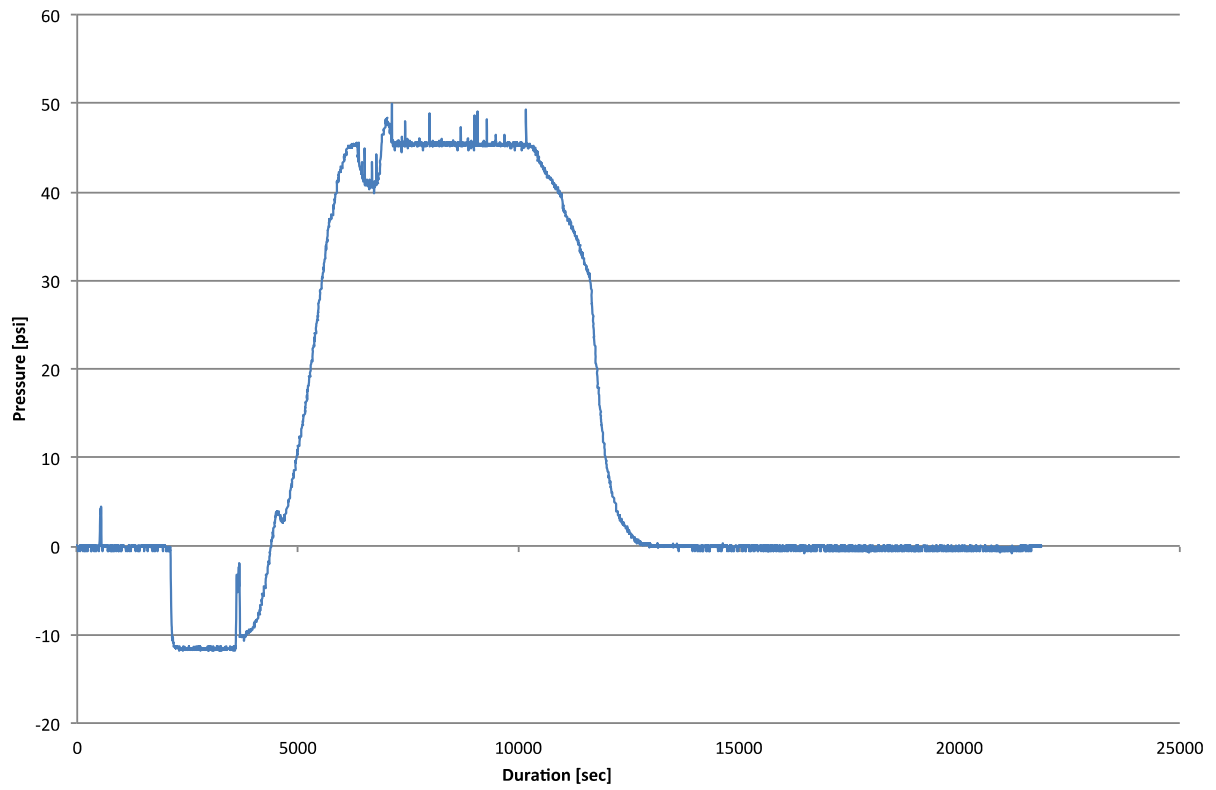


Figure 63. Pressure diagram for Run #36.

VITA

Anton Astner was born in Salzburg, Austria and currently he is a Master student at the University of Tennessee, Knoxville. In year 1993, he graduated from Higher Technical College in Hallein, Austria with concentration in Mechanical Engineering. Subsequently he worked as a technician in the production and planning department for a steel company located in Salzburg, Austria.

In 2009 he graduated from Salzburg University of Applied Sciences with concentration in Forest Products Technology and Management. He designed an undergraduate research thesis evaluating “A Feasibility Study on Energy Supply Conversion to Biomass for a Dairy Plant in Piding, Germany.”

Anton is currently pursuing a Master’s Degree in Forestry with a concentration in Wood Science Technology and Biomaterials in the Department of Forestry, Wildlife and Fisheries, Center for Renewable Carbon under the advisory of Dr. Timothy M. Young and Dr. Joseph J. Bozell. Based on a joint venture program between the University of Tennessee and the Salzburg University of Applied Sciences, introduced by Dr. Young - he is pursuing a Master’s degree at both universities simultaneously. His research is mainly focused on the optimization of the “Organosolv Pretreatment Process” by applying statistical methods.

He plans to graduate from the University of Tennessee with a “Master of Science degree in Wood Science Technology and Biomaterials” in December 2012. Anton is a member of the Forest Products Society (FPS) since 2011.