2009

Dilute Sulfuric Acid Pretreatment of Switchgrass in Microwave Reactor for Biofuel Conversion: An Investigation of Yields, Kinetics,

Oscar Martin
Virginia Commonwealth University

Dilute Sulfuric Acid Pretreatment of Switchgrass in Microwave Reactor for Biofuel Conversion: An Investigation of Yields, Kinetics, and Enzymatic Digestibility of Solids

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Chemical and Life Science Engineering at Virginia Commonwealth University.

by

Oscar L. Martin, Jr.
Master of Business Administration- Tennessee State University, 1999
Master of Science in Chemical Engineering- The University of Tennessee, 1996
Bachelor of Science in Chemical Engineering- The University of Alabama, 1995

Director: Dr. Stephen S. Fong
Assistant Professor, Chemical and Life Science Engineering

Virginia Commonwealth University
Richmond, Virginia
December, 2009

Acknowledgment

The author wishes to thank several people. I would like to thank my wife, Menjiwe, for her love, support and patience during the past four or so years it has taken me to graduate. I would like to thank my parents for their unending love and support. I would also like to thank Dr. Fong for his help and for his direction with this project.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

BTU	British thermal units
CSF	Combined severity factor
HMF	Hydroxy-methyl-furfural
HPLC	High Performance Liquid Chromatography
LAP	Laboratory Analytical Procedure
PSI	Pounds per square inch
SEM	Scanning electron microscope
NPV	Net present value

ABSTRACT

Lignocellulosic materials provide a raw material source for biofuel conversion and offer several advantages over fossil fuels- usage of a renewable resource, reduced greenhouse emissions, a decreased dependence on foreign oil, and stimulation of the agricultural sector. However, a primary technological challenge in converting lignocellulosic biomass into fuel is overcoming the recalcitrance of its matrix to enzymatic hydrolysis. To overcome these problems for chemical processing, naturally occurring cellulose biomass must be pretreated before it can be further processed using enzymatic hydrolysis or bioconversion.

The goal of this work was to develop a model that predicts the glucose yield (pretreatment and enzymatic digestibility) of dilute acid pretreated switchgrass as a function of pretreatment process conditions (acid loading, 0-1.5 vol%, temperature, 165-195^{o}C, and residence time, 1-10 min). This project was the first study that used a multi-variable design experimental series to directly compare the pretreatment effectiveness (product yield, biomass composition and appearance, pH, etc) of using conventional and microwave heated reactors.

Microwave-pretreated switchgrass afforded up to a 100% higher total glucose yield (combined pretreatment and enzymatic-hydrolysis liquor yields) at equivalent pretreatment severity and at one tenth of the reaction time, relative to conventional

pretreatment. Under best pretreatment conditions of 0.75 vol% acid, 195^{o}C, 1 min residence time, 99% glucose yield and 99% hemicellulose removal were achieved.

Kinetic parameters were estimated for the cellulose and xylan hydrolysis reactions in the pretreatment liquor and the solid residue. The kinetic model gave an average correlation coefficient of 0.93 for all reactions. In addition, the combined severity factors (CSF) were also determined for each experiment. Highest observed enzymatic glucose yield corresponded to a CSF of 1.7.

A mass and energy balance, and economic analysis based on production scale was developed for both reactor systems. The microwave pretreatment process theoretically yielded 48% more ethanol relative to the conventional process. For microwave pretreatment to be commercially viable, two criteria must be met. One, the cost for large-scale continuous microwave reactors would need to be significantly lower than current estimates. And second, higher solids content must be used ($\geq$20 wt% in the slurry) to maximize output.

1.0 BACKGROUND AND SIGNIFICANCE

Gasoline is a petroleum-derived liquid mixture consisting of 5-to-12-carbon hydrocarbons, including parrafins, naphthenes, aromatics, olefins, and hazardous chemicals (5 to 35 percent by volume) such as benzene (to increase the octane rating), toluene, naphthalene, trimethylbenzene, and methyl *tert*-butyl ether (MTBE) (Kaufmann and Shiers, 2008).

Global petroleum consumption has reached 84,035,000 barrels per day, with U.S. petroleum consumption at 20,802,000 barrels per day. Current U.S. motor gasoline consumption is 384.7 million gallons per day, or 140 billion gallons annually. The US is set to consume 290 billion gallons of gasoline a year in cars and trucks by 2050. Inflation adjusted gasoline prices have skyrocketed from $1.35 to $3.22 per gallon from 1998 to 2008. (Energy Information Administration, 2008)

Worldwide energy consumption for 2007 was approximately $5x10^{17}$ BTUs (British Thermal Units) according to the Energy Information Administration (Energy Information Administration). The US accounts for about 27% of this consumption (Energy Information Administration, 2008a). The agency projects global energy consumption to surpass $7x10^{17}$ BTUs by 2030. More than 50% of the projected increase in global energy demand over the next twenty years is attributed to the growing economies of China and India, which currently account for approximately 18% of global

energy consumption. This increase offsets the 17% projected decline in the US share of global energy consumption by 2030. (Energy Information Administration, 2008)

In the long term, fossil fuels are not projected to satisfy the growing global energy demand. Many industry experts predict that the world will face a "peak oil" situation within the current century. Estimates on the data for "peak oil" vary from 2010 to 2030. Models by Campbell and Laherrere (1998), USGS (2000), IEA World Energy Outlook (Energy Information Administration, 2008), and Jackson (2007) alternatively project peak oil to arrive by 2010, 2023, 2030, and after 2030, respectively. Differences in the estimated dates for peak oil result from varying estimates of the magnitude of untapped reserves. Current estimates for crude oil long-term availability range from 0.8 to 2.9 $x10^{12}$ barrels (Kaufmann and Shiers, 2008).

There is tremendous interest in the commercialization of alternatives to petroleum-derived fuels. This is a direct result of the increasing global energy demand, uncertainty of crude oil supplies, and environmental impacts from the use of these fossil fuels. In addition, there is also concern about US dependence on the use of foreign oil supplies and the price fluctuations caused by geo-political situations. One example is the 1973 Arab oil embargo, which resulted in spikes in crude oil prices four times over a 12-month period. This resulted in a US recession, and a 3% decline in the US gross domestic product (Hirsch, 2008).

Studies have shown that global climate change is a result of forced warming due to greenhouse-gas emissions (Hegerl et al., 2007). These greenhouse gases (i.e., carbon dioxide, methane, and nitrous oxide), account for more than 50% of the overall greenhouse effect and are liberated by fossil fuel combustion (Schnoor, 2005). Therefore, the projected increase in energy demand will result in an increased use of fossil fuels and greenhouse emissions. Carbon dioxide emissions are projected to increase from $2x10^{10}$ tons in 1990 to over $4x10^{10}$ tons by 2030 (Energy Information Administration, 2008a). Sulfur and nitrous oxide emissions are other byproducts of fossil fuel combustion. These gases are major contributors to acid rain, which is harmful to freshwater sources, forests, soils, and buildings, in addition to adversely affecting human health (Demirbas, 2004).

Coal and crude oil together represented over 60 percent of domestic energy consumption in 2007. Approximately 60% of the total crude oil in the US is refined into motor gasoline. Renewable energy represents less than eight percent, with only half obtained from biomass. However, 9.2 percent of energy usage in Europe is derived from renewable resources, with some countries using as much as 41 percent. (Energy.eu, 2006). The Department of Energy (DOE) and the US Department of Agriculture (USDA) have both reported that over $1x10^{19}$ tons of biomass can be harvested to displace up to 30 percent of current fossil fuel usage (Perlack et al., 2005).

A comprehensive renewable energy plan is necessary to the meet the projected global energy usage and address environmental concerns associated with fossil fuels.

Renewable energy sources such as biomass, geothermal, hydroelectric, solar, and wind are important parts of an environmentally sustainable energy plan.

Biofuels (e.g., bioethanol, biodiesel, and biobutanol) play a key role in this energy plan. Biofuel are produced by the process of converting organic matter into a combustible fuel as a replacement for fossil fuel. This replaces oil and natural gas, focusing on the use of organic matter in the efficient production of liquid and gaseous biofuels, which yield high net energy gains. This alternative fuel source can be derived from biomass, which is a readily renewable energy source, unlike other natural resources such as petroleum, coal, or nuclear fuels. They offer several advantages over fossil fuels: usage of a renewable resource, reduced greenhouse emissions, decreased dependence on foreign oil, and stimulation of the agricultural sector (Sun, 2005). These alternatives have the potential to replace a significant amount of gasoline in the transportation sector.

1.1 Lignocellulosic biomass

Biomass consists of harvested plant-derived materials that are abundant, inexpensive, and potentially convertible to fuel by fermentation processes. The material can be found as starch in corn, wheat, potatoes, cassava, and other agricultural products and as monomeric sugars or soluble oligomers in corn syrup, molasses, raw sugar juice, sulfite waste liquors. (Ng, 1983)

Current energy-crop production competes for fertile land with food (corn, rice, sugar, and wheat) and their residues (e.g., corn stover or soybean hulls). This also increases pollution from fertilizers and pesticides, and is harmful to the biodiversity of the land (Tilman, 2006). One primary objection to food-based energy crop production is that it could divert agricultural production away from food crops. This could lead to greater food shortages in both the poor and developed countries. There was a 20-million-ton increase in world grain consumption in 2007, roughly 1%. A large component of that – 14 million tons – was used to fuel cars in the U.S. This leaves only six million tons to cover growing food needs. (US Department of Agriculture, 2007) The key to lessening demand for grain is to commercialize biofuel production from low-input crops such as lignocellulosic biomass in the form of perennial grasses, wood chips, crop residues, forest and mill residues, and urban refuse. (Ng, 1983).

Naturally occurring lignocellulosic materials, as shown in Figure 1, have carbohydrate-rich cellulose and hemicellulose fibers that are surrounded by a lignin seal. This forms a complex structural matrix that is resistant to enzymatic hydrolysis. The hemicellulose fibers act like a glue that fill the voids between and around cellulose and hemicellulose fibers. The lignin acts as a protective sheath, thus providing the rigid characteristics. This structure reduces accessibility to the polysaccharide molecules. Hence, removal of the hemicellulose and lignin greatly enhances polysaccharide accessibility. The carbohydrate and lignin composition differs based on the plant species (Sun, 2005).

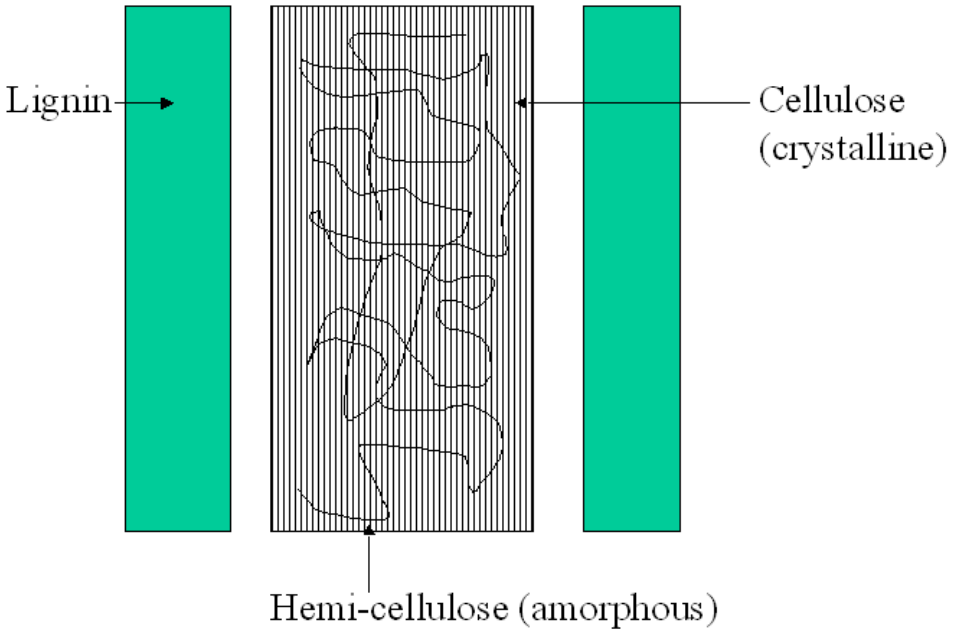

Figure 1: Lignocellulosic structure

In addition to the lignin seal, cellulose chains are held together laterally by intermolecular hydrogen bonds (Fengel and Wegener 1984). These intramolecular hydrogen bonds form between repeating glucose units (Fengel and Wegener 1984). The combined effect of the bonding energies of the hydrogen bonds increases the rigidity of cellulose, causing further insolubility and resistance to hydrolysis.

1.1.1 Cellulose

Cellulose fibers are highly stable homopolymer chains of β-D-glucose units that are linked via β-1-4 glycosidic bonds. The basic repeat unit of cellulose is cellobiose, which consists of two glucose molecules. This linearity of the cellulose chains results in a highly ordered packing of cellulose chains that interact via inter- and intra-molecular

hydrogen bonds involving the hydroxyl groups and hydrogen atoms of adjacent glucose units. As a result, cellulose fibers contain both crystalline fibers and some amorphous regions. In a biomass feedstock, cellulose is the primary reservoir of glucose, the desired fermentation substrate. However, overcoming the crystallinity of the cellulose fibers is a major obstacle for efficient enzymatic hydrolysis (Fengel and Wegener 1984).

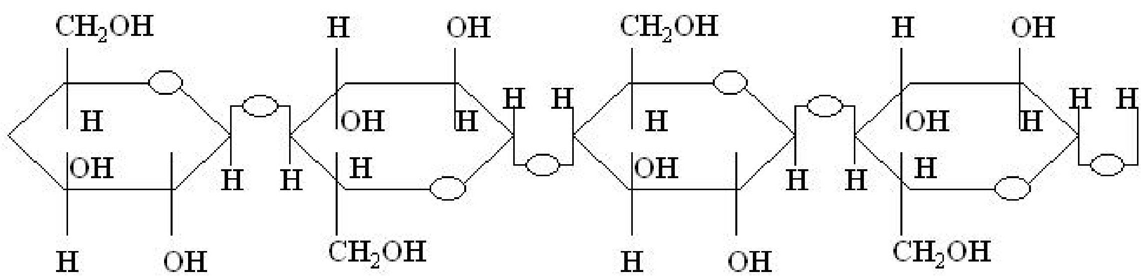

Figure 2. Cellulose structure

1.1.2 Hemicellulose

Hemicellulose is an amorphous biopolymer. These heteropolymer fibers vary in structure and composition, and are composed of five-carbon sugars such as xylose and arabinose, and six-carbon sugars such as galactose and mannose. Switchgrass contains two primary types of hemicellulose: arabinoxylan and glucomannan. Arabinoxylan, which consists of a xylan backbone made up of β-1,4-linked D-xylose units with frequent arabinose side chains, is the dominant hemicellulose component (Fengel and Wegener 1984). The presence of arabinose side chains reduces hydrogen bonding, which contributes to the low crystallinity of hemicellulose. Glucomannan is the minor

hemicellulose component. This component is a copolymeric chain of glucose and mannose units. Intermittent branching in glucomannan also contributes to the low crystallinity (Fengel and Wegener 1984).

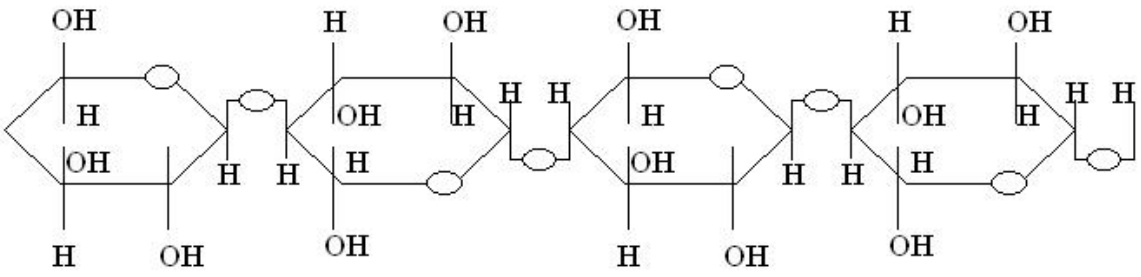

Figure 3. Hemicellulose (xylan) structure

1.1.3 Lignin

Lignin is a stable, high-molecular-weight compound built of phenylpropane units: p-coumaryl alcohol, coniferyl alcohol, and synapyl alcohol. These units are referred to as monolignols. Lignin has a highly complex structure and is difficult to illustrate as basic structural units. The proportions of these components vary based on the type of lignocellulosic material. Switchgrass is comprised of equal portions of all three monolignols. There are many types of carbon-carbon and ether bonds between individual monolignols. As a result, a complex lignin structure consisting of dimers, trimers, and tetramers is formed by random linkages. The carbon-carbon bonds are the strongest, contributing the major part of the barrier nature of lignin (Fengel and Wegener 1984).

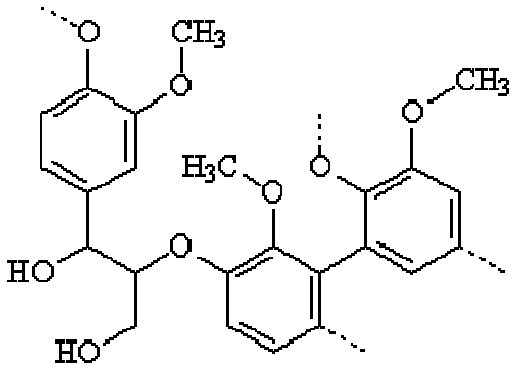

Figure 4. Lignin structure

1.2 Switchgrass

To be sustainable, biomass production must not interfere with existing food-crop production. One means of addressing this is to grow and harvest biomass must be harvested on marginal lands not currently in production. There are approximately 202 million acres of agriculturally abandoned and degraded land in the U.S. that can be used to grow energy crops such as perennial grasses (Tilman, 2006). These grasses are commonly used as fodder crops, and contribute to the energy supply on farms through the use of draft animals (Lewandowski, 2003). Perennial grass is one energy-crop candidate that can be produced on most agricultural land resources, many of which are not suitable for row crops. These grass crops have the potential to achieve high growth rates on more marginally productive croplands where erosion is a concern and soil stabilization is needed (Tolbert, 1998) This development also has the potential for stimulating the agricultural sector by providing a new source of income for farmers (Alizadeh, 2005).

Switchgrass (*Panicum virgatum*, L., Poaceae), as shown in Figure 5, is a warm-season, sod-forming, tall grass, which combines good forage attributes and soil-conservation benefits. This North American native perennial grass belongs to the subfamily Panicoideae of the Gramineae family. This species is commonly associated with the natural vegetation of the Great Plains and the western Corn Belt and is widely distributed in grasslands and non-forested areas throughout North America east of the Rocky Mountains. This grass has been planted in pasture and range-grass mixtures for many years and has become increasingly important as a pasture grass because of its ability to be productive during the hot months of summer, when cool-season grasses are less productive. In southern parts of the US, switchgrass can grow to more than three meters tall and develop roots to a depth of more than 3.5 m (Blake, 2008).

Figure 5. Switchgrass

Source: (Elberson, 2009)

Switchgrass can be harvested in a variety of soil types. Further, it is heat and drought tolerant, while growing well on soils that are shallow and rocky. It is also tolerant to wet areas, environmental restoration, and crop-management treatments. Switchgrass can be easily integrated into existing farming operations because conventional equipment for seeding, crop management, and harvesting can be used. This grass can grow on sand to clay loam soils and can tolerate soils with pH values ranging from 4.9 to 7.6. Annual yields have been reported to be between 11.1 and 34.6 Mg dry mass per hectare (Lewandowski, 2003). Blake (2008) reported that switchgrass can yield between 500 and 1,000 gallons of ethanol per acre using existing technology.

Table 1. Switchgrass forage yield cited in the literature

Reference	Region	Yield, Mg ha^{-1}
Lewandowski et al.	Texas	13.2
Lewandowski et al.	Upper South	12.1
Lewandowski et al.	Alabama	26.0-34.6
Lewandowski et al.	Britain	11.1

1.3 Pretreatment

A primary technological challenge in converting lignocellulosic biomass into fuel is overcoming the recalcitrance of its matrix to enzymatic hydrolysis. To overcome these problems for chemical processing, naturally occurring cellulosic biomass must be pretreated before it can be enzymatically hydrolyzed. Pretreatment is one of the most expensive and least technologically mature conversion steps in the cellulosic ethanol process (Laser, 2001). The purpose is to transform the lignocellulosic structure into a usable fermentation substrate. Economic viability of the pretreatment process depends on

its ability to minimize energy demands and limit costs, such as feedstock size reduction, materials of construction, and treatment of process residues (Mosier, 2003).

To qualify as effective, a pretreatment must meet the following criteria: 1) it maximizes the fermentable glucose yield, 2) it minimizes the formation of fermentation inhibitors from sugar degradation, and 3) it is economically efficient. Principal substrate factors that have been correlated with pretreatment effectiveness include increased cellulose pore volume and hemicellulose and lignin removal.

Pretreatment processes can be loosely grouped into three categories: physical, microbial, and chemical. Physical pretreatments, which demand large amounts of energy, employ purely mechanical means to reduce feedstock particle size, thus increasing surface area available for enzymatic hydrolysis. Examples of such processes include ball milling and compression milling. The primary issue associated with physical pretreatments is the relatively high energy cost. Microbial pretreatment uses microorganisms to remove lignin and improve enzymatic cellulose digestibility. An example of such processes is the use of the fungus *Cyathus stercoreus* to improve hydrolysis. The primary issues associated with microbial pretreatment include slow kinetic and high economic considerations (Hu, 2007). Chemical pretreatments use a variety of chemicals as pretreatment agents: water, acids, alkalis, organic solvents, oxidizing agents, and supercritical fluids. Dilute acid, liquid anhydrous ammonia, lime, and ionic solvent pretreatments have emerged as particularly effective chemical methods (Laser, 2001).

1.3.1 Chemical pretreatment

Chemical pretreatment has been a widely explored approach to overcoming the recalcitrance of natural biomass. Many acids, bases, and other chemicals promote hydrolysis and improve fermentable sugar yield through the removal of hemicellulose and/or lignin. An extensive array of chemical pretreatment options such as the use of oxidizing agents, acids, bases, and other solvents have been investigated. Oxidizing agents tested include alkaline peroxide, sodium and calcium hydroxide, ozone, dioxane, and peroxyacid (organosolv). Acids evaluated include sulfuric acid, hydrochloric acid, phosphoric acid, and nitric acid. Chemical solvents such as ammonia, aprotic solvents (i.e., DMSO), and metal complexes have been explored. These chemicals have shown varying degrees of effectiveness in reducing cellulose crystallinity, disrupting the lignin matrix, and dissolving cellulose (Hu, 2007).

Reaction time, together with temperature and pH, has been reported to influence the pretreatment severity or harshness. Several studies expressed pretreatment severity in terms of a combined severity factor (CSF), that account for multiple process conditions. (Schell, 2003; Kabel, 2006; Chum, 1990) The CSF can be used to determine the best set of experimental parameters required to balance the maximization of hemicellulose and lignin removal with the minimization of glucose degradation, enabling further use of the remaining cellulose (Garrote, 1999). The proposed severity factor is based on an approximation to Arrhenius temperature behavior, but is not limited to first-order kinetics

and allows the well-known reduction in reaction rate with extent of reaction to be accommodated. The formalism presented here linearizes the temperature behavior for convenience, and is equivalent to the Arrhenius formal treatment. The CSF provides a method for consolidating the effects of pretreatment temperature, residence time, and acid concentration into a single parameter, which can be useful for analyzing results. This factor is dependent on process conditions, and does not reflect any physical parameter. CSF is calculated by equation 1:

$$(1) \qquad CSF = \log_{10}\left(t \times e^{\frac{T-100}{14.75}} \right) - pH$$

where t is the reaction time in minutes, T is the reaction temperature in degrees Celsius, and pH is the final pH of the pretreatment liquor. This equation is based on several assumptions. First, the practical operating range span –4 to 3, with highest observed hemicellulose removal at CSF values between 1.4 and 1.7 (Schell, 2003). Low calculated CSF values (-4 to 0) represent less harsh conditions (i.e. relatively low temperatures, residence time, and acidity). High values (0 to 3) represent harsher conditions (i.e. relatively high temperatures, residence time, and acidity). Second, the practical temperature operating range is between 100 and 230^{o}C. Temperatures exceeding 230^{o}C will drive significant thermal degradation of all polysaccharides and monosaccharides, leaving behind mostly lignin in the product (which is not usable for microbial digestion). Third, since the CSF equation is based on the Arrhenius equation for acid catalysis, liquor pH of 7 or less can only be used. (Chum, 1990)

1.3.2 Acid hydrolysis

There are numerous reactions that take place in aqueous sulfuric and other strong acid media. This includes hydrolyses, dehydrations, hydrations, isomerizations, electrophilic substitutions, aromatic rearrangements, carbonyl reactions, and a number of other reactions. (Cox, 1987)

Sulfuric acid has also been added to cellulosic materials for many years, particularly in the pulp-and-paper manufacturing bleaching process (Root et al., 1959; Zeitsch, 2000). This acid has been widely used and studied for pretreatment. In this work, sulfuric acid was used to catalyze the hydrolysis of polysaccharides found in biomass.

The molecular mechanism of acid-catalyzed cellulose hydrolysis is represented by the cleavage of the β-1-4-glycosidic bond (Xiang, 2003). This is a homogeneous reaction in which the acid catalyzes the breakdown of cellulose to produce oligomers (cellobiose) and monosaccharides (glucose). The rate of thermal induced degradation is accelerated in the presence of water, acids and oxygen. As the temperature increase, the degree of polymerization of cellulose decreases further, free radicals appear and carbonyl, carboxyl and hydroperoxide groups are formed. This undesirable and independent reaction involves the breakdown of glucose to form degradation products, such as xylitol, succinic acid, L-lactic acid, glycerol, acetic acid, ethanol, 5-hydroxy-2-furaldehyde, and furfural

(Hu, 2008). Excessively severe conditions such as high acid loading or high temperatures can result in oxidative degradation of carbohydrates, yielding fermentation inhibitors (Mosier, 2003).

Kinetic modeling plays a key role in the design, development, and operation of reactors. Kinetic data are also vital in the design and evaluation of processes to hydrolyze cellulosic materials to glucose for ethanol conversion (Conner, 1985).

Cellulose hydrolysis depends on the reaction rates for glucose formation and degradation. The overall system can be modeled as two consecutive pseudo-first-order reactions proceeding independently. The rate constants are functions of the acid loading and reaction temperature (Conner, 1985).

(2) $A \rightarrow B \rightarrow C$

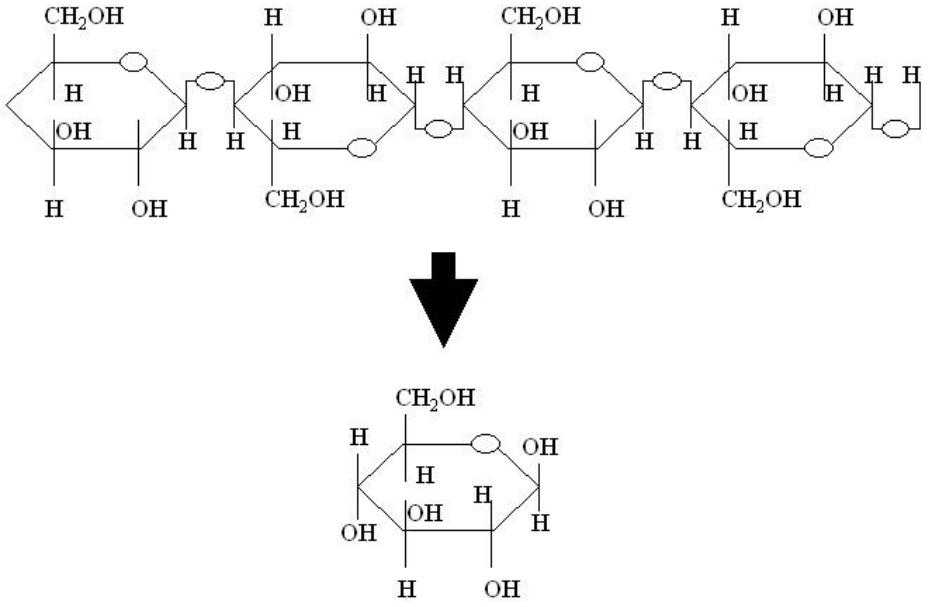

Figure 6. Cellulose hydrolysis reaction

where

- A represents crystalline cellulose
- B represents glucose monomers
- C represents glucose degradation products

The challenge arises because the processing conditions required for the breakdown of crystalline cellulose (A→ B) also contribute to glucose degradation (B → C) (Grethlein, 1975).

1.4 Conventional heating

Conventional chemical process heating is based on conduction, i.e., superficial heat transfer from a region of higher temperature to a region of lower temperature. An external heating source must be used (e.g., a Bunsen burner, electric plate heater, oil bath, or heating mantle). Most batch-pretreatment reactors use conduction to heat the biomass contents to reaction temperature. The contents are typically fed into a corrosion-resistant vessel (e.g., stainless steel or glass) and heated using a steam- or electrically heated jacket. These vessels are typically sealed, allowing for high internal pressure generation (Kappe, 2005).

Conductive heating is reported to be a relatively slow and inefficient method for transferring energy into the reaction system. This process depends on convection currents and on the thermal conductivity of the penetrated materials. The temperature of the reactor is often higher than that of the contents. This process does not offer precise temperature control, and energy transfer is not uniform. For steam-jacketed systems, this creates uneven distribution. As a result, superheated steam typically collects in the upper portion of the jacket, with cooler condensate collecting near the bottom. Internal hot spots also develop around hot steam inlet nozzles, adding to the problem of uneven product heating. This increases the likelihood of product burn-on and local overheating. Further, a temperature gradient can develop within the contents. This can result in local overheating causing product decomposition (Kappe, 2005).

1.4.1 Mechanism

Conduction is the transfer of heat or electricity through a substance, resulting from a difference in temperature between different parts of the substance, in the case of heat, or from a difference in electric potential, in the case of electricity. Since heat is energy associated with the motions of the molecules making up the substance, it is transferred by such motions, shifting from regions of higher temperature, where the particles are more energetic, to regions of lower temperature. The rate of heat flow between two regions is proportional to the temperature difference between them and the thermal conductivity of the substance. In solids, the molecules themselves are bound and contribute to conduction of heat mainly by vibrating against neighboring molecules; a more important mechanism, however, is the migration of energetic free electrons through the solid (The Columbia Encyclopedia, 2008).

1.4.2 Pretreated switchgrass using conventionally heated reactors

There are numerous cases of conduction-heated (conventional) switchgrass pretreatments in the literature. For example, Alizadeh (2005) pretreated switchgrass in a 300-mL stainless steel bench-top pressure vessel (PARR Instrument Co., IL) using liquid anhydrous ammonia. Different biomass moisture levels (40 to 100 weight percent), ammonia loading (0.8 to 1.25 kg ammonia:kg biomass), and reaction temperatures (80 to 100^{o}C) were investigated. The highest observed pretreatment conditions (80 weight

percent biomass moisture, 100°C reactor temperature, and 1:1 kg ammonia: kg switchgrass) resulted in up to a fivefold increase in cellulose saccharification relative to non-pretreated biomass. Dilute-acid pretreated switchgrass examples in the literature are shown in Table 2.

Table 2. Conventional pretreated switchgrass in the literature

Reference	Pretreatment	Condition	Result
Alizadeh 2005	Switchgrass Ammonia	40-100 wt % solids Amm 0.8-1.25 vol.% 80-100^{o}C	93% cellulose conversion
Wyman 1992	Switchgrass Sulfuric acid	140^{o}C 1 hour 0-0.5 vol.% acid	70% cellulose conversion
Dien 2006	Switchgrass Sulfuric acid	10 wt% solids 0-2.5 vol.% acid 150^{o}C	76% cellulose conversion

1.5 Microwave heating

Microwave irradiation is an alternative approach to conduction heating, and has proved to be a highly effective heating source in chemical reactions. Irradiation uses direct interaction between the heated object and an applied electromagnetic field to generate heat. This heating mechanism can accelerate the reaction rate, provide better yields and uniform and selective heating, and achieve greater reproducibility of reactions (Kappe, 2005). Other cited advantages include reduction of process-energy requirements and the ability to instantaneously start and stop the process (Datta, 2001; Gabriel et al., 1998).

1.5.1 Mechanism

Microwaves fall between the infrared and radio-frequency region of the electromagnetic spectrum. This region corresponds to a frequency range of 300 MHz to 30 GHz. Most domestic and industrial microwave systems operate at either 900 MHz or 2.45 GHz to avoid interference with RADAR transmissions and telecommunications. (Sridar, 1998)

A microwave photon carries only 1 joule per mole of energy, which is not enough to induce any chemical activity in materials. As a result, microwave radiation by itself cannot render any significant reactions in materials. However, microwaves interact with polar molecules and ions in a material, causing acceleration in chemical, biological, and physical processes. Depending on the dipole moment, individual polar molecules will react differently to microwave radiation. These interactions result in both thermal and non-thermal effects that drive physical, chemical, or biological reactions. (Sridar, 1998)

Thermal effects are driven by the oscillating nature of the microwaves. This causes the polar molecules to vibrate at a rapid rate (Figure 7). The molecules realign themselves to match that of the electric field. The repeated vibration induces friction between the polar molecules, and the entire system, generating heat within the system. The rate of change of the electric field is relatively close to the response time of the polar molecules at the microwave-frequency range. Polar molecules are not able to respond fast

enough at higher frequencies, hence no vibration or heat generation. Conversely, polar molecules realign themselves at a slow rate at lower frequencies, resulting in little heat generation. (Sridar, 1998)

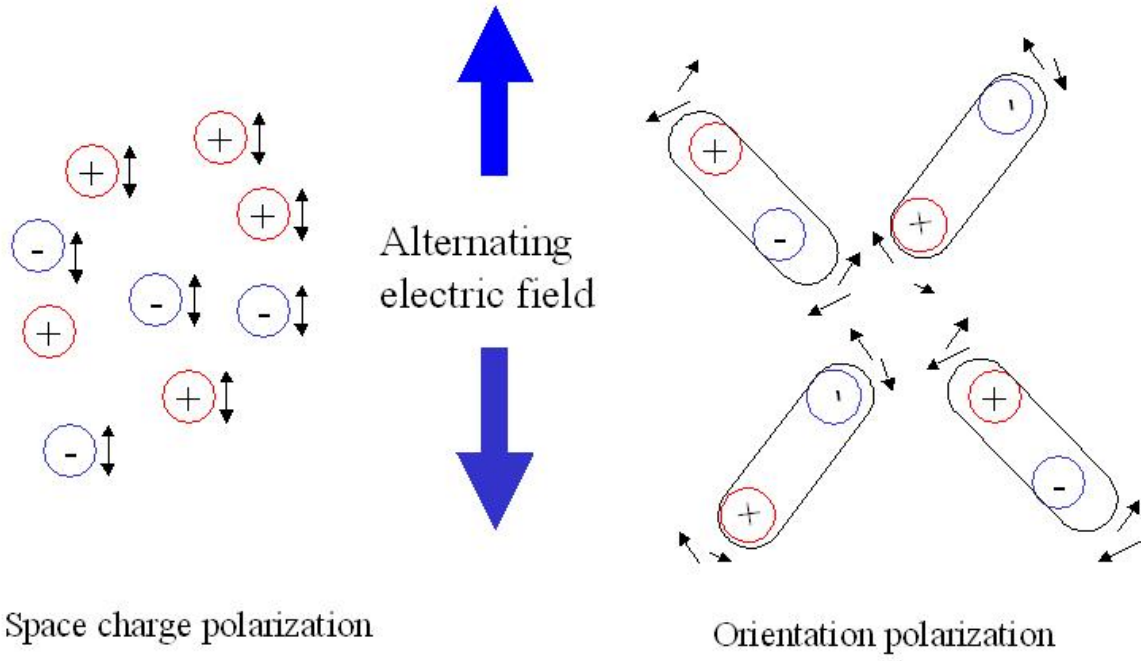

Figure 7. Molecular oscillations of polarizable substances under the influence of an alternating electric field.

Ionic conduction is another mechanism that induces thermal effects. Ionic species that are dissolved in liquids or solids are excited, and orient themselves with the changing direction of the electric field. The ions collide with one another, generating heat within the system. (Sridar, 1998)

Ooshima (1984) reported that cellulosic materials are heated internally upon microwave irradiation. The lignocellulosic structure – cellulose, hemicellulose, water,

and other low–molecular-weight compounds such as organic acids –absorb microwave radiation as kinetic energy. The polar molecules and their neighboring clusters are forced to orient themselves to a specific direction, followed by a shock of the polar molecules when the field is reversed (Ooshima, 1984).

Non-thermal effects are also believed to complement the thermal effects of microwaves. Hu (2007) reported that microwave irradiation causes a physical "explosion" effect among the microfibers, causing the disintegration of the recalcitrant structures of the biomass. Further, the electromagnetic field used in microwaves is believed to produce physico-chemical effects that also accelerate the breakdown of the crystalline regions.

Figure 8 shows a model of an inverted temperature gradient in microwave (left) versus oil bath (right) heating. The model assumes contents in the test tube that requires a target reaction temperature of 475^{o}C. As illustrated, a temperature gradient can develop within the test tube and contents. Since the test tube on the left is transparent to microwaves, only the sample is heated, and not the test tube walls. However, the test tube and the sample are both directly heated in the conventional heated system (right). This is evident by the entire test tube showing temperatures near 500^{o}C. This leads to high localized overheating (hot spots), which can cause product decomposition (Kappe, 2005).

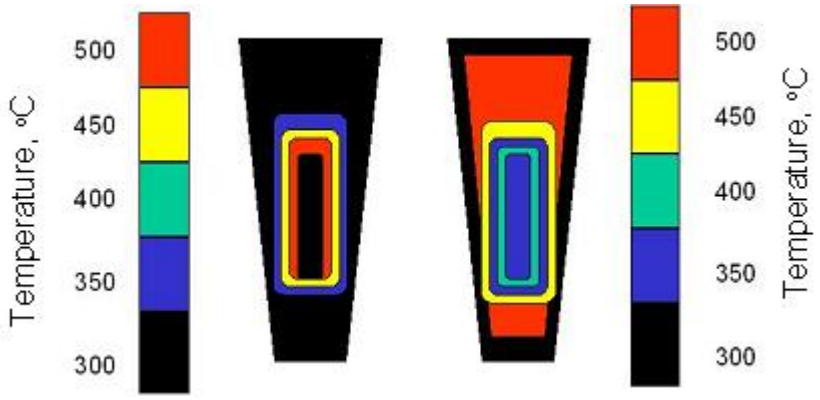

Figure 8. Inverted temperature gradient in microwave (left) versus oil bath (right) heating (Source: Kappe, 2005)

1.5.2 Switchgrass pretreatment using microwave reactors

The first reported use of microwave pretreatment of lignocellulose was Ooshima et al. (1984). Ooshima showed the benefit of microwave-assisted water pretreatment of rice straw and bagasse relative to untreated biomass. Zhu et al (2006) investigated microwave-assisted stepwise alkali/acid/peroxide pretreatment of rice and wheat straw. However, the sugar yield based on dry weight of untreated original materials was not provided. Therefore, it is not possible to compare these results with other pretreatment methods. Zhu et al. also used an uncovered beaker to boil the straw-alkali solution in the microwave. Here, volume loss due to evaporation may be significant since a relatively long reaction time of 60 minutes was used (Hu, 2007). Table 3 summarizes microwave pretreated biomass reported in the literature to date.

Hu (2007) investigated microwave-assisted alkali pretreatment of switchgrass, comparing conventional and microwave heating by varying the alkali loading, but using a fixed temperature (190^{o}C) and residence time (5 minutes). Therefore, the effects of temperature and time, and interactions thereof, were not directly compared for both reactors. In addition, dilute acid pretreatment has been proven to be a more effective method for hemicellulose removal relative to alkali pretreatment. Studies done by Eggeman (2005) showed xylose yields of 89.7% and 0.8% for dilute acid and alkali, respectively.

Table 3. Microwave-pretreated switchgrass in the literature

Reference	Pretreatment	Condition	Result
Ooshima 1984	Rice straw Sealed vessel Water	5 wt% solids 170-230^{o}C	Increased enzymatic hydrolysis by 2.3 vs. untreated
Hu 2008	Switchgrass Sealed vessel Sodium hydroxide	5 wt% solids 0.05 to 0.3 g alkali/g 70-90^{o}C	Increased enzymatic hydrolysis by 5.1 vs. untreated

A more thorough and direct comparison of conventional heated vs. microwave irradiated reactors would be necessary for determining the highest observed and most cost effective pretreatment approach. This information can be used for the development of a large-scale microwave-based pretreatment process. The hypothesis is that microwave pretreatment requires lower pretreatment severity (and energy consumption) to achieve comparable glucose yields relative to conventionally heated pretreatment.

2.0 MATERIALS AND METHODS

2.1 Materials

Cellulose and lignocellulosic substrates were pretreated in conventional and microwave-heated reactors, using the specific materials and methods as follows.

2.1.1 Substrates

Avicel® micro-crystalline cellulose (Sigma Aldrich; St Louis, MO) was used as a pure cellulose control. Microcrystalline cellulose is cellulose derived from high-quality wood pulp. While cellulose is the most abundant organic material, microcrystalline cellulose can only be derived from a special grade of alpha cellulose.

Whatman paper (Piscataway, NJ) was also used as a pure cellulose control. These cellulose filters are comprised of high-quality cotton linters that have been treated to achieve a minimum alpha cellulose content of 98%. The paper samples were ground to a powder using a household coffee grinder prior to pretreatment.

Switchgrass (National Renewable Energy Laboratory, Golden, CO) was used as the experimental biomass. The air-dried and pre-cut switchgrass was also ground to a powder using a household coffee grinder prior to pretreatment. The composition of the switchgrass (on a dry basis) from an average of three randomly selected samples from the

lot was 30.1±0.4% cellulose, 29.3±0.6% xylan, and 23.8±0.8% lignin (acid soluble and insoluble). Figure 9 shows the untreated experimental switchgrass.

Figure 9. Experimental switchgrass

2.1.2 Acid

Dilute sulfuric acid solutions (0, 0.75, and 1.5 vol.%) were prepared and used as the pretreatment catalyst.

2.1.3 Cellulase Enzyme

A cellulase enzyme from *Trichoderma reesei* organism (Sigma Aldrich; St Louis, MO) was used for enzymatic hydrolysis of the solid residue for glucose production.

2.2 Pretreatment

Conventionally and microwave heated reactors were used to pretreat the substrates prior to enzymatic-hydrolysis.

2.2.1 Conventionally heated reactor

Conventional heating pretreatment was performed using a 500-mL stainless-steel reactor vessel (PARR® High-Temperature, High-Pressure Reactor Model 4575A; Parr Instrument, Moline, IL). This fixed-head reactor (Figure 10) has a 1,500-Watt / 115 V electric heater and is capable of heating contents up to 500°C and 5,000 psi. The head is equipped with a gas inlet/liquid sampling port with valves and a dip tube, pressure gauge (SS, 0-7,500 psi), gas-release valve, single-loop serpentine cooling coil, thermowell with type J thermocouple, and a footless magnetic stirrer. The reactor is constructed of T316SS stainless steel and has dimensions of 16.5” in width, 23.5” in diameter, and 43” in height. The conventionally heated reactor was charged with 4 weight-percent solids (10 grams of ground switchgrass immersed in 250 mL of solution).

Figure 10. PARR® High-Temperature, High-Pressure Reactor Model 4575A

2.2.2 Microwave-heated reactor

Microwave irradiation pretreatment was conducted using a CEM Explorer 48 (CEM, Inc., Matthews, NC). The microwave reactor (Figure 11) contains 48 positions for 10-mL vessels or 24 positions for 35-mL vessels. The reactor is capable of using up to 300 Watts of power, obtaining a 300-^{o}C maximum temperature, and a 300-psi maximum pressure. The biomass and contents were sealed in 35-mL glass vessels and irradiated to the specified process conditions. The microwave reactor was also charged with 4 weight-percent solids (0.6 grams of ground switchgrass immersed in 15 mL of solution).

Figure 11. CEM Explorer 48 Microwave Reactor

2.3 Enzyme hydrolysis

In accordance with National Renewable Energy Laboratory Procedure 009 for "Enzymatic Saccharification of Lignocellulosic Biomass", pretreated samples (0.1 gram cellulose equivalent) were hydrolyzed batchwise with 60 FPU/gram cellulose in a jacketed cylindrical glass vessel under agitation (150 rpm) at 50°C and at pH 4.8. Samples (0.5 mL) were taken continuously from the bioreactor over a three-day period at eight-hour intervals and the glucose concentrations determined.

2.4 Analysis

A High-Performance Liquid Chromatograph (HPLC; Dionex, Sunnyvale, CA) was used for chemical analysis. This HPLC uses a 0.005 M sulfuric acid solution as the mobile phase, flowing at 0.6 mL per minute at 30^{o}C. Biomass carbohydrates, acid-soluble lignin, and acid-insoluble lignin were measured using the methods described in NREL Laboratory Analytical Procedure (LAP #002) for ''Determination of Structural Carbohydrates and Lignin in Biomass''. Carbohydrates (monomeric sugars) and other chemical species (acetic acid, 5-hydroxymethanol furfural, and furfural) in the pretreatment liquor were measured in accordance with NREL Laboratory Procedure entitled "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples". These methods are outlined in Appendix 2.

A scanning electron microscope was used to assess the porosity of the samples. The LEO 435 Variable Pressure SEM offers high-performance with a resolution of 4 nm. Its 5 axis computer controlled stage is mounted in a specimen chamber measuring 300 x 265 x 190 mm. The samples were sputter coated with gold and imaged with secondary electrons at 10mm working distance and 45 degrees of specimen tilt. The beam conditions were 30Kv and 25 picoamps. The original images were stored in TIFF format. They were converted to JPEG format and corrected for brightness, contrast, and gamma for electronic transmission. No other image enhancement or modifications were applied.

3.0 EXPERIMENTAL PLAN

The goal was to develop a model that predicts combined glucose yield (pretreatment and enzymatic hydrolysis) of dilute acid-pretreated switchgrass as a function of pretreatment process conditions. A direct comparison of the pretreatment effectiveness of conduction heating and microwave irradiation heating was made.

Our hypothesis was that microwave pretreatment can enhance glucose yields relative to conventionally heated pretreatment. Previous reports in the literature suggest that microwave irradiation contributes to a reduction in cellulose crystallinity caused by more efficient heating and a physical separation between the fibers. The increased cellulose porosity is believed to allow for increased microbial access and digestion, which contributes to increased glucose yields (Hu et al. 2008, Ooshima et al. 1984).

A flow diagram of the proposed pretreatment process is illustrated in Figure 12. Experimentally, precut switchgrass samples were pretreated followed by filtering of the slurry through a Whatman nylon membrane filter, separating residues and liquid. The filtered cakes were dried at 35^{o}C and stored for enzymatic hydrolysis. The liquid fraction was collected to determine the glucose, xylose, and degradation product yields obtained in the conventional and microwave pretreatment process. The filtered cakes were digested using the cellulase enzyme to assess glucose yield.

A three-variable, three-level Taguchi design experiment (Table 4) was used to generate experimental data, and gain an understanding of the relationships between reactor conditions and their responses. A total of nine runs (plus two replicates) were

conducted. Minitab® software (Minitab; State College, PA) was used to analyze the multi-variable design experiment results and make a direct comparison between the conventional reactor and the microwave reactor.

Table 4: Pretreatment experimental design

Condition	Acid Loading Vol%	Temperature ^{o}C	Residence Time Minutes
1	0	165	1
2	0	180	5
3	0	195	10
4	0.75	165	5
5	0.75	180	10
6	0.75	195	1
7	1.5	165	10
8	1.5	180	1
9	1.5	195	5

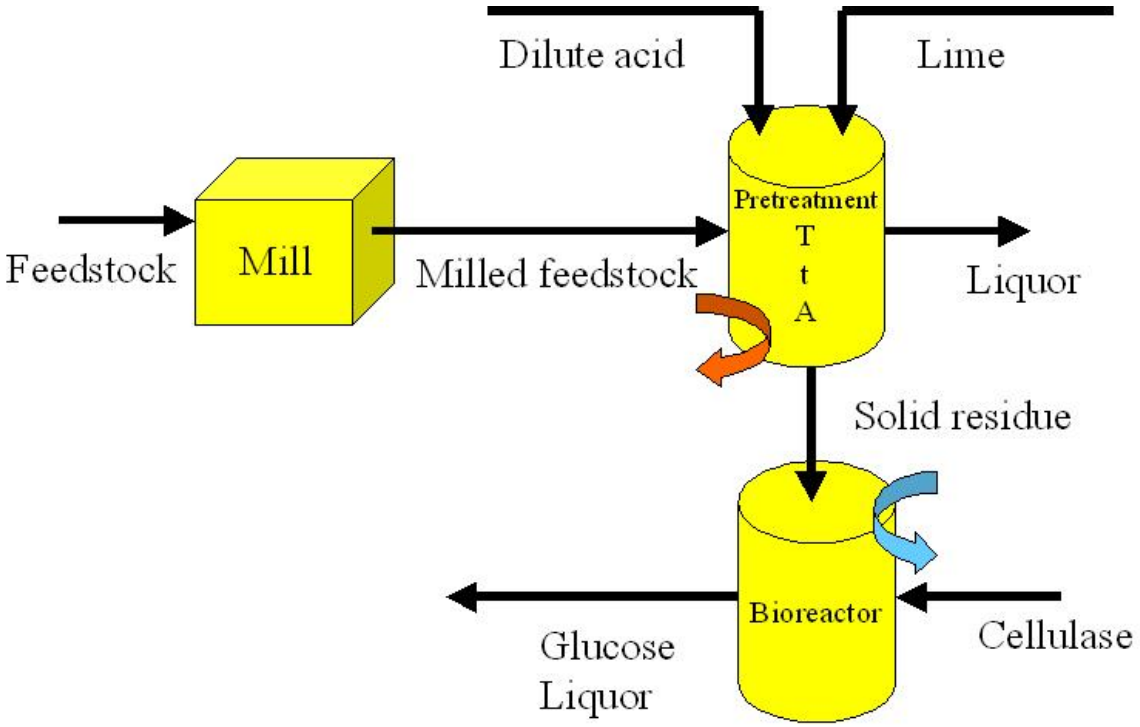

Figure 12. Process-flow diagram

where

- T (temperature, ^{o}C)
- t (residence time, min)
- A (acid loading, vol%)

4.0 RESULTS AND DISCUSSION

The pretreatment reactor responses (pressure, biomass composition, pretreatment liquor composition, and enzymatic hydrolysis glucose yield) as a function of acid loading, temperature, and residence time are presented for the three substrates (Avicel®, Whatman paper, and switchgrass) and reactor types (conventional and microwave reactors).

4.1 Pressure

The microwave reactor reached final pressures ten times faster than the conventionally heated reactor. This is related to the faster heat generation, which is due to the direct interaction between the heated object and the applied electromagnetic field as opposed to the gradient heating mechanism for the conventional reactor.

The conventionally heated reactor vessel, which was charged with 10 grams of biomass and 250 mL of solution), reached 195^{o}C and 300 psi after a 60-minute ramp time. The microwave reactor vessel, which was charged with 0.6 grams of switchgrass and 15 mL of water, reached 195^{o}C and a 300-psi pressure after a six-minute ramp time. Reactor pressures as a function of temperature and ramp time are shown in Table 5 and illustrated in Figures A1 and A2.

Table 5: Final reactor pressure obtained during experimentation

Condition	Temperature ^{o}C	Pressure psi Conventional	Ramp time, Min	Pressure Psi Microwave	Ramp time, min
1	165	100±5	28±1	100±5	3±1
2	180	150±5	38±1	154±8	5±1
3	195	200±6	49±2	240±8	7±1
4	165	100±4	27±2	100±3	3±1
5	180	150±6	39±1	151±7	5±1
6	195	200±4	50±2	220±10	7±1
7	165	100±3	30±1	100±4	3±1
8	180	150±4	40±2	160±6	5±1
9	195	200±5	49±2	230±5	7±1

4.2 Biomass

The biomass substrates were assessed for mass loss and discoloration due to pretreatment.

4.2.1 Mass loss

Mass loss is the ratio of the change in mass before and after pretreatment to the initial mass charged to the reactor. Mass loss is due to polysaccharide hydrolysis, decomposition, and lignin removal. Experimental results are presented in Table 6.

Figures A4 through A6 display the Minitab® data means summary analysis output. There was no performance difference in mass loss between the two reactors. The

analysis shows the influence of acid loading, temperature, and residence time on mass loss for the three substrates and both reactors.

The mass loss for all three substrates increased with acid loading and temperature for both reactors over the experimental range. As the acid loading and temperature increase, the degree of polymerization of the polysaccharides decrease further, free radicals appear and carbonyl, carboxyl and hydroperoxide groups are formed, thus resulting in more mass loss. Avicel® micro-crystalline cellulose particles are the most crystalline of the three substrates (Harris, 2008). As a result, this substrate requires the highest amount of pretreatment severity to initiate cellulose hydrolysis; the lower-crystallinity materials require slightly less severity for cellulose hydrolysis. Switchgrass, which contains lower-molecular-weight polymers (hemicellulose), requiring less severity for hemicellulose removal.

Increasing acid loading from 0 to 1.5 vol.% resulted in a significant mass loss. Avicel®, Whatman paper, and switchgrass lost up to 50, 75, and 90 wt% mass, respectively. Increasing temperature from 165 to 195^{o}C resulted in mass loss increasing from 12 to 50, 25 to 50, and 50 to 80 wt% for Avicel®, Whatman paper, and switchgrass, respectively.

The cellulose and xylan fractions in the switchgrass as a function of pretreatment conditions are shown in Figures A6 and A7. Experimental results are presented in Table 7. The cellulose fraction peaks at 0.75 vol% due to complete hemicellulose removal, and

decreases at higher loading thereafter due to cellulose hydrolysis. There was no clear relationship between temperature and cellulose fraction, and residence time and cellulose fraction. Complete xylan removal occurs at temperatures lower than for cellulose removal. This is due to rapid hydrolysis of the more amorphous and lower molecular weight hemicellulose. Results show the xylan fraction to rapidly decreases to zero in the presence of acid (0.75 vol% and greater), at least 180^{o}C and 5 min residence time.

Table 6: Mass loss result summary

Condition	Acid loading, Vol%	Temperature °C	Time, Min	Avicel		Whatman paper		Switchgrass	
				Mass loss, % Conventional	Mass loss, % Microwave	Mass loss, % Conventional	Mass loss, % Microwave	Mass loss, % Conventional	Mass loss, % Microwave
1	0	165	1	8 ± 1	8 ±1	4 ± 1	8 ± 2	37 ± 2	24 ± 2
2	0	180	5	5 ± 1	1 ±1	11 ± 2	18 ± 1	34 ± 2	39 ± 1
3	0	195	10	4 ± 1	13 ± 1	5 ± 1	1 ± 0	0 ± 0	0 ± 0
4	0.75	165	5	16 ± 1	3 ±1	28 ± 2	40 ± 3	56 ± 3	69 ± 2
5	0.75	180	10	50 ± 3	47 ± 3	46 ± 3	56 ± 2	81 ± 3	59 ± 1
6	0.75	195	1	68 ± 4	48 ± 4	64 ± 2	30 ± 1	81 ± 4	61 ± 2
7	1.5	165	10	21 ± 3	42 ± 3	45 ± 3	60 ± 3	71 ± 3	47 ± 2
8	1.5	180	1	37 ± 4	47 ± 4	75 ± 2	70 ± 3	83 ± 3	52 ± 1
9	1.5	195	5	90 ± 8	95 ± 8	79 ± 2	68 ± 3	83 ± 2	99 ± 1

Table 7: Pretreated biomass composition result summary

				Conventional Reactor			Microwave Reactor		
Condition	Acid loading, vol%	Temp °C	Time, Min	Cellulose Wt%	Xylan Wt%	Lignin Wt%	Cellulose Wt%	Xylan Wt%	Lignin Wt%
1	0	165	1	29.3 ± 0.8	39.8 ± 0.5	39.1 ± 1.2	32.5 ± 0.9	27.5 ± 0.6	26.7 ± 0.6
2	0	180	5	31.4 ± 0.5	32.9 ± 0.4	34.0 ± 0.8	38.4 ± 0.6	30.9 ± 0.8	37.7 ± 1.0
3	0	195	10	84.7 ± 0.3	0.0 ± 0.0	19.5 ± 0.2	63.6 ± 1.0	0.0 ± 0.0	39.3 ± 0.2
4	0.75	165	5	84.4 ± 0.5	0.0 ± 0.0	26.9 ± 0.8	62.7 ± 1.0	0.0 ± 0.0	20.0 ± 0.2
5	0.75	180	10	61.4 ± 0.6	0.0 ± 0.0	41.0 ± 1.1	65.0 ± 0.9	0.0 ± 0.0	41.3 ± 0.3
6	0.75	195	1	56.3 ± 0.3	0.0 ± 0.0	53.4 ± 0.8	54.1 ± 0.5	0.0 ± 0.0	50.8 ± 0.8
7	1.5	165	10	64.9 ± 0.8	0.0 ± 0.0	36.0 ± 0.9	65.0 ± 1.0	0.0 ± 0.0	41.0 ± 0.2
8	1.5	180	1	66.6 ± 0.7	0.0 ± 0.0	39.7 ± 0.5	59.7 ± 0.9	0.0 ± 0.0	25.5 ± 0.1
9	1.5	195	5	6.6 ± 0.3	0.0 ± 0.0	86.7 ± 1.6	0.0 ± 0.0	0.0 ± 0.0	91.1 ± 0.8
Unpretreated				30.1 ± 0.4	29.4 ± 0.6	23.8 ± 0.8	30.1 ± 0.4	29.37 ± 0.6	23.8 ± 0.8

Lignin removal was more difficult in the dilute-acid system due to its insolubility in acidic conditions. Increasing acid loading and temperature resulted in higher lignin percentage of lignin remaining in the pretreated solids. Since polysaccharide hydrolysis is acid- and temperature-driven, this leaves behind a higher portion of lignin in the remaining solids. No correlation was found between mass loss and residence time over the operating range.

4.2.2 Color

Color changes are a result of substrate decomposition. It is understood that lignicellulosic materials contain water-soluble wood extractives that oxidize (under acidic and high temperature conditions) and polymerize to form a brown coloration. Experimental findings suggest that pretreatment severity influence the final substrate color. Figure 13 illustrates switchgrass discoloration due to pretreatment. The color shift was negligible under low-severity pretreatment conditions (low ends of the acid and temperature experimental ranges). Moderate-severity pretreatment conditions (middle of the acid and temperature experimental ranges) shifted the color from natural to brown, while high-severity conditions (high ends of the acid and temperature experimental ranges) shifted the final color from natural to dark brown.

Figure 13. Switchgrass discoloration due to pretreatment

4.2.3 Porosity

As previously stated, substrate porosity and microbial digestion are directly related. The goal was to visually assess the openness within the structure of the samples, as an indicator of porosity. A scanning electron microscope (SEM) was used to obtain photographs of the unpretreated, conventional, and microwave pretreated switchgrass (Figures 14 through 16). The same magnification was used for each sample (with a $3x10^{-5}$ scale). The unpretreated sample appears rigid and contains a hard, rope-like outer shell (Figure 14). The conventional-pretreated sample does not show a rigid outer shell, in which the fibers appear to have separated in one direction (Figure 15). The microwave-pretreated sample appears to be even more open than the conventional-pretreated samples, with fiber separation in two different directions (Figure 16). The increased fiber separation within the structure can be attributed to the non-thermal effects caused by microwave-pretreatment. (Hu 2008, Ooshima 1984) This phenomenon should contribute to higher microbial digestion and glucose yield. Glucose yield results will be reported later in this study.

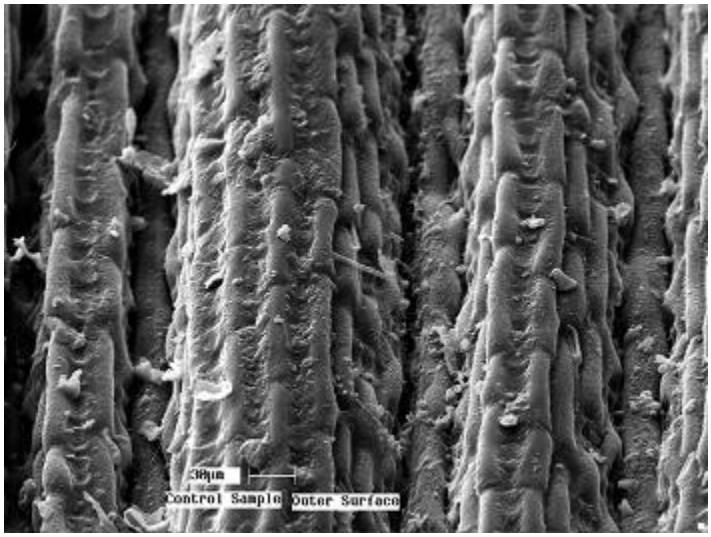

Figure 14: SEM photograph of unpretreated switchgrass

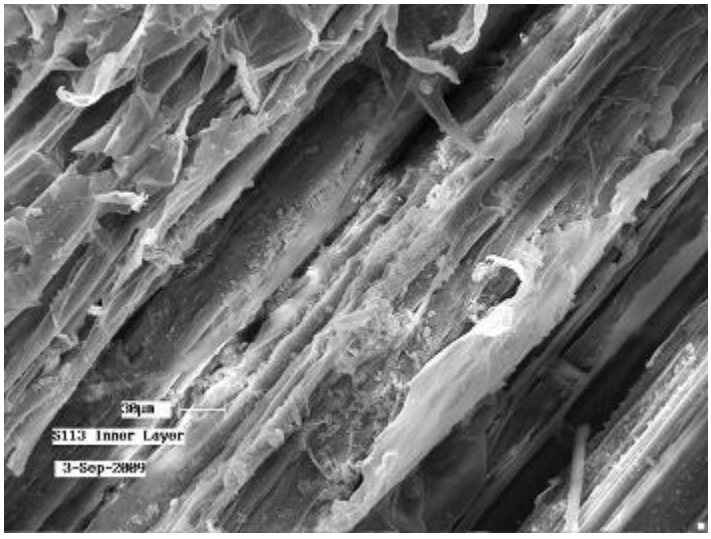

Figure 15: SEM photograph of conventional-pretreated switchgrass

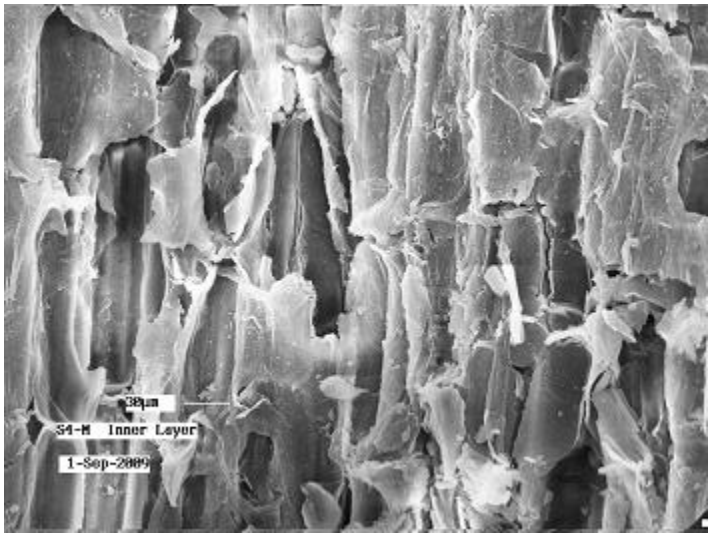

Figure 16: SEM photograph of microwave-pretreated switchgrass

4.3 Pretreatment liquor

The pretreatment liquor was characterized using measurements for pH, glucose, xylose, and degradation product yields.

4.3.1 pH

The pH of the pretreatment liquor is an indicator for the presence of sugar-degradation products and fermentation inhibitors. Since sulfuric acid was added to the reactor, our objective was to observe deviations from the sulfuric-acid baseline.

Figures A8 through A10 display the Minitab® data means summary analysis output. The analysis shows the influence of acid loading, temperature, and residence time

on liquor pH. A decrease in pH is usually a result of the formation of acidic degradation products such as succinic acid, acetic acid, lactic acid, etc.

Performance differences in pH were insignificant between the two reactors. The pH obviously decreased with increasing acid loading. As expected, the pH of the three substrate liquors significantly decreased with increasing acid loading. However, temperature and residence only slightly affected the liquor pH for all substrates and both reactors. Experimental pH results are presented in Table 8. The negative pH shift induced by temperature is supported by the formation of acetic acid (Figure 18A) and succinic acid (Figure 20A) at elevated temperature conditions. This is a result of the formation of free radicals, carbonyl, carboxyl, and hydroperoxide groups.

Table 8: pH result summary

Condition	Acid loading, Vol%	Temperature ^{o}C	Time, Min	Avicel		Whatman paper		Switchgrass	
				pH Conventional	pH Microwave	pH Conventional	pH Microwave	pH Conventional	pH Microwave
1	0	165	1	4.8 ± 0.1	4.8 ± 0.1	5.6 ± 0.2	5.8 ± 0.2	5.5 ± 0.1	5.5 ± 0.2
2	0	180	5	4.7 ± 0.2	4.8 ± 0.1	3.9 ± 0.2	5.0 ± 0.1	5.2 ± 0.1	5.0 ± 0.1
3	0	195	10	4.4 ± 0.1	4.6 ± 0.1	4.4 ± 0.1	5.0 ± 0.1	4.7 ± 0.2	4.1 ± 0.1
4	0.75	160	5	1.3 ± 0.1	1.0 ± 0.0	1.2 ± 0.1	1.0 ± 0.0	1.4 ± 0.0	1.0 ± 0.1
5	0.75	185	10	1.3 ± 0.1	1.2 ± 0.0	1.5 ± 0.0	1.0 ± 0.0	1.3 ± 0.1	1.1 ± 0.0
6	0.75	190	1	1.3 ± 0.1	0.9 ± 0.0	1.6 ± 0.0	0.8 ± 0.0	1.3 ± 0.0	1.0 ± 0.0
7	1.5	165	10	1.0 ± 0.0	1.1 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	1.0 ± 0.0	0.7 ± 0.0
8	1.5	180	1	1.1 ± 0.0	0.9 ± 0.0	1.2 ± 0.0	0.5 ± 0.0	1.1 ± 0.0	0.9 ± 0.0
9	1.5	195	5	1.2 ± 0.0	0.5 ± 0.0	1.0 ± 0.0	0.5 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

Table 9 shows the change in liquor pH as the acid loading, temperature, and residence is elevated from the low end to the high end of the operating range. Acid loading by far has the predominant effect on pH (4.0 shift), followed by temperature (0.8 shift) and residence time (0.8 shift).

Table 9: Change in pretreatment liquor pH as a function of pretreatment parameters

Pretreatment Liquor	Acid loading Increasing from 0 to 1.5 vol%	Temperature Increasing from 165 to 195^{o}C	Residence time Increasing from 1 to 10 min
Avicel®	-4.5	-1.1	-0.9
Whatman paper	-3.5	-0.4	-0.8
Switchgrass	-4.0	-0.6	-0.6

4.3.2 Glucose

Glucose present in the pretreatment liquor was liberated by the acid/temperature-catalyzed cellulose hydrolysis reaction. Experimental results are shown in Table 10. Figures A11 through A13 display the Minitab® data means summary analysis output. This analysis shows glucose yields in the pretreatment liquor as a function of acid loading, temperature, and residence time for the conventional and microwave reactors, respectively.

The microwave reactor liberated more glucose in the Avicel® liquor relative to the conventional reactor. Because Avicel® is a pure cellulose substrate the reaction is not impeded by the presence of hemicellulose and lignin. Glucose in the Avicel® liquor increased with acid loading and temperature. The microwave reactor produced on average 7 g L^{-1} higher glucose concentrations in the liquor for all process parameters –

acid loading (up to 4 g L^{-1} higher), temperature (from 3 to 6 g L^{-1} higher), and residence time (from 3 to 7 g L^{-1} higher) – relative to the conventionally heated reactor. The higher glucose yields can be attributed to the direct interaction of microwaves with the cellulose and more efficient heating.

The interaction of pretreatment process conditions on glucose yield in the switchgrass liquor is shown in Figure A13A. The highest observed glucose yield occurred during combination of 0.75 vol% acid and 195^{o}C (for both reactors), and combination of low residence time (1 min), 195^{o}C, and 0.75 vol% acid. The lowest observed glucose yields occurred at low temperatures (165^{o}C), and combination of 1.5 vol% acid and 195^{o}C (for both reactors). Higher acid loading and residence time results in glucose degradation.

Table 10: Pretreatment liquor glucose result summary

Condition	Acid loading	Temperature	Time	Avicel		Whatman paper		Switchgrass	
				Glucose g L^{-1} Conventional	Glucose g L^{-1} Microwave	Glucose g L^{-1} Conventional	Glucose g L^{-1} Microwave	Glucose g L^{-1} Conventional	Glucose g L^{-1} Microwave
1	0	165	1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0	180	5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	1.0 ± 0.1
3	0	195	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	0.75	165	5	1.9 ± 0.3	5.1 ± 0.1	3.3 ± 0.1	8.6 ± 0.1	0.4 ± 0.1	0.0 ± 0.0
5	0.75	180	10	3.9 ± 0.3	12.4 ± 0.1	3.3 ± 0.1	8.3 ± 0.1	5.7 ± 0.1	0.0 ± 0.0
6	0.75	195	1	1.1 ± 0.1	11.9 ± 0.1	3.4 ± 0.1	1.4 ± 0.1	6.1 ± 0.8	8.8 ± 0.1
7	1.5	165	10	2.9 ± 0.1	7.6 ± 0.1	3.0 ± 0.1	6.9 ± 0.1	2.6 ± 0.1	5.5 ± 0.1
8	1.5	180	1	0.0 ± 0.0	8.7 ± 0.1	4.7 ± 0.1	0.0 ± 0.0	5.6 ± 0.6	6.8 ± 0.1
9	1.5	195	5	0.0 ± 0.0	3.1 ± 0.1	2.3 ± 0.1	3.4 ± 0.1	0.8 ± 0.1	1.0 ± 0.1

Table 11: Pretreatment liquor xylose result summary

Condition	Acid loading	Temperature	Time	Avicel		Whatman paper		Switchgrass	
				Xylose g L^{-1} Conventional	Xylose g L^{-1} Microwave	Xylose g L^{-1} Conventional	Xylose g L^{-1} Microwave	Xylose g L^{-1} Conventional	Xylose g L^{-1} Microwave
1	0	165	1	-	-	-	-	0.9 ± 0.1	2.7 ± 0.1
2	0	180	5	-	-	-	-	1.1 ± 0.1	0.9 ± 0.1
3	0	195	10	-	-	-	-	0.0 ± 0.0	0.0 ± 0.0
4	0.75	165	5	-	-	-	-	10.3 ± 0.3	4.2 ± 0.3
5	0.75	180	10	-	-	-	-	0.0 ± 0.0	6.0 ± 0.7
6	0.75	195	1	-	-	-	-	0.0 ± 0.1	0.4 ± 0.0
7	1.5	165	10	-	-	-	-	2.9 ± 0.2	0.0 ± 0.0
8	1.5	180	1	-	-	-	-	0.1 ± 0.0	0.0 ± 0.0
9	1.5	195	5	-	-	-	-	0.0 ± 0.0	0.0 ± 0.0

The microwave reactor also liberated more glucose in the Whatman-paper liquor relative to the conventional reactor. Similarly to the Avicel® reactor, the reaction was not impeded by the presence of hemicellulose and lignin. The glucose level increased with rising acid loading, but averaged 2.25 and 3.25 g L^{-1} for the conventional and microwave reactors, respectively.

Glucose levels in the switchgrass-pretreatment liquor were similar for both reactors. The relationships between glucose and pretreatment conditions were similar as well for both reactors. Glucose increased from 0 to 4.5 g L^{-1} as the acid loading was increased from 0 to 0.75 vol.%. However, polysaccharide degradation resulted at acid loading beyond 0.75 vol.%. This was evidenced by the formation of acetic acid (up to 6 g L^{-1}) and furfural (up to 0.5 g L^{-1}) in the liquor. Increasing temperature from 165 to 195^{o} resulted in a positive shift in glucose yield (from 1.5 to 4.0 g L^{-1}). No correlation between residence time and glucose production was found.

4.3.3 Xylose

Table 11 and Figure A14 shows the xylose level in the switchgrass-pretreatment liquor as a function of pretreatment conditions. Xylose levels in the liquor peaked at 0.75 vol% acid loading, and decreases to zero at higher loading levels. Xylose levels decreased with increasing temperatures, and were non existent at 195^{o}C. This is attributed to a fast xylan-hydrolysis reaction rate, in addition to degradation of the lower-molecular-weight simple sugar at moderate and high pretreatment severity conditions.

4.3.4 Degradation Products

Overall, the microwave reactor yielded more degradation products relative to the conventional reactor. This can be attributed to the direct interaction of microwaves with the polysaccharides causing faster cellulose and xylan hydrolysis and degradation reaction rates.

Hydroxy-methyl-furfual

Hydroxymethylfurfual (HMF) is an aldehyde and a furan compound formed during the thermal decomposition of sugars and carbohydrates and is also a fermentation inhibitor. This compound can be used to synthesize a broad range of chemicals currently derived from petroleum. Liquid fuels that are potential alternatives to ethanol obtained by fermentation processes can also be derived from HMF using chemical processes (Su, 2009).

Experimental results of the hydroxy-methyl furfural (HMF) measured in the pretreatment liquor are presented in Table 12. The microwave reactor and the conventional reactor produced comparable amounts of HMF. Average HMF levels were 0.37, 0.25, and 0.24 g L^{-1} for the Avicel®, Whatman paper, and switchgrass, respectively. Figures A15 through A17 show HMF levels as a function of pretreatment conditions.

HMF levels increased with acid loading and temperature due to an increase in thermal degradation rates, which drive the formation of free radicals and carbonyl groups.

The interaction of pretreatment process conditions on HMF yield in the switchgrass liquor is shown in Figure A17A. The highest observed HMF yield occurred during the combination of 0.75 vol% acid and 195^{o}C (for both reactors), and the combination of low residence time (1 min), 195^{o}C, and 0.75 vol% acid. The lowest observed HMF yields occurred at combination low temperatures (165^{o}C), acid, and residence time, and combination of 1.5 vol% acid and 195^{o}C (for both reactors). HMF is degraded and totally consumed at high acidic and temperature conditions (a combination of 1.5 vol% and 195^{o}C) as levulinic acid is formed.

Acetic acid

Acetic acid is a weak carboxylic acid and also a fermentation inhibitor. Acetic acid is produced as a result of the hydrolysis of acetyl groups present in the hemicellulose. Gizenia et al. (2008) noted that concentrations as low as 0.25 g L^{-1} can affect microbial growth and reduce the rate of ethanol production.

Experimental results of the acetic acid measured in the pretreatment liquor are presented in Table 13. The microwave reactor generated on average 5 g L^{-1} more acetic acid in the switchgrass liquor than the conventional reactor. Figure A18 shows acetic acid levels in the switchgrass liquor as a function of pretreatment conditions. The

conventionally and microwave-pretreated liquor yielded up to 6.4 and 59.8 g L^{-1} of acetic acid, respectively. This corresponded to pretreatment conditions of at least 0.75 vol.% and 180^{o}C. The relatively high acetic acid formation yielded in the microwave reactor can be attributed to its higher reaction rates.

Xylitol

Xylitol is a sugar polyalcohol of great interest in the food (as a sweetener), odontological and medical-pharmaceutical industries. At present, it is industrially obtained by a chemical hydrogenation of D-xylose recovered from hydrolyzates of lignocellulosic wastes (Sampaio, 2006).

Xylitol was generated in the switchgrass-pretreatment liquor. This chemical was only detected in the conventional reactor. A comparison of Figures A14 and A19 shows the indirect relationship between xylose consumption and xylitol formation. This is indicative of the acid and temperature induced chemical hydrogenation of xylose. Xylitol levels increases with acid loading and temperature. Increasing acid from 0 to 1.5 vol.% resulted in up to 48 g L^{-1} of xylitol formation. Elevating temperature from 165 to 195^{o}C also resulted in up to 48 g L^{-1} of xylitol formation.

Succinic acid

Succinic acid is a dicarboxylic acid that can be used as a precursor for many chemicals of industrial importance including adipic acid, 1,4-butanediol, tetrahydrofuran, N-methyl pyrrolidinone, 2-pyrrolidinone, succinate salts, and gamma-butyrolactone. In addition to applications in the agricultural, food, and pharmaceutical industries, succinic acid could also be used in the synthesis of biodegradable polymers such as polybutyrate succinate, polyamides, and various "green" solvents. Presently, succinic acid is produced commercially by catalytic hydrogenation of petrochemical-derived maleic acid or maleic anhydride, but can also be generated through carbohydrate degradation caused during cellulose and lignocellulose pretreatment. (Zheng, 2009)

The microwave reactor produced more succinic acid relative to the conventional reactor. Up to 10.6 g L^{-1} was detected in the Avicel®-pretreatment liquor, corresponding to an acid loading and temperature of 0.75 vol.% and 195^{o}C. Succinic acid was also detected in the switchgrass-pretreatment liquor. Figure A20 shows succinic acid levels in the switchgrass liquor as a function of pretreatment conditions. The conventional and microwave reactors yielded averages of 6 to 30 g L^{-1} of succinic acid, respectively. Peak levels (95 g L^{-1}) corresponded to a high acid loading (1.5 vol.%), which induced rapid polysaccharide degradation.

Table 12: Pretreatment liquor hydroxy-methyl furfural (HMF) result summary

Condition	Acid loading Vol%	Temperature ^{o}C	Time Min	Avicel		Whatman paper		Switchgrass	
				HMF g L^{-1} Conventional	HMF g L^{-1} Microwave	HMF g L^{-1} Conventional	HMF g L^{-1} Microwave	HMF g L^{-1} Conventional	HMF g L^{-1} Microwave
1	0	165	1	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
2	0	180	5	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
3	0	195	10	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
4	0.75	165	5	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.01	0.68 ± 0.02	0.20 ± 0.02	0.34 ± 0.00
5	0.75	180	10	0.03 ± 0.00	0.83 ± 0.00	0.83 ± 0.00	0.86 ± 0.00	0.41 ± 0.00	0.59 ± 0.01
6	0.75	195	1	1.43 ± 0.02	1.01 ± 0.02	1.52 ± 0.00	0.03 ± 0.00	0.57 ± 0.01	0.50 ± 0.00
7	1.5	165	10	0.13 ± 0.00	0.30 ± 0.00	0.32 ± 0.00	0.44 ± 0.02	0.03 ± 0.00	0.35 ± 0.00
8	1.5	180	1	0.03 ± 0.00	0.67 ± 0.00	0.73 ± 0.03	0.03 ± 0.00	0.32 ± 0.01	0.53 ± 0.01
9	1.5	195	5	0.03 ± 0.00	0.36 ± 0.00	0.97 ± 0.00	0.03 ± 0.00	0.26 ± 0.01	0.03 ± 0.00

Table 13: Pretreatment liquor acetic acid result summary

Condition	Acid loading Vol%	Temperature ^{o}C	Time Min	Avicel		Whatman paper		Switchgrass	
				Acetic acid g L^{-1} Conventional	Acetic acid g L^{-1} Microwave	Acetic acid g L^{-1} Conventional	Acetic acid g L^{-1} Microwave	Acetic acid g L^{-1} Conventional	Acetic acid g L^{-1} Microwave
1	0	165	1	-	-	-	-	0.14 ± 0.00	0.14 ± 0.00
2	0	180	5	-	-	-	-	0.14 ± 0.00	0.14 ± 0.00
3	0	195	10	-	-	-	-	3.45 ± 0.00	0.14 ± 0.00
4	0.75	165	5	-	-	-	-	0.14 ± 0.00	0.14 ± 0.00
5	0.75	180	10	-	-	-	-	6.40 ± 0.00	12.7 ± 9.83
6	0.75	195	1	-	-	-	-	5.93 ± 0.01	4.89 ± 1.63
7	1.5	165	10	-	-	-	-	5.85 ± 0.05	12.8 ± 0.07
8	1.5	180	1	-	-	-	-	5.68 ± 0.01	5.13 ± 0.17
9	1.5	195	5	-	-	-	-	6.40 ± 0.02	7.38 ± 1.16

4.4 Enzyme hydrolysis liquor

The enzyme-hydrolysis liquor of the pretreated switchgrass was analyzed for glucose. Figure A21 displays the Minitab® data means summary analysis output. This analysis show glucose yields from enzymatic hydrolysis as function of acid loading, temperature, and residence time for the conventional and microwave reactors, respectively.

4.4.1 Glucose yield as a function of pretreatment conditions

Glucose measured in the pretreated-switchgrass enzymatic hydrolysis liquor is summarized in Table 14. Microwave-pretreated switchgrass yielded more glucose in the enzymatic-hydrolysis liquor than the conventionally pretreated switchgrass. The performance advantage is likely attributed to the nonthermal effects associated with microwave treatment (Hu et al 2008, Ooshima 1984). This is evident in the SEM photos shown earlier. The average glucose yields across all acid loading were 7.0 g L^{-1} and 4.0 g L^{-1} for the microwave and conventionally pretreated substrates, respectively. The relationship between pretreatment conditions and glucose yields were similar for both reactors. It is well known that acid opens the biomass pores, allowing for greater microbial digestion. Increasing acid loading from 0 to 0.75 vol.% contributed to hemicellulose removal, resulting in higher cellulose content in the pretreated biomass. The higher cellulose loading offers more substrate for microbial digestion. Highest observed glucose yields (6 g L^{-1}) were obtained at 0.75 vol.% acid loading and

temperatures between 180 and 195^{o}C. However, higher acidity and temperature conditions drove cellulose hydrolysis, leaving higher ratios of lignin in the remaining solid (>71 wt%), which impedes enzymatic hydrolysis. Pretreatment residence time had no significance influence on glucose yield for either reactor.

The interaction of pretreatment process conditions on glucose yield in the enzymatic hydrolysis liquor is shown in Figure A21A. Overall, moderate pretreatment severity, which provided the best balance between complete hemicellulose removal and minimal cellulose degradation, resulted in the highest observed glucose yield. This corresponded to a combination of 1.5 vol% acid and 180^{o}C (for the conventional reactor), and combination of 0.75 vol% acid and 195^{o}C (for the microwave reactor). The lowest observed glucose yields based on conventional pretreatment occurred at high acid loading (1.5 vol%), and a combination of high temperature (195^{o}C) and high residence time ($\geq$5 min). The glucose yields were decreased from its peak as the acid loading was increased. These conditions produced a pretreated substrate that contained low cellulose ratio. Hence, this was less cellulose for the microbes to digest.

The interaction of pretreatment process conditions on normalized glucose yield (g glucose g biomass^{-1}) in the enzymatic hydrolysis liquor is shown in Figure A22A. The highest observed glucose yield based on conventional pretreatment occurred during combination of 0.75 vol% acid and all temperatures and residence times, and combination of 1.5 vol% acid and 165-180^{o}C. The highest observed glucose yield based on microwave pretreatment occurred during a combination of 0.75 vol% acid and 195^{o}C, and a combination of 1.5 vol% acid and 165-180^{o}C temperatures. The lowest observed

glucose yields based on conventional pretreatment occurred at combination of high acid loading (1.5 vol% acid), temperature (195^{o}C) and residence time ($\geq$5 min). These conditions also produced a pretreated substrate that contained a low cellulose ratio. Hence, less cellulose for the microbes to digest.

4.4.2 Glucose yield as a function of biomass composition

Glucose yield from enzymatic hydrolysis is also dependent on the pretreated biomass composition: cellulose, xylan, and lignin content. The microwave reactor yielded up to 166 percent more glucose at equivalent cellulose and xylan portions in the pretreated biomass relative to conventional pretreated samples. Figures A22 and A23 exhibit glucose yields as functions of cellulose and xylan contents for the conventional and microwave reactors, respectively. Higher enzymatic glucose yields were directly related to higher cellulose contents and lower xylan contents in the pretreated biomass. This is due to the fact that hemicellulose hydrolysis increases pore volume in plant cells, and is therefore beneficial for subsequent cellulose hydrolysis.

As previously stated, unpretreated switchgrass contained 30.1 wt% cellulose, 29.3 wt% xylan, and 23.8 wt% lignin. For the conventional reactor, a maximum glucose level of 6 g L^{-1} was found when the cellulose was greater than 70 weight percent and the xylose was less than 10 weight percent. For the microwave reactor, a maximum glucose level of 10 g L^{-1} occurred when the cellulose was greater than 40 weight percent and the xylose was less than 15 weight percent.

The microwave reactor yielded up to 100 percent more glucose at equivalent cellulose and lignin portions in the pretreated biomass. Figure A25 illustrates glucose yields as functions of cellulose and lignin contents for the conventional and microwave reactors, respectively. Higher enzymatic glucose yields were directly related to higher cellulose contents and lower lignin fractions in the pretreated biomass. For the conventional reactor, a maximum glucose level of 7 g L^{-1} was seen when the cellulose was greater than 70 weight percent and the lignin was less than 10 weight percent. For the microwave reactor, a maximum glucose level of 10 g L^{-1} occurred when the cellulose was greater than 60 weight percent and the lignin was less than 10 weight percent.

Table 14: Enzymatic hydrolysis liquor glucose result summary

Condition	Acid loading Vol%	Temperature ^{o}C	Time Min	Conventional Reactor		Microwave Reactor	
				% digestion	g L^{-1}	% digestion	g L^{-1}
1	0	165	1	16 ± 0	1.8 ± 0.1	50 ± 1	5.6 ± 0.2
2	0	180	5	18 ± 0	2.0 ± 0.1	99 ± 1	10.0 ± 0.1
3	0	195	10	23 ± 1	2.6 ± 0.1	96 ± 2	10.0 ± 0.2
4	0.75	165	5	56 ± 1	6.2 ± 0.1	58 ± 1	6.4 ± 0.1
5	0.75	180	10	60 ± 1	6.7 ± 0.1	63 ± 1	7.0 ± 0.1
6	0.75	195	1	45 ± 0	5.0 ± 0.1	99 ± 1	11.0 ± 0.1
7	1.5	165	10	59 ± 1	6.6 ± 0.1	56 ± 0	6.2 ± 0.0
8	1.5	180	1	76 ± 2	8.4 ± 0.2	46 ± 1	5.1 ± 0.1
9	1.5	195	5	4 ± 1	0.4 ± 0.0	0 ± 0	0.0 ± 0.0
Unpretreated				21 ± 1	2.3 ± 0.1	21 ± 1	2.3 ± 0.1

5.0 MODEL

A model that predicts product yield was developed using calculated severity factors and reaction kinetics.

5.1 Combined severity factor

The Combined Severity Factor (CSF) was determined based on reactor temperature, residence time, and pretreatment liquor pH, as outlined earlier in equation 1. This factor is dependent on process conditions, and does not reflect any physical parameter.

5.1.1 Combined glucose yield as a function of combined severity factor

Tables 15 and 16 summarize measured glucose in pretreated switchgrass liquor and hydrolysis liquors, as a function of combined severity factor, respectively. Microwave-pretreated substrates produced higher glucose yields at comparable CSF values in the switchgrass-pretreatment liquor, relative to conventional pretreatment. Glucose increased with CSF, up to a point beyond which glucose levels eroded for both reactors. Highest observed CSF was between 1 and 2, resulting in a 6.3 and 8.8 g l^{-1} glucose yield in the pretreatment liquor for the conventional and microwave reactors, respectively (Figure A24). Glucose degradation predominated when CSF exceeded 2.0.

Table 15: Glucose in switchgrass pretreatment-liquor as a function of combined severity factor

CSF	Conventional Reactor, g/L	Microwave Reactor, g/L
-3.6	0.7 ± 0.0	0.0 ± 0.0
-2.0	0.5 ± 0.0	1.0 ± 0.1
-0.6	0.0 ± 0.0	0 ± 0
1.5	4.5 ± 0.2	5.2 ± 0.0
2.1	2.6 ± 0.1	5.5 ± 0.1
2.5	0.8 ± 0.1	1.0 ± 0.1

Microwave-pretreated substrates also produced higher glucose yields at comparable CSF values in the switchgrass-enzymatic hydrolysis liquor, relative to conventional pretreatment. Highest observed CSF was between –1.0 and 2.0, resulting in yields of 8.0 and 12.2 g L^{-1} of glucose yield in the enzyme-hydrolysis liquor for the conventional and microwave reactors, respectively (Figure A25). The glucose yield also declined once CSF exceeded 2.0 due to low cellulose content in the pretreated biomass.

Table 16: Glucose from switchgrass enzymatic hydrolysis as a function of combined severity factor

CSF	Conventional Reactor, g/L	Microwave Reactor, g/L
-3.6	1.6 ± 0.1	5.0 ± 0.2
-2.0	1.8 ± 0.1	10.9 ± 0.1
-0.6	2.3 ± 0.1	9.6 ± 0.2
1.8	5.9 ± 0.1	12.2 ± 0.2
2.1	6.0 ± 0.1	6.3 ± 0.0
2.5	0.4 ± 0.0	0.0 ± 0.0

The combined glucose yields (pretreatment plus enzymatic-hydrolysis liquor) for both reactors are shown in Figure A26. The glucose reported here is defined as weight of glucose divided by the original biomass weight. This takes into account mass loss in the pretreatment step. Total glucose yield for the conventional reactor is highest observed at

0.20 g glucose g biomass^{-1} (corresponding to a CSF of 1.8). This compares to a highest observed total glucose of 0.31 g glucose g biomass^{-1} (corresponding to a CSF of 1.7) for the microwave reactor.

5.1.2 Xylose yield as a function of combined severity factor

Figure A27 exhibits xylose levels in the switchgrass-pretreatment liquor as functions of pretreatment conditions for both reactors. The xylan hydrolysis reaction requires lower activation energy relative to cellulose hydrolysis. As a result, the hemicellulose is easily removed. A strong relationship was not found between CSF and xylose yield in the pretreatment liquor. However, the peak xylose yield of 6.0 g L^{-1} corresponded to a CSF between 1.5 and 2.0. CSF lower than 1.5 and greater than 2.0 resulted in xylose yields lower than 2.0 g L^{-1}.

5.1.3 Degradation product yield as a function of combined severity factor

Hydroxymethylfurfual

No relationship was evident between CSF and hydroxymethylfurfual (HMF) levels for all three substrates (Figure A28). Peak HMF yields (up to 2.0 g L^{-1}) corresponded to a CSF between 1.2 and 2.8. In contrast, the lowest HMF yield (0.25 g L^{-1} or less) corresponded to a CSF lower than 1.2.

Acetic Acid

Similarly to the hydroxy-methyl-furfual findings, no relationship was evident between CSF and acetic acid yield for the switchgrass liquor. Peak acetic acid yields (greater than 6.0 g L^{-1}) corresponded to a CSF greater than 1.2 (Figure A29). However, even higher acetic acid yields resulted under certain conventional and microwave reactor conditions (CSF- 2.2). The lowest acetic acid yield (3.4 g L^{-1} less) corresponded to a CSF lower than 1.2.

5.2 Kinetic model

Development of a kinetic model for predicting the glucose yield is important for reactor design, understanding reaction parameters, and estimating costs.

The Arrhenius relationship for general acid-base catalysis was used to determine the kinetic parameters and model the cellulose and xylan hydrolysis to glucose, as shown in equation 3:

(3) $$k = \left[k^{o} + k^{H}\left(\left[H^{+}\right]\right) + k^{OH}\left(\left[OH^{-}\right]\right)\right]e^{\frac{-E}{RT}}$$

where

- k_i (min^{-1}) is the overall reaction constant
- k^o_i (min^{-1}) is the solvent factor
- k^H_i (min^{-1} M) is the acid factor
- k^{OH}_i (min^{-1} M) is the base factor
- $[H^+]$ is the molal hydrogen-ion concentration,
- E_i (kcal / g mol) is the activation energy (energy that must be overcome in order for a chemical reaction to occur)
- R is the gas constant, 1.98 cal K^{-1} mol^{-1}
- T is the reaction temperature (Kelvin)

Most lignocellulosic pretreatment references in the literature have focused on determining only xylan-hydrolysis kinetics (Schell, 2003). Experimental mass-balance and chemical-composition data were used to determine the kinetic parameters for the cellulose and xylan hydrolysis reactions (and resulting degradation reactions). Since we focused here on acidic pretreatment conditions (pH <2) the hydroxyl-ion term was assumed to be minimal and rewritten as the hydrogen-ion concentration in terms of the pH. Liquor pH has been shown to be more appropriate than using the effective acid concentration, which could effectively be zero if there is insufficient acid. The final pH takes into account the absorption capacity of the substrate. (Schell, 2003)

(4) $k = \left[k^o + k^H \left(10^{-pH}\right)\right] e^{\frac{-E}{RT}}$

The rate constant represents a transformation between two states (the reaction) that is controlled by an intermediate high-energy excited state, it can be said that the activation energy (E) represents the energy difference between the initial state and the intermediate state (activated species). The $\left[k^{o}+k^{H}\left(10^{-pH}\right)\right]$component corresponds to the conventionally used "pre-exponential factor". In this case, the parameter $\left[k^{o}+k^{H}\left(10^{-pH}\right)\right]$ represents the frequency of collisions between the reactants and their orientation. It is often taken as constant across small temperature ranges (Schwaab, 2007).

In this study, several assumptions were made. First, we assumed that the reaction is biphasic cellulose and hemicellulose hydrolysis, therefore focusing on the rate-limiting step (conversion of the slow crystalline polysaccharide). Second, we assumed that there was a single activation energy for the reaction. Results from this study show pre-exponential factors as high as 10^{17} min^{-1}, which represents relatively high collisions, but comparable to factors reported in similar and previous studies found in the literature. Schell (2003) reported pre exponential factors as high as 10^{30} min^{-1}. Maloney (1984) reported pre exponential factors as high as 10^{19} min^{-1}.

The model was developed using a nonlinear-regression analysis software (LAB Fit Curve Fitting Software; Paraiba, Brazil).

5.2.1 Glucose yield in the pretreatment liquor

Table 17 summarizes the kinetic constants for glucose formation in the switchgrass-pretreatment liquor. Fitting experimental glucose yield results to equation 4 resulted in a correlation coefficient of 0.96. The solvent factor and acid factor for the 0.75 vol.% acid loading conditions were 4.65×10^{17} and $6.11\text{x}10^{17}$ min^{-1}, and 6.26×10^{17} and 7.20×10^{17} min^{-1} for the conventional and microwave reactor, respectively. The solvent factor and acid factor for the 1.5 vol.% acid loading conditions are 8.06×10^{9} and $6.54\text{x}10^{12}$ min^{-1}, and -7.67×10^{10} and -3.16×10^{13} min^{-1} for the conventional and microwave reactors, respectively. The activation energies for the 0.75 and 1.5 vol% acid catalyst conditions are 35.2 and 23.5 kcal / g mol, respectively.

Table 17: Kinetic constants for the glucose formation in the switchgrass pretreatment liquor

Acid loading	Reactor	k^o min^{-1}	k^H $min^{-1}\cdot M^{-1}$	R^2
0.75	Parr	$4.65\text{x}10^{17}$	$6.26\text{x}10^{17}$	0.962
0.75	CEM	$6.11\text{x}10^{17}$	$7.20\text{x}10^{17}$	0.963
1.5	Parr	$8.06\text{x}10^{9}$	$-7.67\text{x}10^{10}$	0.981
1.5	CEM	$6.54\text{x}10^{12}$	$-3.16\text{x}10^{13}$	0.886

The model suggests that the microwave reactor theoretically release glucose at a faster rate than the conventional reactor at comparable process conditions. This coincides with the reports that the kinetics of acid hydrolysis of cellulose are strongly dependent on the state of hydrogen bonding (Xiang, 2003). The nonthermal microwave effects provide

additional energy required to overcome the hydrogen bonding within the glucan chain, thus easier glucose release (Hu, 2007).

5.2.2 Xylose yield in the pretreatment liquor

Table 18 summarizes the kinetic constants for the xylan hydrolysis in the switchgrass-pretreatment liquor. Fitting experimental cellulose yield results to equation 4 resulted in a correlation coefficient of 0.91. The solvent factor and acid factor for the 0.75 vol.% acid loading conditions were 5.39×10^{4} and $5.39x10^{3}$ min^{-1}, and $-9.82x10^{5}$ and $-2.53x10^{4}$ min^{-1} for the conventional and microwave reactor, respectively. The activation energies for the 0.75 and 1.5vol% acid catalyst conditions are 10.0 and 0 kcal / g mol respectively. Yat (2008) reported an activation energy of 10.0 kcal / g mol for similar acid catalyst to switchgrass loading.

The xylan hydrolysis has a significantly lower activation energy requirement relative to cellulose hydrolysis, which explains its relatively easy removal from the biomass.

Table 18: Kinetic constants for the xylose formation in the switchgrass pretreatment liquor

Acid loading	Reactor	k^{o} min^{-1}	k^{H} $min^{-1}\cdot M^{-1}$	R^2
0.75	Parr	$5.39x10^{4}$	$-9.82x10^{5}$	0.912
0.75	CEM	$5.39x10^{3}$	$-2.53x10^{4}$	0.924

5.2.3 Degradation product levels in the pretreatment liquor

Hydroxymethylfurfual

Table 19 summarizes the kinetic constants for hydroxymethylfurfual (HMF) formation in the switchgrass-pretreatment liquor. Fitting experimental HMF yield results to equation 4 resulted in a correlation coefficient of 0.96. The solvent factor and acid factor for the 0.75 vol.% acid loading conditions were $1.20x10^{13}$ and $3.85x10^{13}$ min^{-1}, and $–1.43x10^{14}$ and $–4.19x10^{14}$ min^{-1} for the conventional and microwave reactor, respectively. The solvent factor and acid factor for the 1.5 vol.% acid loading conditions are $3.25x10^{12}$ and $4.91x10^{12}$ min^{-1}, and $–3.05x10^{13}$ and $–2.38x10^{13}$ min^{-1} for the conventional and microwave reactors, respectively. The activation energies for the 0.75 and 1.5 vol% acid catalyst conditions are 30.2 and 28.1 kcal / g mol, respectively.

Table 19: Kinetic constants for the HMF formation in the switchgrass pretreatment liquor

Acid loading	Reactor	k^o min^{-1}	k^H $min^{-1} \cdot M^{-1}$	R^2
0.75	Parr	$1.21x10^{13}$	$-1.43x10^{14}$	0.973
0.75	CEM	$3.85x10^{13}$	$-4.19x10^{14}$	0.927
1.5	Parr	$3.25x10^{12}$	$-3.05x10^{13}$	0.961
1.5	CEM	$4.91x10^{12}$	$-2.38x10^{13}$	0.917

The model suggests that the microwave reactor theoretically produced HMF at a faster rate than the conventional reactor at comparable process conditions. This can be attributed to the overall faster reaction rates associated with microwave heating.

Acetic acid

Table 20 summarizes the kinetic constants for acetic acid formation in the switchgrass-pretreatment liquor. Fitting experimental acetic acid yield results to equation 4 resulted in a correlation coefficient of 0.93. The solvent factor and acid factor for the 0.75 vol.% acid loading conditions were 5.37×10^{10} and 1.98×10^{12} min^{-1}, and 2.99×10^{12} and -1.97×10^{13} min^{-1}, for the conventional and microwave reactors, respectively. The solvent factor and acid factor for the 1.5 vol.% acid loading conditions were 2.88×10^{7} and 1.87×10^{9} min^{-1}, and -2.65×10^{8} and -8.58×10^{9} min^{-1} for the conventional and microwave reactors, respectively. The activation energies for the 0.75 and 1.5 vol% acid catalyst conditions are 25.0 and 17.5 kcal / g mol, respectively.

Table 20: Kinetic constants for the acetic acid formation in the switchgrass pretreatment liquor

Acid loading	Reactor	k^o min^{-1}	k^H $min^{-1}\cdot M^{-1}$	R^2
0.75	Parr	$5.37x10^{10}$	$-2.99x10^{12}$	0.936
0.75	CEM	$1.98x10^{12}$	$-1.97x10^{13}$	0.836
1.5	Parr	$2.88x10^{7}$	$-2.65x10^{8}$	0.991
1.5	CEM	$1.87x10^{9}$	$-8.58x10^{9}$	0.908

The model suggests that the microwave reactor theoretically yielded acetic acid at a faster rate than the conventional reactor at comparable process conditions. This can also be attributed to the overall faster reaction rates associated with microwave heating.

6.0 OVERALL MASS, ENERGY, AND ECONOMIC ANALYSES

A mass-and-energy balance of the flows entering and exiting each step of the pretreatment process and bioreactor was conducted (Figure A30). Switchgrass, at a 100 kg hr^{-1} feed basis, is delivered to the feed-handling area for storage and size reduction. Next, the biomass is conveyed to pretreatment and conditioning. Here, the biomass is fed at 4 wt% and treated with dilute sulfuric acid (0.75 vol.%) at a high temperature (195^{o}C) for a very short residence time (1 minute), liberating the hemicellulose sugars and other compounds. Next, ion exchange and/or over-liming are required to remove compounds liberated in the pretreatment that will be toxic to the fermenting organism(s). The pretreated solids are fed to the hydrolysis step for glucose recovery and microbial digestion.

6.1 Mass Balance

The products yielded – polysaccharides, monosaccharides, and degradation products – were assessed for the pretreatment liquor and the solid residue. Polysaccharides included cellulose and hemicellulose (xylan). Monosaccharides included glucose and xylose. Degradation products included xylitol, succinic acid, acetic acid, and hydroxymethylfurfual. Acid-soluble and -insoluble lignin were also quantified. Table 21 summarizes the mass flows entering and exiting the pretreatment process.

Table 21. Mass balance for the pretreatment process

Flow	Component	Mass kg hr^{-1}
A	Raw switchgrass	100
B	Milled switchgrass	100
C	Sulfuric acid solution	2,500
D	Lime	15
E	Pretreated slurry	2,611
F	Cellulase enzyme solution	38
H	Hydrolysis solution	2,649

6.2 Energy Balance

An energy balance on the pretreatment process was conducted using equation 5:

(5) $\Delta H = \Delta E_p + \Delta E_k = Q + W_s$

where

- ΔH is the change in enthalpy
- ΔE_k is the change in potential energy due to motion of the system
- ΔE_p is the change in kinetic energy due to the position of the system
- Q is the energy flow due to temperature difference
- W_s is the energy flow due to the driving force other than temperature difference (force, torque, voltage, etc.)

Since the process involves chemical equipment (i.e., reactor, distillation column, evaporator, heat exchanger, etc.), we assumed the following:

- Heat flow and internal energy changes (enthalpy change) are the most important; and
- Shaft work, kinetic energy, and potential-energy changes are negligible.

$$Q = \Delta H$$

$$Q = m_i C_p \Delta T$$

where

- m_i is mass flow rate for stream *i*
- C_p is the specific heat capacity for stream *i*
- ΔT is the temperature difference

Table 22 summarizes the energy content for each flow, and overall energy balance (3.09×10^5 kJ hr^{-1}). The heating value for switchgrass based on elementary composition was estimated to be 1.85×10^4 kJ kg^{-1}.

Table 22. Energy balance for the pretreatment process

Stream	Mass Kg hr^{-1}	Specific Heat kJ (kg K)$^{-1}$	Temperature K	Q kJ hr^{-1}
A	100	1.85	298	
B	100	1.85	298	0
C	2,481	4.18	298	
D	15	1.18	298	
E	2,611	4.06	468	1,810,271
F	38	4.18	298	
G	2,649	4.10	323	-1,500,373
Total				309,899

6.3 Economic Analysis

The feasibility of new energy crops will depend largely on production costs, costs of converting the biomass to usable energy, and costs of competing fuels. For biomass crops to compete with other fuels, they must be grown in the least costly manner so farmers can derive a benefit equal to or greater than with food crops.

An economic analysis using a 100 kg hr^{-1} biomass feed rate as the basis for the pretreatment system is presented. The cost assessment considered the following process steps:

- Harvest
- Delivery
- Milling
- Pretreatment
- Enzymatic hydrolysis

The microwave pretreatment process has a higher investment, lower operating cost, and higher operating income, relative to the conventional pretreatment process. The investment cost for the conventional-batch, conventional-continuous, microwave-batch, and microwave-continuous pretreatment process was estimated at $1.38, $1.53, $1,88, and $1.88 million dollars, respectively. The annual operating cost for the conventional-batch, conventional-continuous, microwave-batch, and microwave-continuous

pretreatment process was estimated at $689,294, 576,907, $741,564, and $626,177 respectively. The operating income for the conventional-batch, conventional-continuous, microwave-batch, and microwave-continuous pretreatment process was estimated at ($465,266), ($343,668), ($405,631), and ($276,493) respectively. The operating income does not include co-product credits such as excess electricity, use of lignin as boiler fuel, use of recycle water, etc. Comprehensive investment and operating costs for both reactor systems are outlined in sections 6.3.1 through 6.3.7 and summarized in Figure A31.

6.3.1 Harvest

Maintaining high forage yields and keeping costs low results in the best economic returns. Switchgrass is not commonly grown as an energy crop but can be harvested in high yields. The seeds for switchgrass are estimated to cost $7.72 kg^{-1}. Seed prices for other perennial grasses are shown in Table 23. (Hallam, 2001)

Table 23. Seed price for selected perennial grasses

	Unit	$
Switchgrass	Kg	7.72
Sweet sorghum	Kg	1.10
Forage sorghum	Kg	0.77
Maize	100 kernels	0.90
Big bluestem	Kg	19.84
Reed canarygrass	Kg	9.92
Alfalfa	Kg	5.51

The rents for grasslands and croplands were assumed to be $124 ha^{-1} $year^{-1}$ and $185 ha^{-1} $year^{-1}$, respectively. Hence, the land rents per dry Mg switchgrass used in this

study were $11.27 and $16.82 for grasslands and croplands, respectively, assuming a switchgrass-production yield of 11 dry Mg ha^{-1} $year^{-1}$. The production costs, excluding the harvest and storage, for switchgrass planted in croplands and grasslands were $44.24 dry Mg^{-1} and $36.83 dry Mg^{-1}, respectively, at the same yield of 9 dry Mg ha^{-1} $year^{-1}$. These production costs were then adjusted to $36.17 dry Mg^{-1} and $30.10 dry Mg^{-1}, respectively, for the yield of 11 dry -Mg ha^{-1} $year^{-1}$. The switchgrass harvest cost at the yield of 11 dry Mg ha^{-1} in square bales was assumed to be $24.10 dry Mg^{-1}. This includes mowing, raking, baling, transporting the bales to the edge of field and stacking, etc. (Kumar and Sokhansanj, 2007).

6.3.2 Delivery

The delivered cost for switchgrass is composed of land costs (or farmer premium), production/farming, harvest, storage, and transportation costs. Switchgrass (at 15 wt% moisture) is typically delivered in bales. The transportation cost is comprised of fixed and variable distance costs. Fixed distance cost includes the costs associated with loading, uploading and stacking; variable distance cost is dependent on hauling distance.

. Table 24 summarizes the total costs for delivered switchgrass. The storage costs for switchgrass were estimated to be $8 dry Mg^{-1} per year assuming that the switchgrass is stored in dense, square bales. The fixed distance cost of transportation covering the costs of loading, unloading, and stacking is $3.74 dry Mg^{-1}. The approximate total delivered cost is then $77.21 dry Mg^{-1} (Huang, 2008).

Table 24. Total feedstock cost

	$ dry Mg^{-1}
Farmer premium/land rent	11.27
Fertilizer cost	-
Production/farming/stumpage	30.10
Collection/harvest	24.10
Storage	8.0
Grinding/chipping	-
Distance fixed cost	3.74
Total cost	77.21

6.3.3 Milling

Natural switchgrass must be milled to less than 10 mm in size for highest observed conversion. The finer size is necessary to maximize the surface area for microbial digestion. (Jannasch et al. 2001)

A Schutte-Buffalo Hammer Mill Model 1320 was quoted by Schutte-Buffalo (Orlando, Florida). This unit can be used to mill one-meter-tall switchgrass down to 5 mm. This unit operates on 40 HP, 3/60/460/3,600 rpm TEFC motor, direct-connected with guard, and is manufactured from ½" A-36 plate steel mounted on a structural steel sub-base. The bottom pan for connection is integrally mounted, 16" in diameter, and has a 3,000-CFM fan. The estimated capital cost for this equipment is $17,725. The operating cost for a 40-HP unit operating 24 hours per day at $0.0935 kWhr^{-1} is $2.71 hr^{-1}.

6.3.4 Pretreatment

The material costs for pretreatment are presented in Table 25. Sulfuric acid is used as the pretreatment catalyst for converting the hemicellulose to xylose. Lime is used to neutralize the pretreatment liquor. At a 100-kg hr^{-1} biomass feed rate, approximately 8.2 $x10^3$ kg hr^{-1} of pure sulfuric acid (making a 2,500-Gallon, 0.75 vol% sulfuric acid solution) and 6.6 $x10^3$ kg hr^{-1} of calcium hydroxide is required for pretreating a 4 wt% biomass slurry.

Table 25. Pretreatment chemical cost

	Source	Cost, \$ kg^{-1}
Sulfuric acid (99%) cost, \$ kg^{-1}	Chemical Marketing Reporter, 2009	0.242
Lime cost, \$ kg^{-1}	Chemical Marketing Reporter, 2009	0.154
Water	Chemical Marketing Reporter, 2009	0.0004

Investment and Utility Cost

The lignocellulose-to-ethanol process requires electricity, steam, and a cooling-water supply. Steam is required in the pretreatment step to deliver heat and in distillation. The temperature of the biomass slurry must be elevated from room temperature to the target temperature (195^oC). Cooling and chilled water is used to adjust the temperature of the process streams. The pretreatment liquor can be cooled to room temperature before off-site separation. There are two different reactor types for consideration- batch and continuous.

Batch vs. Continuous Reactor

Continuous flow reactors are used to mix and heat ingredients continuously in a reactor in a single pass. In a continuous reactor, the weighing, loading, mixing, heating, and discharge steps occur continuously and simultaneously. Continuous heating is preferred for applications where:

- Large quantities of a single product are to be mixed.
- In a continuous process line requiring high production rate.
- Strict batch integrity is not critical.
- Smoothing out batch product variations is required.

The advantages of the continuous heating operation, continuous reactor are as follows:

- High Capacity - Compared to batch reactors, continuous reactors of smaller volumes and power can be used to produce large quantities of uniform mix. Hence for a given capacity they are more compact than batch reactor.
- Lower Mixing Time - The residence time in continuous reactor is lower than in batch reactor.
- Consistent Mixing Performance – With proper feeding arrangements, online instrumentation and operation controls, a consistent mixing performance and uniform product quality can be achieved

- Suitability for Automatic Control - Operation of continuous reactor can be automated using online monitoring and measuring instruments
- Minimum Segregation – Continuous reactors can reduce and control segregation of products as they can be located in proximity of the next processing station.
- Lower Cost of Mixers - Continuous reactors tend to be cheaper than the equivalent batch mixers because they are compact and require less space. However the cost of feeders for metering the product into the reactor, instrumentation and control may result in a higher overall cost of the system.
- Minimum Labor – Since material feeding and discharging processes are automated, minimal labor is required for continuous reactions.

(Tekchandaney, 2009)

Tables 27 and 28 summarize the investment and operating utility requirements to support the pretreatment step for the conventional and microwave reactor systems, respectively.

Conventional Reactor

Investment and operating cost for conventional batch and continuous pretreatment are presented in Tables 26 and 27. A 1,320-Gallon, 316 stainless steel, steam jacketed and agitated reactor vessel can be used to react the contents in the batch reactor. This vessel (3 ft radius, 6 ft height, and 1 ft wall thickness) is capable of withstanding 600-psi internal pressure, and allows for up to 50% volume expansion. The estimated capital cost for this system is $500,000. A shell-tube, fixed U/large 316 stainless steel heat exchanger

(600-psi internal pressure) with 8,333 ft^2 of heat transfer area was used for the continuous reactor. The estimated capital cost for this system is $649,000. A 1,000 lb. hr^{-1} boiler capable of producing 600-psi, 230^oC steam was estimated at $447,000. A forced-draft cooling tower with a 1.7-million BTU hr^{-1} cooling load was estimated at $126,000. (Matte, 2009)

Table 26- Investment and operating cost for conventional batch pretreatment

	Specifics	Capital	Operating Cost $ yr^{-1}
Reactor vessel	1,320 gallon SS 316 600 psi	$500,000	-
Boiler	1,000 lb. hr^{-1} 600 psi steam	$447,000	21,725
Cooling	1.7 million BTU hr^{-1}	$126,000	2,540
Electricity			453,518

Table 27- Investment and operating cost for conventional continuous pretreatment

	Specifics	Capital	Operating Cost $ yr^{-1}
Reactor vessel	Shell-tube 8,333 ft^2 SS 316 600 psi	$649,000	-
Boiler	1,000 lb hr^{-1} 600 psi steam	$447,000	21,725
Cooling	1.7 million BTU hr^{-1}	$126,000	2,540
Electricity			453,518

Assumptions (McAloon, 2000):

- Steam @ 230^oC, Enthalpy 1,205 BTU lb^{-1}

  Estimated cost $2.12 $(1{,}000\ lb)^{-1}$

- Cooling water @ 15^{o}C, Enthalpy 30 BTU lb^{-1}

  Estimated cost \$0.05 (1,000 lb)$^{-1}$
- Electricity cost, \$0.08 per kilowatt-hour (kWh) with a 70% efficiency
- The continuous reactor's product throughput was estimated to be at least 50% higher relative to the batch reactor (Moseley, 2009).

Microwave Reactor

Industrial Microwave Systems (Morrisville, NC) quoted a batch and continuous microwave reactor. The batch reactor uses a 1,300-Gallon ceramic vessel for reacting the contents. The continuous reactor is based on 6-Gallon min^{-1} (1,308 kg hr^{-1}) total feed rate system, and is one of the largest continuous microwave reactor available. Heating these contents to the reaction temperature (195^{o}C) would require 250 kW. To provide 250 kW of absorbed microwave power, this would require three 100 kW generators. The estimated price for the both systems are \$650,000, which includes a control system, three 100 kW microwave generators, three stainless steel applicators with high-pressure 2"-diameter ceramic tubes, and a support frame. When scaling of equipment, the new cost of the scaled equipment can be determined according to the following scaling expression:

$$(6)\ C_{new} = C_o \times \left(\frac{S_{new}}{S_o} \right)^f$$

where

- C_{new} and C_o are the new cost and the original cost, respectively
- S_{new} and S_o are the new size and the original size, respectively
- f is the capital cost scaling factor or exponent.

In this analysis f = 0.6.

$$C_{new} = \$650{,}000 \times \left(\frac{5{,}000kg}{1{,}308kg} \right)^{0.6}$$

$$C_{new} = \$1{,}453{,}000$$

The investment and operating cost for the microwave batch and continuous pretreatment reactors are shown in Tables 28 and 29. The continuous reactor's product throughput was estimated to be at least 50% higher relative to the batch reactor (Moseley, 2009). The microwave's electricity is assumed to be 90%.

Table 28: Investment and operating cost for microwave batch pretreatment

	Specifics	Capital	Operating Cost $ yr^{-1}
Reactor vessel	1,300-Gallon Ceramic vessel	$1,453,000	-
Cooling	1.7 million BTU hr^{-1}	$126,000	$2,540
Electricity			$352,736

Table 29: Investment and operating cost for microwave continuous pretreatment

	Specifics	Capital	Operating Cost $ yr^{-1}
Reactor vessel	5,000 kg hr^{-1} Ceramic tube	$1,453,000	-
Cooling	1.7 million BTU hr^{-1}	$126,000	$2,540
Electricity			$352,736

6.3.5 Enzymatic Hydrolysis

Cellulase enzyme is required to drive the cellulose to glucose reaction (enzymatic hydrolysis). The operating conditions are shown in Table 30. At a 100 kg hr^{-1} biomass feed rate, approximately 16 kg hr^{-1} of *Trichoderma reesei* cellulase is required for operation. The current estimate cost for cellulase ranges from 30 to 50 cents per gallon of ethanol produced. Research is underway with the objective of reducing cellulase cost to less than 5 cents per gallon of ethanol (US Department of Energy, 2005). Suszkiw (2008) reports that one ton of switchgrass produces approximately 90 gallons of ethanol. This corresponds to a long term cellulase cost of $0.0727 kg^{-1}.

Table 30: Major operating conditions for enzymatic hydrolysis

	Condition	
Enzymatic hydrolysis	Cellulase loading	60 FPU g^{-1}
	Initial saccharification	4% total solids
	Temperature	323 K (50^{o}C)
	Total residence time	36 hours

The capital cost for five 1,000-Gallon 316 stainless steel tanks is $290,000. (Matche, 2009) Investment and operating costs for the enzymatic hydrolysis step are shown in Table 31.

Table 31: Investment and operating cost for enzymatic hydrolysis

	Specifics	Capital ($)	Operating Cost ($ yr^{-1})
Hydrolysis vessels	Five 1,000 gallon tanks	290,000	-
T reesei cellulase	16 kg hr^{-1}	-	26,111

6.3.6 Waste-stream outlet

The pretreatment liquor contains numerous constituents, such as unconverted polysaccharides, monosaccharides (e.g., xylose), acid-neutralization salts, and other byproducts. An assessment of the product separation cost, outlet opportunities, and product value (i.e. xylose fermentation) was performed. Table 32 summarizes the waste stream outlet potential.

Lignin can be used for boiler fuel, in addition to conversion to a higher-value co-product (i.e. fuel or chemical). To be beneficial, the value of the lignin-derived co-product must be enough to cover the costs of the upgrade process and still supply revenue to the plant to offset the biofuel production costs (Das, 2000).

The most effective approach for recovering the various lignin fractions involves cooling the liquor and filtering out the soluble lignin that precipitates upon cooling. This

accounts for approximately one-third of the total soluble lignin. The remaining lignin can then be removed using an adsorbent. The adsorbed lignin can be removed by treating the adsorbent in a furnace. This allows for recovery of the heat content of the solubilized lignin and regenerates the catalyst for reuse. Conventional extractive methods can be used to remove the adsorbed lignin compounds in a manner such that the compounds can eventually be upgraded to fuel components (Das, 2000).

The other constituents, such as the cell matter, xylose, xylitol, furfural, and acetic acid, have been identified as potential co-products of the biofuel process. Interstitial cell matter could be valuable, but might require significant purification. Markets for xylose (xylose fermentation to ethanol), furfural (petrochemical refining solvent), xylitol (sweetener) and acetic acid (vinegar) are in place. Traditional methods for recovering low-volatility acetic acid and other carboxylic acids involve formation of the insoluble calcium carboxylate salt (Grzenia, 2008). Succinic acid can be recovered using amine-based extraction (Hong, 2005).

Gypsum is a very soft mineral composed of calcium sulfate dihydrate. This compound is formed when lime reacts with the sulfuric acid, and can be used as a finish for walls and ceilings, fertilizer, or soil conditioner.

Table 32: Waste stream potential based on a 100-kg hr^{-1} feed-rate plant

By-product	Potential, kg yr^{-1}	Market price \$ kg^{-1}	Potential revenue \$ yr^{-1}
Lignin	210,240	Varied	\$126,144
Xylose	44,676	0.08	3,574
Xylitol	-	20.6	-
Furfural	10,249	1.70	17,424
Acetic acid	134,116	0.90	120,704
Gypsum	236,520	0.14	15,374

6.3.7 Financial summary

The financial attractiveness of the different pretreatment projects was assessed using the payback period and net present value methods.

The payback method of financial appraisal, used to evaluate capital projects, calculates the return per year from the start of the project until the accumulated returns are equal to the cost of the investment, at which time the investment is said to have been paid back. The time taken to achieve this payback is termed the payback period. Under this method the required payback period sets the hurdle rate (threshold barrier) for project acceptance. (Lefley, 1996) Equation 7 shows the payback period calculation.

(7) $$Payback.period = \frac{\text{Investment required}}{\text{Net annual savings}}$$

Here, the investment required is the capital cost differential between the proposed pretreatment process and the conventional-batch process. The net annual saving is the net cost differential between the two processes.

A project's financial benefit can also be measured by its net present value (NPV), which is determined by discounting all arising cash flows (at an assumed cost of capital) to the start time of the project. As such, the NPV can be regarded as the 'cash equivalent' of undertaking the project. (Wiesemann, 2009) Equation 8 shows the net present value calculation.

$$(8) \qquad NPV = \frac{\text{Net cash flow (or relative savings)}_t}{(1+i)^t}$$

Here, t is the year, and *i* is the cost of capital. A six year time horizon was used for the net present value (NPV) analysis. We assumed that the cost savings relative to the conventional-batch pretreatment process to be the net cash flow, and a cost of capital of 8 percent. (Table 33).

The detailed financial summaries for the four pretreatment processes are shown in Table 34.

Table 33: Financial summary for the pretreatment reactor systems.

		Conventional-Batch		Microwave-Batch		Conventional-Continuous		Microwave-Continuous	
	Cost per unit	*Flow Rate*	*Operating Cost*	*Flow Rate*	*Operating Cost*	*Flow Rate*	*Operating Cost*	*Flow Rate*	*Operating Cost*
		kg/hr	*$/yr*	*Kg/hr*	*$/yr*	*kg/hr*	*$/yr*	*kg/hr*	*$/yr*
Revenue	$/kg								
Ethanol	$0.61	7.8	$35,160	9.9	$44,332	11.8	$52,714	14.8	$66,465
Xylose	$0.08	3.4	$2,384	3.4	$2,384	5.1	$3,574	5.1	$3,574
Acetic acid	$0.90	10.2	$80,510	10.2	$80,510	15.31	$120,704	15.31	$120,704
HMF	$1.70	0.8	$11,622	0.8	$11,622	1.17	$17,424	1.17	$17,424
Gypsum	$0.07	18.0	$10,254	18.0	$10,254	27	$15,374	27	$15,374
Lignin	$0.60	16.0	$84,138	16.0	$84,138	24	$126,144	24	$126,144
Total Revenue			$224,067		$233,240		$335,933		$349,685
Operating Cost									
Fixed	$/hr								
Labor	$5	1.0	$43,350	1.0	$43,350	1	$43,350	1	$43,350
Administration	$10	0.0	$0	0.0	$0	0	$0	0	$0
Depreciation			$27,600		$37,720		$30,600		$37,720
Variable	$/kg								
Switchgrass	$0.08	66.7	$45,113	66.7	$45,113	100	$67,636	100	$67,636
Sulfuric acid	$0.24	12.5	$26,583	12.5	$26,583	18.8	$39,854	18.8	$39,854
Lime	$0.15	10.7	$14,397	10.7	$14,397	16	$21,585	16	$21,585
Water	$0.0004	1654.8	$5,799	1654.8	$5,799	2481	$8,693	2481	$8,693
Cellulase	$0.07	10.7	$6,796	10.7	$6,796	16	$10,190	16	$10,190
Electricity (Hammer mill)	$0.08		$23,652		$23,652		$23,652		$23,652
Electricity (Microwave)	$0.08				$352,736				$352,736
Electricity (Boiler)	$0.08		$453,518				$453,518		
Steam	$0.0021		$21,725		$0		$21,725		$0
Cooling water	$0.0500		$20,761		$20,761		$20,761		$20,761
Total Cost			$689,294		$576,907		$741,564		$626,177
Net			-$465,226		-$343,668		-$405,631		-$276,493

Assumptions:

- Feed: 4 wt% solids
- Throughput: Continuous processes produce at 50% higher throughput relative to batch processes
- Revenue: based on market price estimates for ethanol and waste stream products
- Energy efficiency: Microwave heating processes are 90% energy efficient. Conventional heating processes are 70% energy efficient
- Labor: shared labor
- Inflation: 3%
- Depreciation: straight line over 50 years

Table 34 shows the payback period relative to the conventional-batch pretreatment process. Overall, the microwave pretreatment processes yielded lower payback periods (2.6 years average) relative to the conventional pretreatment process (4.2 years). This is attributed to two factors: higher revenue (due to relatively higher glucose/ethanol throughput), and lower cost (due to microwave's lower energy usage). A payback period of less than 3 years is typically the approval threshold for most industry capital projects.

Table 34: Payback period analysis

	Payback period, Years
Conventional-batch	Baseline
Conventional-continuous	4.2
Microwave-batch	2.5
Microwave-continuous	2.7

Table 35 shows the net present value relative to the conventional-batch pretreatment process. Overall, the microwave pretreatment process yielded higher net present values relative to the conventional pretreatment processes. The microwave-continuous processes had the highest NPV of all designs ($366,941). This is attributed to the 50% higher throughput associated with continuous vs. batch processes, 26% higher glucose/ethanol yield and 20% higher energy efficiency associated with microwave vs. conventional processes.

Table 35: Net present value analysis

	NPV $
Conventional-batch	Baseline
Conventional-continuous	$55,948
Microwave-batch	$125,501
Microwave-continuous	$366,941

6.3.8 Outlook

The outlook and scale-up potential for microwave pretreatment is still in its infancy. Commercial outlook is best realized through the scale up of a continuous microwave reactor system.

The scalability of the microwave technology has been limited. Presently, the manufacturers are directing their research to develop products that can increase the yield

volume substantially. These new products have been successful in augmenting the scale of reactions from the level of 0.2 mL to 1,000 mL. Design concepts, although not commercially available, have shown promise to achieve volumes near 1,500 kg hr^{-1}. However, scalability and cost effectiveness to the level of industrial production has still not been achieved, which questions the commercial viability of microwave chemistry.

In addition, there is a demand for a further increase in the rate of reaction. Consequently, instrument manufacturers are developing prototypes that will be able to achieve high-pressure conditions inside the reaction vessel, resulting in an increased rate of reaction. Other areas of research include design modifications in the existing equipment, to provide safer reaction conditions; and development of equipment that can be used for chemical analysis as well as chemical synthesis.

7.0 CONCLUSIONS

Switchgrass and other lignocellulosic feedstocks offer promise as a renewable energy source for biofuel production. However, a primary technological challenge in converting switchgrass into fuel is overcoming the recalcitrance of its matrix to enzymatic hydrolysis. To overcome these problems for chemical processing, naturally occurring lignocellulosic biomass must be pretreated before it can be further processed using enzymatic hydrolysis or bioconversion. Two pretreatment reactor types were evaluated for effectiveness- conventional heated and microwave radiation.

Conventional chemical heating, which is based on conduction mechanisms, has been reported to be a slow and inefficient heating method. Microwave radiation, which is based on direct interaction between the heated object and an applied electromagnetic field, has been reported to offer more uniform heating, good temperature control, and better yields. This project thoroughly and directly compared the effectiveness of these two pretreatment reactors. A Taguchi design experiment was useful in evaluating the effect of process conditions (sulfuric acid loading, temperature, and residence time) on desirable and undesirable product yield for both reactor types. The primary conclusions from this study are:

1. Microwave pretreatment is a more effective cellulose and switchgrass pretreatment technique than conventional heating chemical pretreatment due to the acceleration of

reactions during the pretreatment process. Target reaction temperatures were reached up to ten times faster than conventional heating. This offers the potential for higher throughput upon scale-up.

2. Microwave pretreatment offered up 100 percent higher total glucose yield (in the pretreatment and enzymatic hydrolysis steps) at comparable pretreatment severity relative to conventional heating. This could translate into higher fuel output at lower power and energy requirements relative to conventional heating.
3. Microwave's more efficient and target heating contributed to rapid cleavage of the glycosidic bonds, resulting in higher glucose yield in the pretreatment step.
4. Microwave pretreated switchgrass samples were more porous relative to conventional pretreated samples (as observed from SEM photographs). These findings support literature reported microwave induced non-thermal effects, which cause fiber separation and expose more accessible surface area of cellulose to cellulase.
5. Acid loading had the greatest influence on final glucose yield, followed by temperature and residence time. Increasing acid loading drove polysaccharide hydrolysis, resulting in higher glucose yield and hemicellulose removal in the pretreatment step, higher cellulose ratio in pretreated samples, and the potential for higher degradation product yield at 1.5 vol%. Best acid loading over the experimental range was at 0.75 vol%.
6. Temperature assisted the cellulose hydrolysis reaction, but also drove thermal degradation. High temperature ($\geq 180^{o}C$) and low residence time (1 min) was more effective on releasing glucose than low temperature ($<180^{o}C$) and high residence time ($\geq$5 min).

The highest observed total glucose yield (99% conversion) was found under 0.75 vol% sulfuric acid, 195^{o}C temperature, and 1 min residence time conditions. Based on these conditions, theoretical ethanol yields for microwave-pretreated switchgrass were calculated using NREL's ethanol yield calculator. Theoretical ethanol yields are 50 gallons per dry ton harvested, based on fermentation of only glucose.

The models developed in this study were useful in predicting the glucose yield as a function of pretreatment conditions for both reactor types. The first model involved determining kinetic parameters for cellulose and xylan hydrolysis reactions based on the Arrhenius relationship and general acid-base catalysis. Correlation coefficients for this model type were favorable over the experimental range. The second model was based on determining combined severity factors. Although correlation coefficients for this model type were low, this model can be a supplemental method for highlighting general areas of interest and of concern.

Further investigation must be done to demonstrate the commercial applicability of microwave pretreatment. This study highlighted four opportunities for bridging the gap to industrial scale and potential. One, a continuous process must be employed to maximize throughput. Batch processes are throughput limited due to additional steps involved in the process. We recommend partnering with Industrial Microwave, Inc. for design and evaluation of a pilot-scale continuous process. Another potential partner would be Cambrex Corporation, who was the recipient of the Silver Innovation Award at the 2009

CPhI Event for its Continuous-Flow Microwave-Assisted Organic Synthesis (CFMAOS) technology. Their CaMWave^{TM} KiloLAB flow reactor is capable of manufacturing up to 12 kg hr^{-1} of product based on current designs. Their technology platforms are touted as being more versatile, faster, cleaner, offering more reliable reactions, which can lead to improved productivity and lower manufacturing costs.

Second, solids loading of at least 20 wt% must be demonstrated on the pilot unit. Bench-top units (typically 500 mL) are only able to process solids up to 10 wt% due to equipment constraints. Larger units must be utilized for processes higher solids loading. The higher solids loading is required to achieve at least break-even economics, by taking advantage of higher throughput and incremental energy usage relative to lower solids slurry. Third, a direct comparison of conventional and microwave continuous pretreatment processes at higher solid loading conditions would be beneficial. Fourth, an investigation of other energy crops, such as wheat straw, corn stover, and soybean waste would be valuable.

The potential for obtaining an application or process patent is achievable for processing lignicellulosic biomass using continuous microwave technology for biofuel. The novelty would be a process that yields higher fuel throughput at lower energy usage. A comprehensive patent search rendered no patents or applications in this area.

REFERENCES

Alizadeh, H (2005). Pretreatment of Switchgrass by AFEX; *Applied Biochemistry and Biology* ,121-124, 1133-1142.

Azuma J.I.; Tanaka F; Koshijima T (1984). Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation, *J. Fermentation Technol.*, 62, 377–384.

Blake, C; (2008). Plethora of crops holds potential for Western biofuels production boom.; *Western Farm Press* 30, 1525-1217.

Chum, Helena (1990). Pretreatment-Catalyst Effectsand the Combined Severity Parameter; *Applied Blochernistry and Biotechnology* (25) 1-14

Campbell, C.J., Laherrere, J.H., (1998). Preventing the next oil crunch - the end of cheap oil. *Sci. Am.* 278 (3), 77-83.

Conner, Anthony (1985). Kinetic Model for the Dilute Sulfuric Acid Saccharification of Lignocellulose; *Journal of Wood Chemistry*; 5, 461-489.

Cox, Robin, (1987) Organic Reactions in Sulfuric Acid: The Excess Acidity Method; *Acc. Chem. Res*. 20, 27-31.

Das, C. P. (2000) Removal of Lignin by Industrial Solid Wastes*; Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, Vol. 4, No. 4, 156-161.

Datta, A.K, (2001). Fundamentals of heat and moisture transport for microwaveable food product and process development, in: Datta, A. K., Anantheswaran, R. C. (Eds.), Handbook of microwave technology for food applications. Marcel Dekker Inc, New York.

Demirbas, A., (2004). Bioenergy, global warming, and environmental impacts. *Energy Sources*. 26 (3), 225-236.

Dien, Bruce (2006). Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass and Bioenergy* (30) 880–891

Elberson, Ir, (2009) Business Unit Biobased Products; http://www.switchgrass.nl

Eggeman, Tim (2005) Process and Economic Analysis of Pretreatment Technologies; *Bioresource Technology* 96 (2005) 2019-2025.

Energy Information Administration (EIA), 2007. Biofuels in the US transportation sector. Available from http://www.EIA.doe.gov/oiaf/analysispaper/biomass.html

Energy Information Administration (EIA), 2008a. International energy outlook 2008. Available from http://www.EIA.doe.gov/oiaf/ieo/

Energy Information Administration (EIA), 2008b. Renewable energy consumption and electricity preliminary 2007 statistics. Available from http://www.Energy Information Administration.doe.gov/cneaf/alternate/page/renew_energy_consump/rea_prereport.html

Energy.eu, 2005. Renewable energy in final energy consumption. Available from http://energy.eu/#renewable

Esteghlalian, A., Hashimoto, A.G., Fenske, J.J., Penner, M.H., (1997). Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. *Bioresour. Technol*. 59 (2-3), 129-136.

Fengel, D., Wegener, G. (1984). Wood: Chemistry, Ultrastructure, Reactions. Berlin: Walter de Gruyter.

Gabriel, C., Gabriel, S., Grant, E.H., Halstead, B.S.J., Mingos, D.M.P., (1998). Dielectric parameters relevant to microwave dielectric heating. *Chem. Soc. Rev*. 27 (3), 213-223.

Garrote, G., Dominguez, H., Parajo, J.C., (2002). Interpretation of deacetylation and hemicellulose hydrolysis during hydrothermal treatments on the basis of the severity factor. *Proc. Biochem*. 37, 1067–1073.

Gizenia, David (2008); Membrane extraction for removal of acetic acid from biomass hydrolysates; *Journal of Membrane Science*, 322, 189–195

Grethlein, H. E. 1991. Common Aspects of Acid Prehydrolysis and Steam Explosion for Pretreating Wood. Bioresource Technology 36: 77 - 82.

Citation: Harris D, DeBolt S (2008) Relative Crystallinity of Plant Biomass: Studies on Assembly, Adaptation and Acclimation. PLoS ONE 3(8): e2897. doi:10.1371/journal.pone.0002897

Hegerl, G.C., Zwiers, F.W., Braconnot, P., Gillett, N.P, Luo, Y., Marengo Orsini, J.A., Nicholls, N., Penner, J.E, Stott P.A., 2007: Understanding and attributing climate change. In Solomon,S., Qin, D., Manning, M., Chen, Z., Marquism M., Averyt, K.B., Tignor, M., Miller H.L. (Eds.) Climate Change 2007: The physical science basis. Contribution of working proup I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press,

Cambridge, United Kingdom and New York, NY, USA.

Hirsch, R.L., (2008). Mitigation of maximum world oil production: Shortage scenarios. *Energy Policy*. 36 (2), 881-889.

Hong, Yeon (2005); Removal of acetic acid from aqueous solutions containing succinic acid and acetic acid by tri-n-octylamine; *Separation and Purification Technology*, 42, 151–157.

Hu, Zhenhu (2007); Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment; *Biochemical Engineering Journal*

Huang, Hua-Jiang (2008); Effect of biomass species and plant size on cellulosic ethanol: A comparative process and economic analysis; *Biomass and Bioenergy*,

Jackson, P.M. (2007). Peak oil theory could distort energy policy and debate. Journal of Petroleum Technology. 59 (2).

Jannasch, R., Quan, Y., Samson, R., 2001. A process and energy analysis of pelletizing switchgrass. Available from http://www.reapcanada. com/library.htm.

Kabel, Mijam (2007); Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw; *Bioresource Technology*, 98, 2034–2042

Kappe, C. Oliver (2005) Microwaves in Organic and Medicinal Chemistry, Wiley-VCH, Weinheim 45 1677-1678

Kaufmann, R.K., Shiers, L.D., (2008). Alternatives to conventional crude oil: When, how quickly, and market driven? *Ecol. Econ*. 67 (3), 405-411.

Kumar A, Sokhansanj S. (2007). Switchgrass (Panicum virgatum, L.) delivery to a biorefinery using integrated biomass supply analysis and logistics (IBSAL) model. Bioresource Technology; 98:1033–44.

Laser, Mark (2002) A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol; *Bioresource Technology* 81, 33-44

Lefley, Frank (1996). The payback method of investment appraisal: A review and synthesis; *Int. J. Production Economics* (44) 207 -224

Lewandowski, Iris; (2003). The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe; *Biomass and Bioenergy*, 25, 335 – 361.

Matche Chemical Equipment Cost Estimation. Available from http://www.matche.com

McAloon, Andrew (2000); Determining the Cost of Producing Ethanol from Corn Starch and Lignocellulosic Feedstock; National Renewables Energy Laboratory

Moseley, Jonathan (2009); Energy Efficiency of Microwave- and Conventionally Heated Reactors Compared at meso Scale for Organic Reactions; *Energy & Fuels*;

Mosier, Nathan; (2005); Features of promising technologies for pretreatment of lignocellulosic biomass; Bioresource Technology; 96; 673-686.

Ng, T K (1983); Production of Feedstock Chemicals; *Science*, 219, 733-740

Ooshima, H (1984) Microwave treatment of cellulosic materials for enzymatic hydrolysis; *Biotechnology Letters*, 6 289-294.

Parrish, D.J., Fike, J.H. (2005). The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Sci. 24 (5-6), 423-459.

Perlack, R.D., Wright, L.L., Turhollow, A.F, Graham, R.L., Stokes B.J., Erbach, D.C. 2005. Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. Available from http://www.osti.gov/bridge

Sampaio, Fabio et al (2006); Influence of temperature and pH on xylitol production from xylose by Debaryomyces hansenii UFV-170; *Process Biochemistry*, 41, 675–681.

Sanderson, M.A., Adler, P.R., Boateng, A.A., Casler, M.D., Sarath, G. (2006). Switchgrass as a biofuels feedstock in the USA. *Canadian Journal of Plant Science*. 86 (5), 1315-1325.

Schell, D (2003). Dilute–Sulfuric Acid Pretreatment of Corn Stover in Pilot-Scale Reactor; *Applied Biochemistry and Biotechnology*; Vol. 105–108, 69-84.

Schmer, M.R., Vogel, K.P., Mitchell, R.B., Perrin, R.K., (2008). Net energy of cellulosic ethanol from switchgrass. *Proc. Natl. Acad. Sci. U. S.* A. 105 (2), 464-469.

Schnoor, J.L. (2005). Global warming: A consequence of human activities rivaling earth's biogeochemical processes. *Hum. Ecol. Risk Assess.* 11 (6), 1105-1110.

Schwaab, Marcio (2007). Optimum reference temperature for reparameterization of the Arrhenius equation. Part 1: Problems involving one kinetic constant. *Chemical Engineering Science* (62) 2750 – 2764.

Sridar, V., (1998). Microwave radiation as a catalyst for chemical reactions. *Curr. Sci*. 74 (5), 446-450.

Su, Yu (2009); Single-step conversion of cellulose to 5-hydroxymethylfurfural (HMF), a versatile platform chemical; *Applied Catalysis A*: General 361, 117–122.

Sun, Ye; (2005) Dilute acid pretreatment of rye straw and bermudagrass for ethanol production; *Bioresource Technology*, 96 1599–1606.

Suszkiw, Jan (2008); Scientists Determine Farm Costs of Producing Switchgrass for Ethanol; US Department of Agriculture- Agriculture Research Service

The Columbia Encyclopedia, Sixth Edition. 2008. Retrieved October 07, 2009 from Encyclopedia.com: http://www.encyclopedia.com/doc/1E1-conducton.html

Tekchandaney, Jayesh. 2009. Retrieved October 09, 2009 from: http://www.brighthub.com

US Geological Survey (USGS), 2000. World petroleum assessment 2000. US geological survey digital data series–DDS-60. Available from http://greenwood.cr.usgs.gov/WorldEnergy/

Xiang, Qian, Heterogeneous Aspects of Acid Hydrolysis of α-Cellulose, *Applied Biochemistry and Biotechnology*, Vol. 105–108, 2003,

US Department of Energy (2005); http://www1.eere.energy.gov/biomass/printable_versions/cellulase_enzyme.html

Wiesemann, Wolfram (2009). Maximizing the net present value of a project under uncertainty. *European Journal of Operational Research* (202) 356–367

Win, David (Apr. 2005); Furfural – Gold from Garbage; AU J.T. 8(4): 185-190

Wu, Z.W., Lee, Y.Y., (1997). Ammonia recycled percolation as a complementary pretreatment to the dilute-acid process. *Appl. Biochem. Biotechnol*. 63 (5), 21-34.

Wyman, C.E., Spindler, D.D., Grohmann, K., (1992). Simultaneous saccharification and fermentation of several lignocellulosic feedstocks to fuel ethanol. *Biomass Bioenerg.* 3 (5), 301-307.

Yat, Shu Chiang (2008). Kinetic characterization for dilute sulfuric acid hydrolysis of timber varieties and switchgrass; Bioresource Technology, 99, 3855–3863.

Zheng, Pu (2009). Fermentative production of succinic acid from straw hydrolysate by Actinobacillus succinogenes; *Bioresource Technology*, 100, 2425–2429.

Zhu, Shengdong (2006). Comparison of Three Microwave/Chemical Pretreatment Processes for Enzymatic Hydrolysis of Rice Straw; *Biosystems Engineering* 93 (3), 279–283.

APPENDIX 1

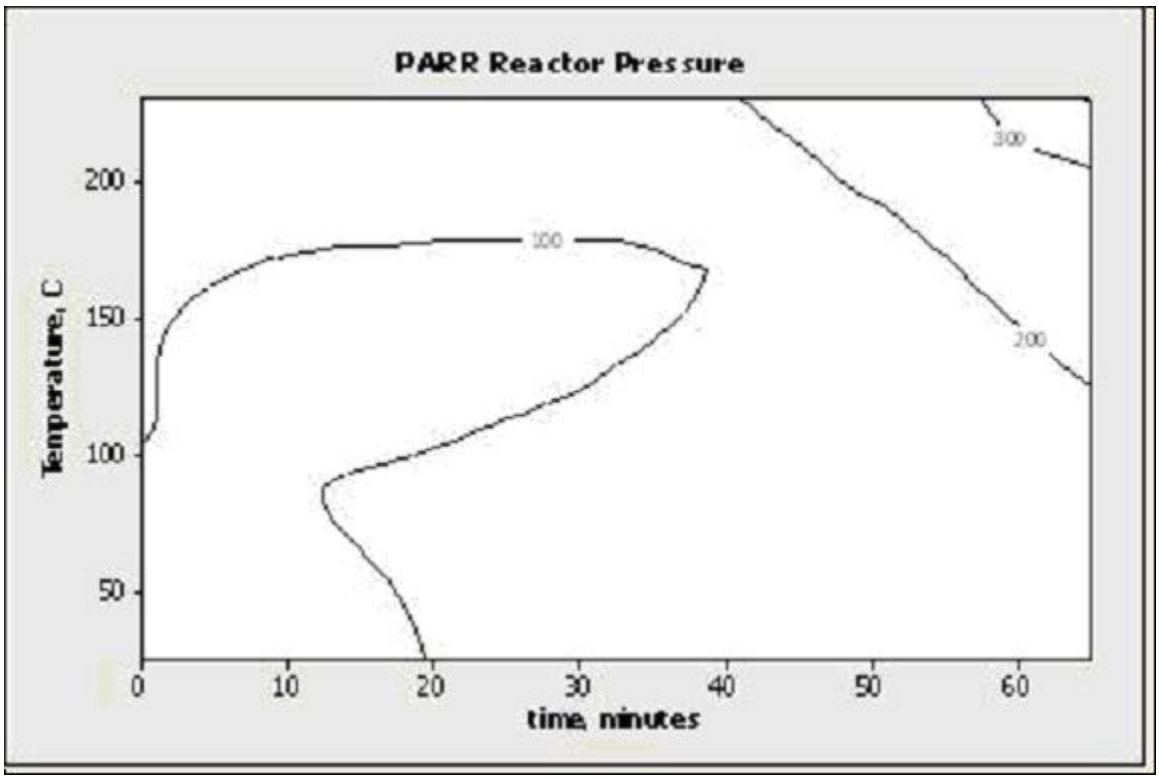

Figure A1: PARR® reactor pressure as a function of temperature and ramp time

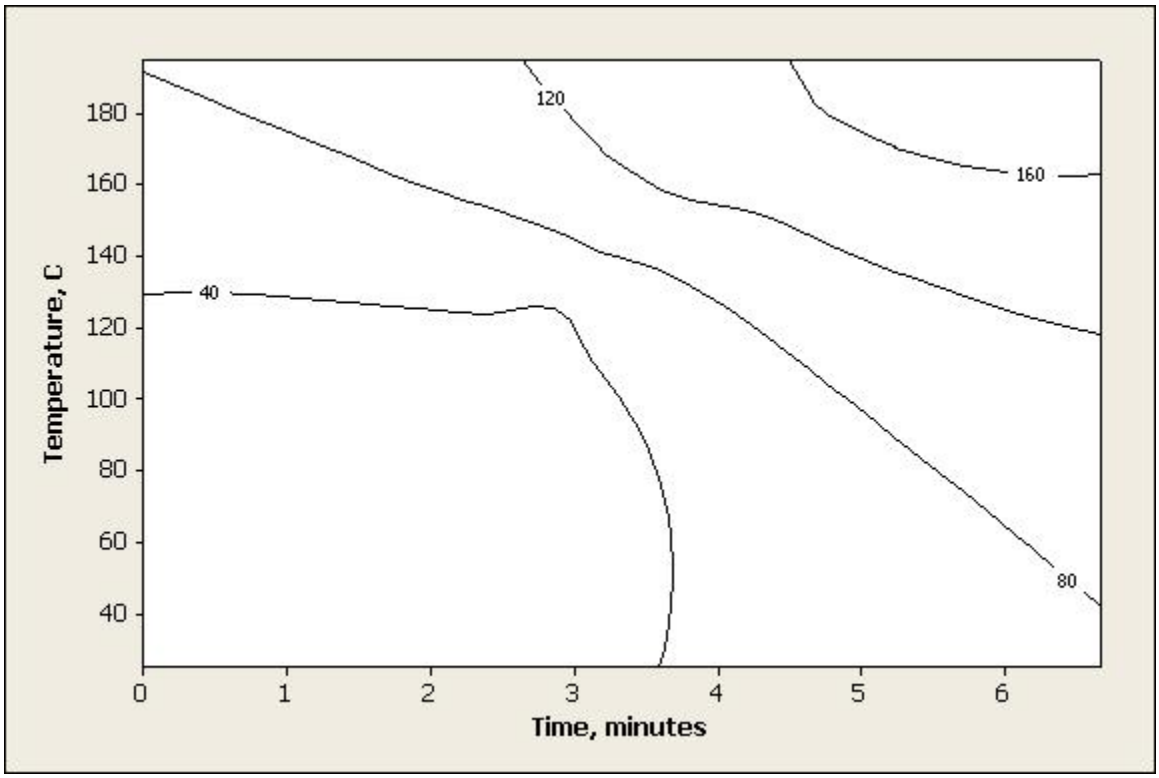

Figure A2: CEM Explorer reactor pressure as a function of temperature and ramp time

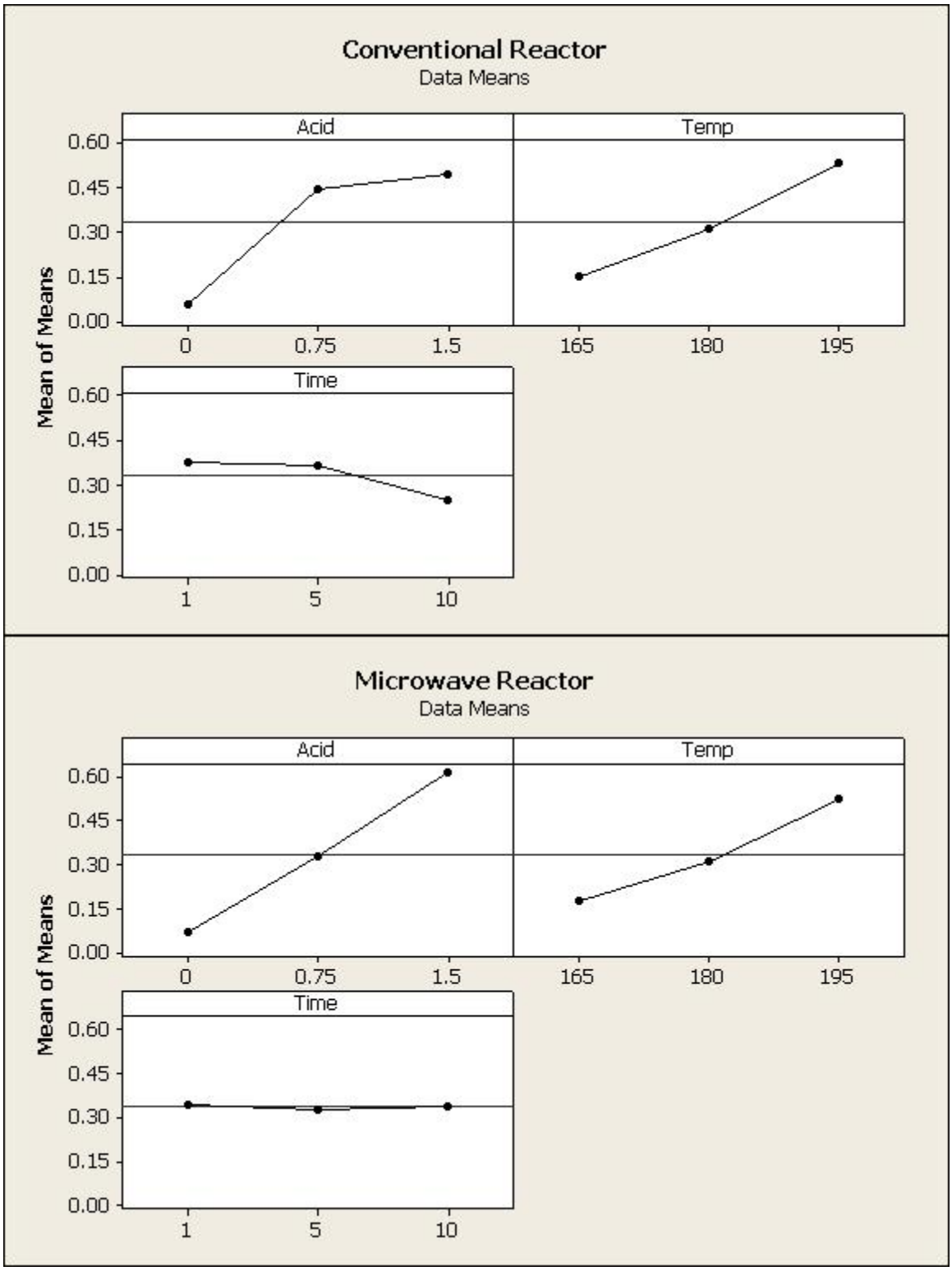

Figure A3: Avicel® mass loss fraction as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

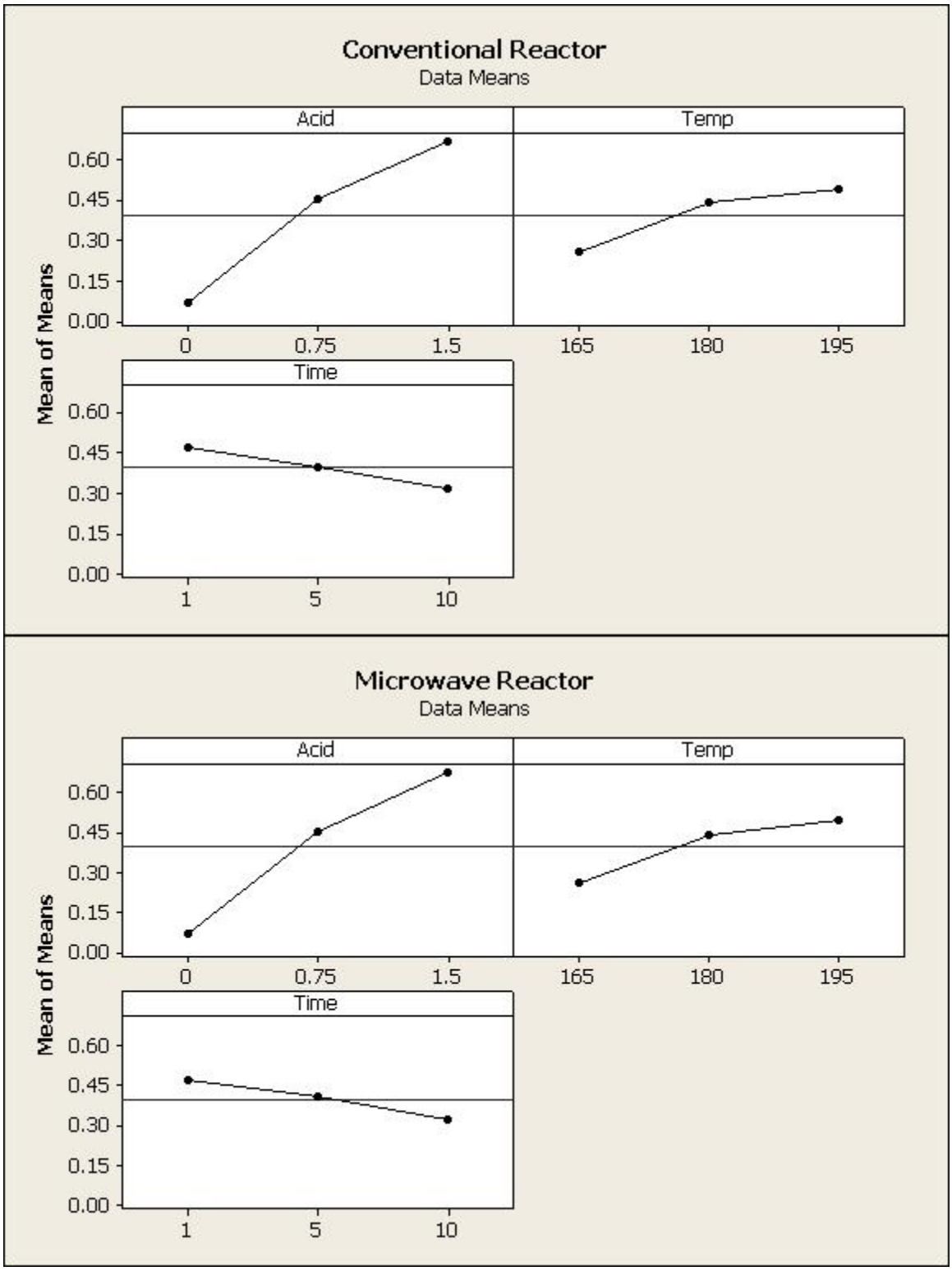

Figure A4: Whatman paper mass loss fraction as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

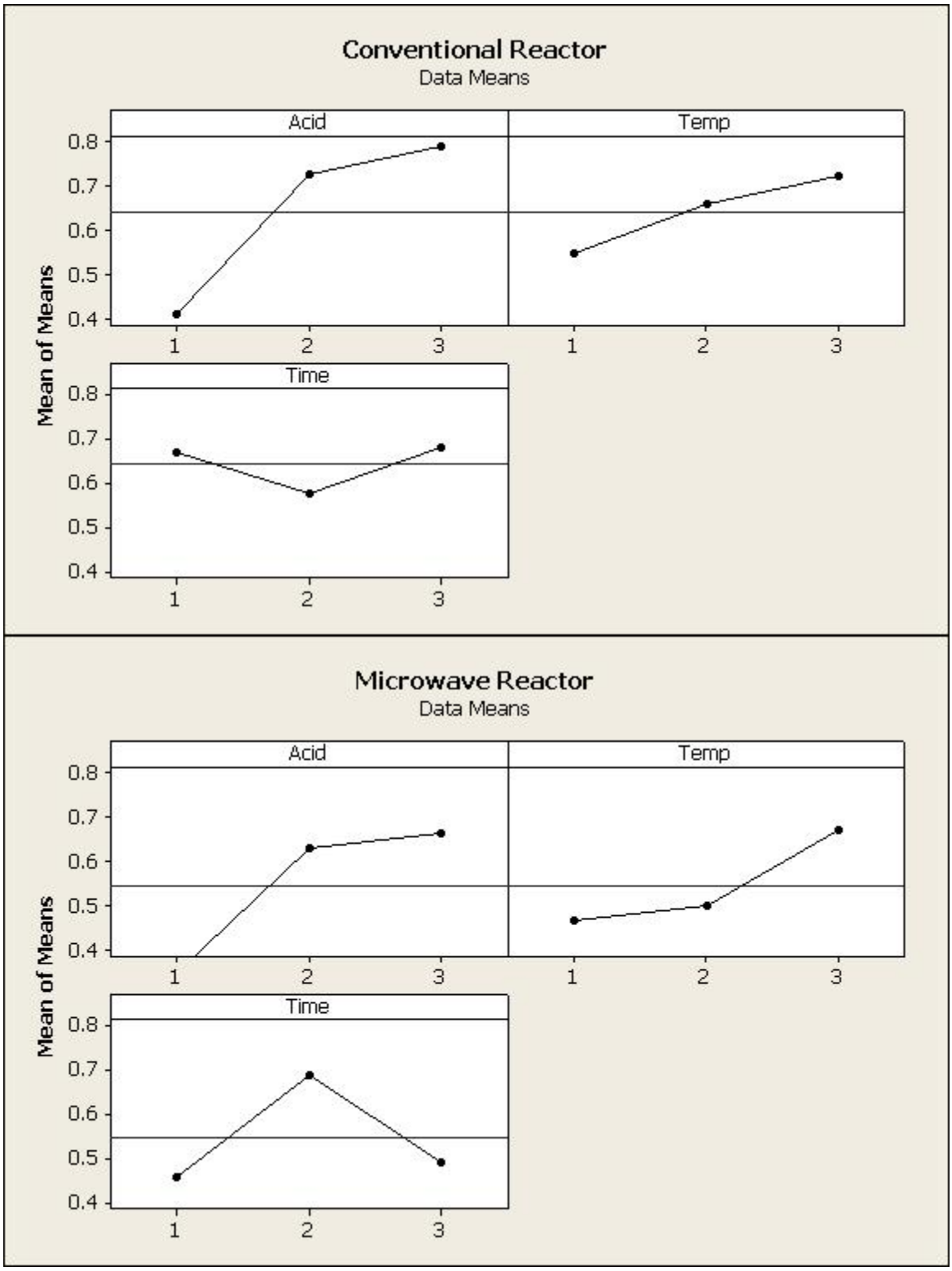

Figure A5: Switchgrass mass loss fraction as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

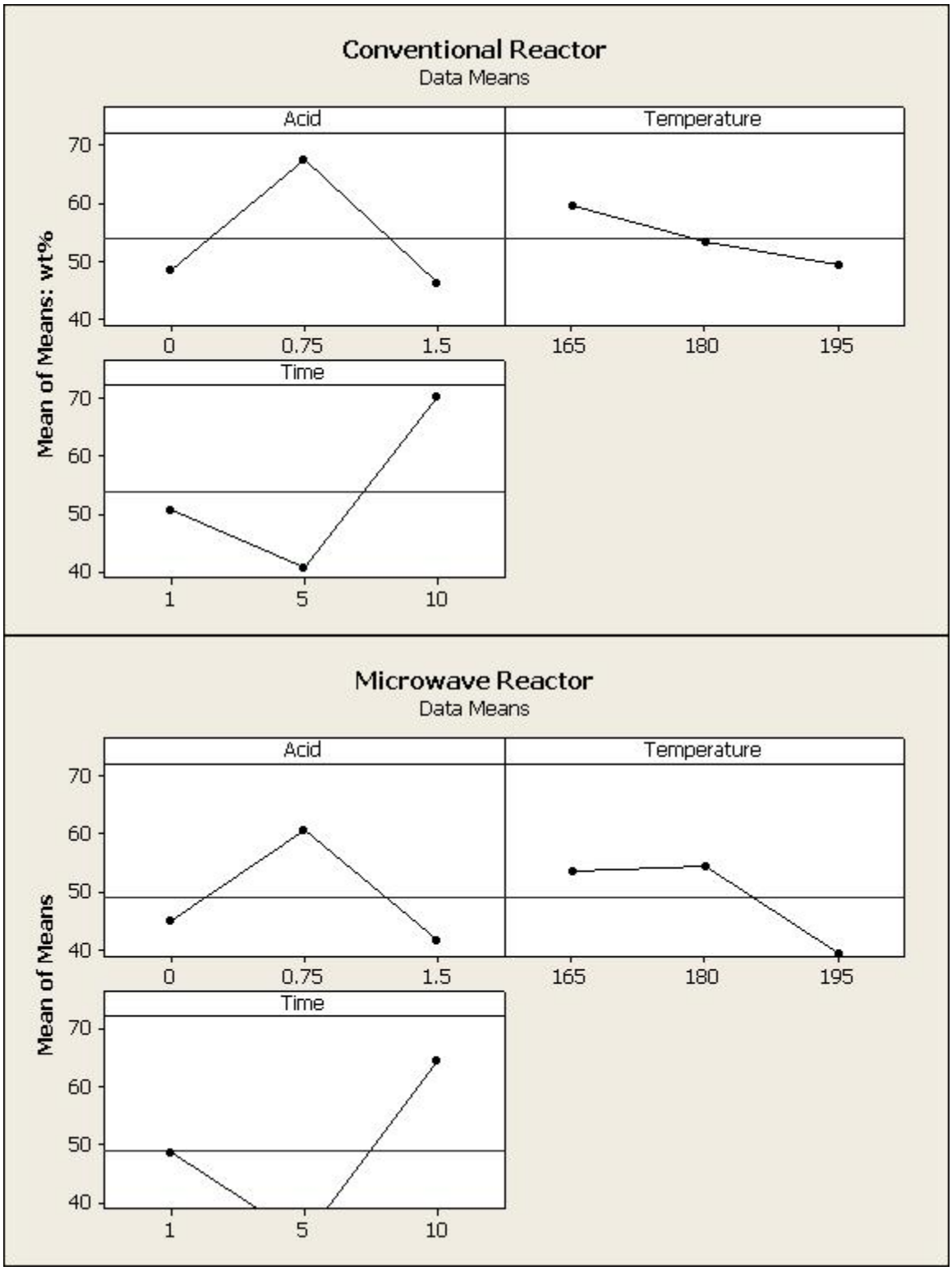

Figure A6: Switchgrass cellulose wt% as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

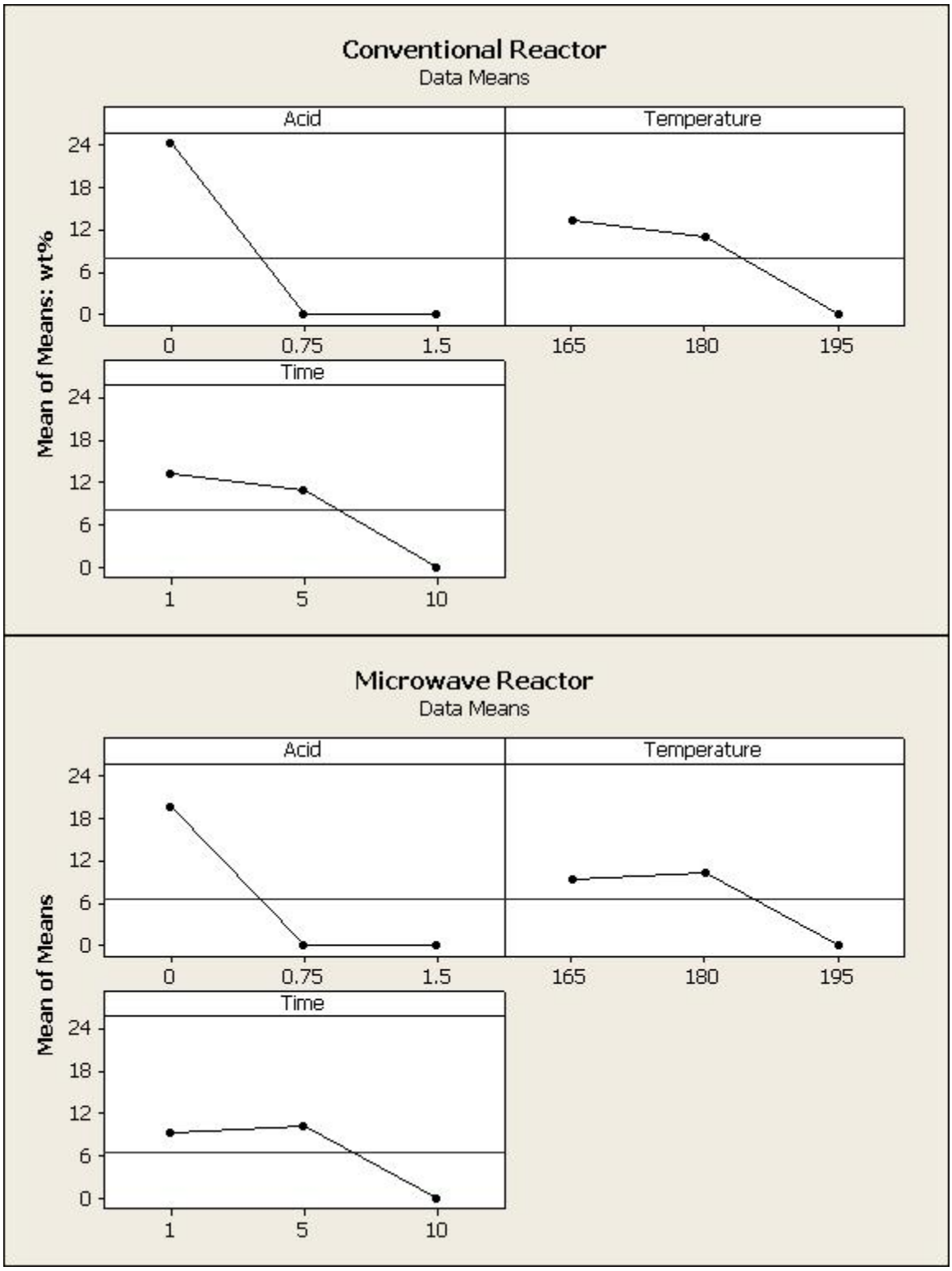

Figure A7: Switchgrass xylan wt% as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

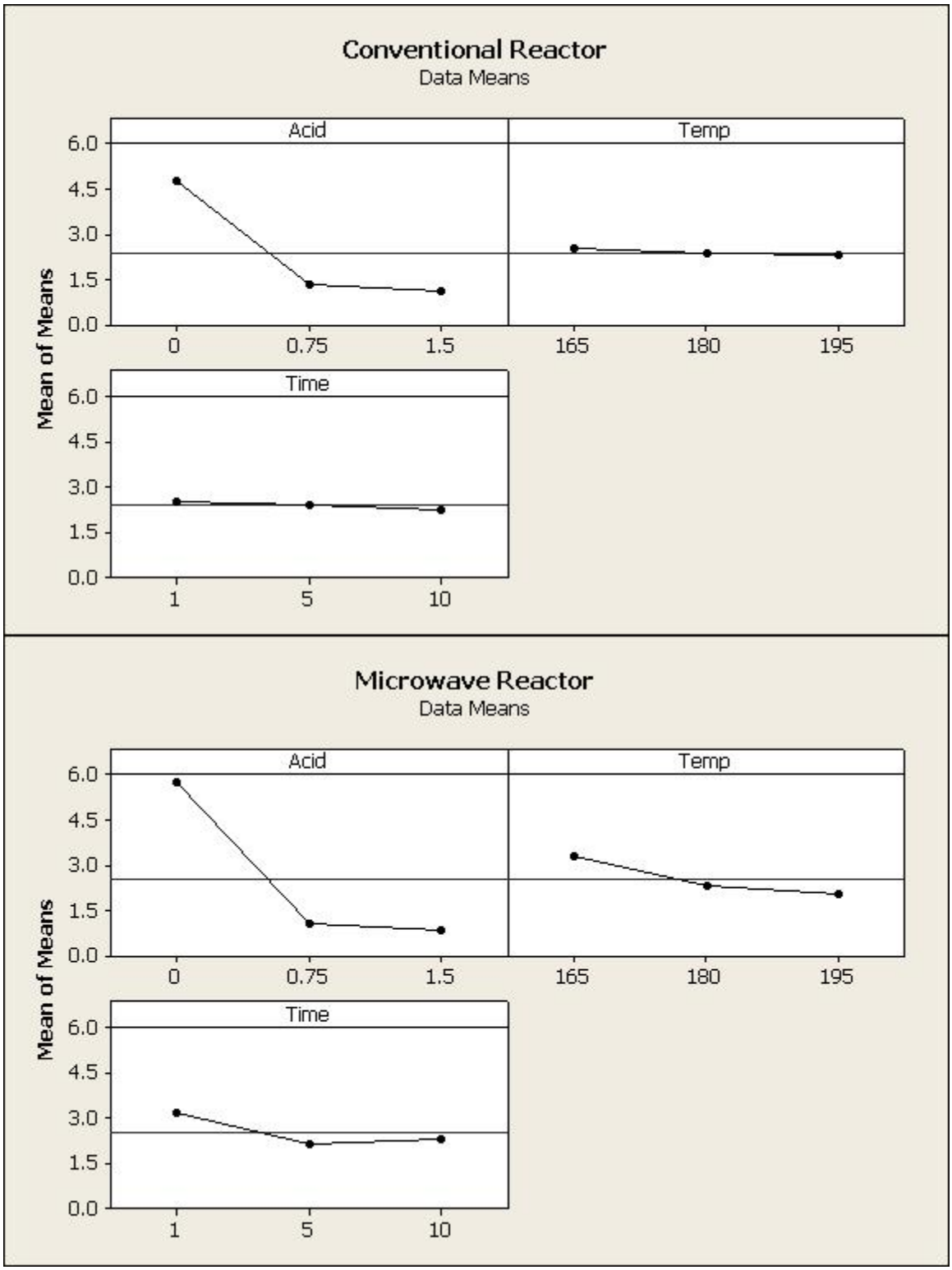

Figure A8: Avicel® liquor pH as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

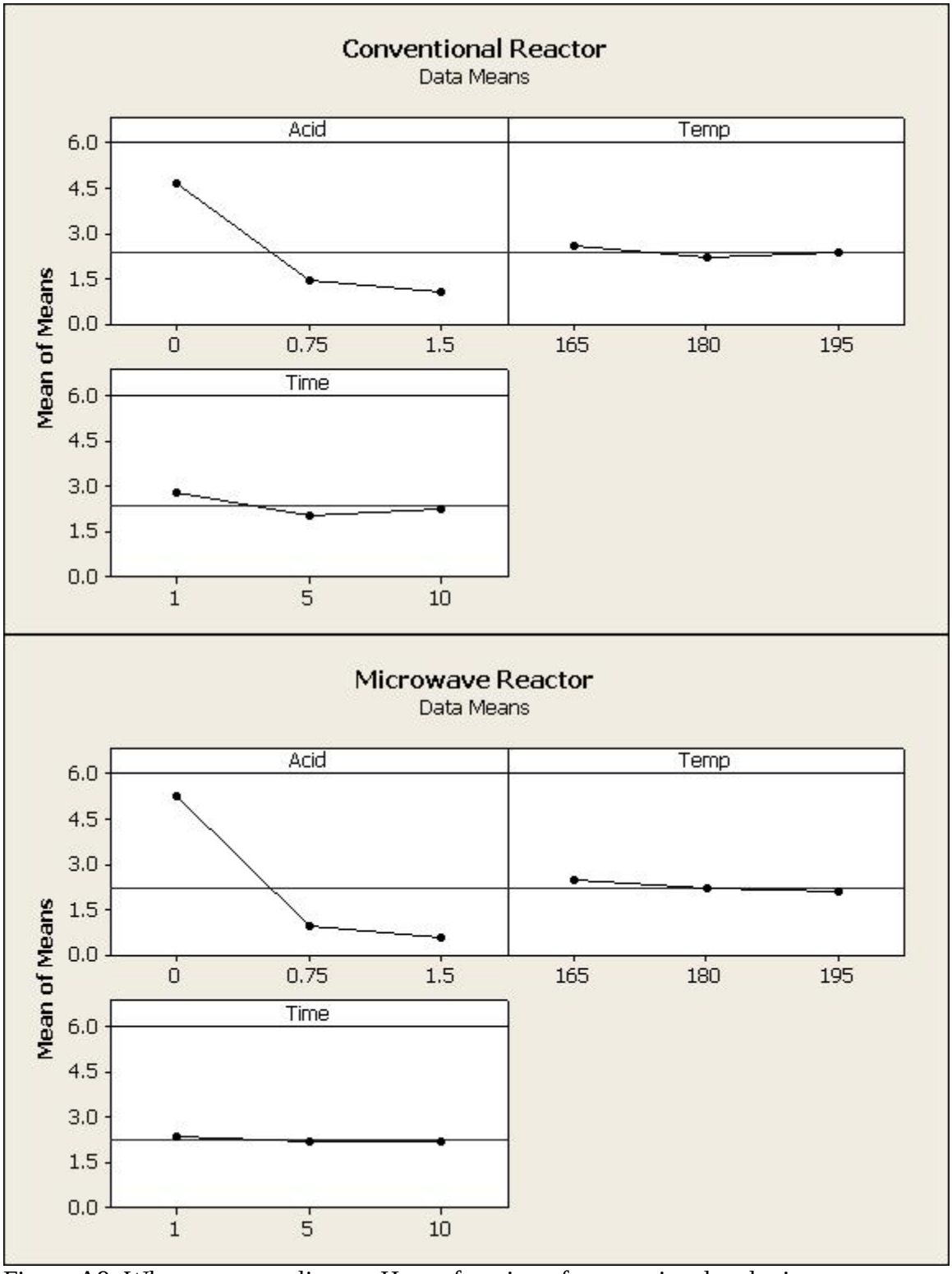

Figure A9: Whatman paper liquor pH as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

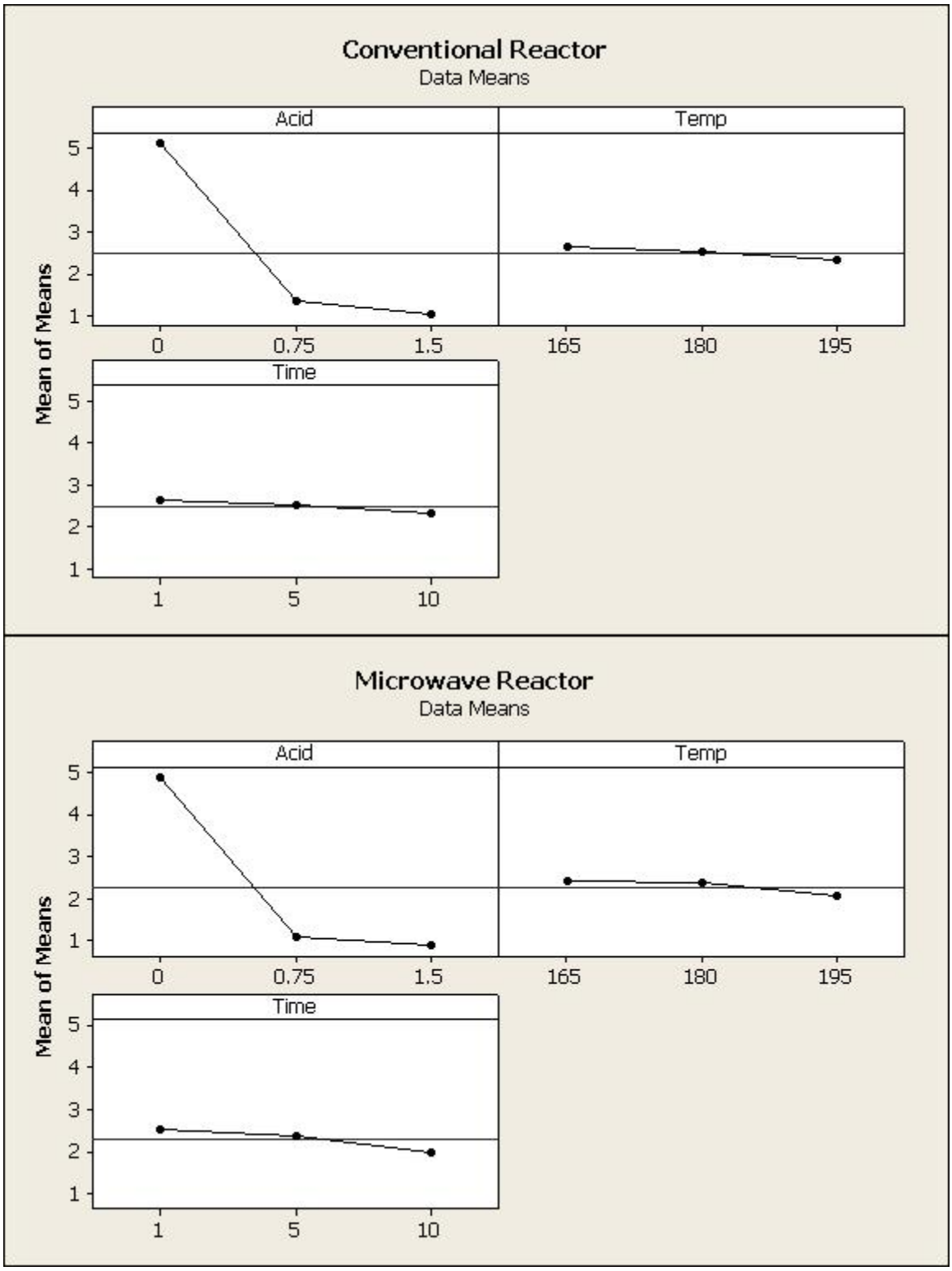

Figure A10: Switchgrass liquor pH as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

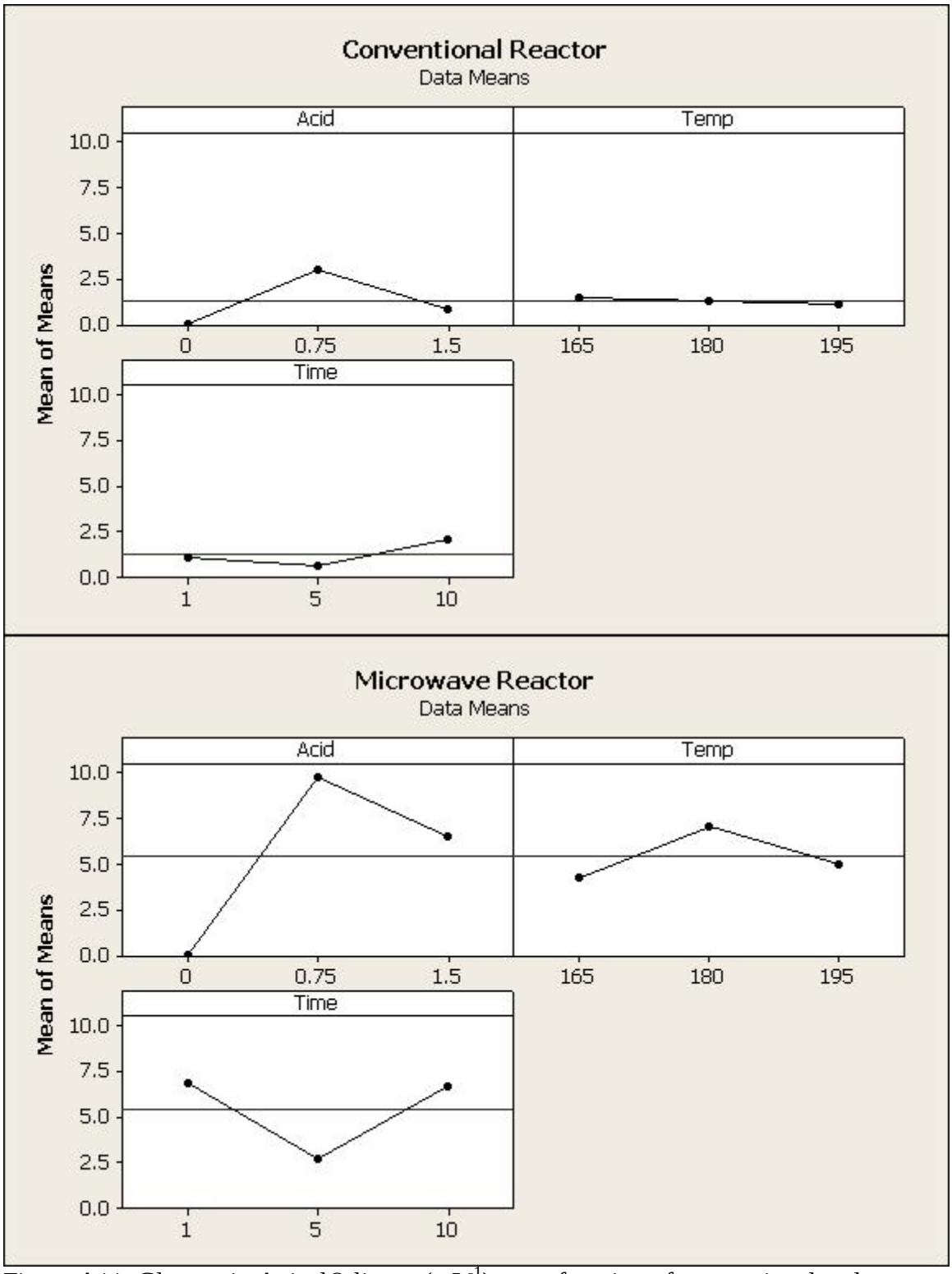

Figure A11: Glucose in Avicel® liquor (g L^{-1}) vas a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

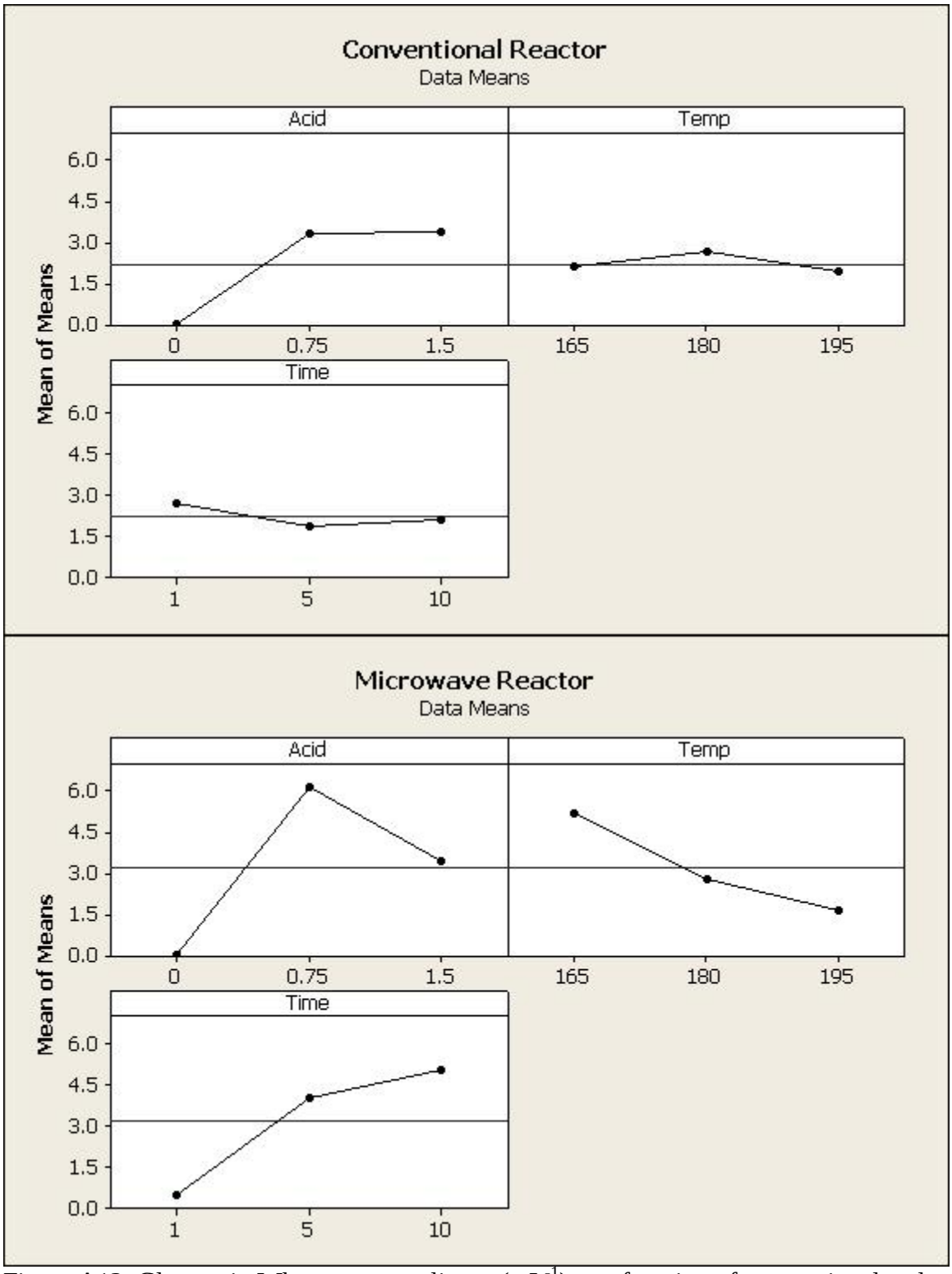

Figure A12: Glucose in Whatman paper liquor (g L^{-1}) as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

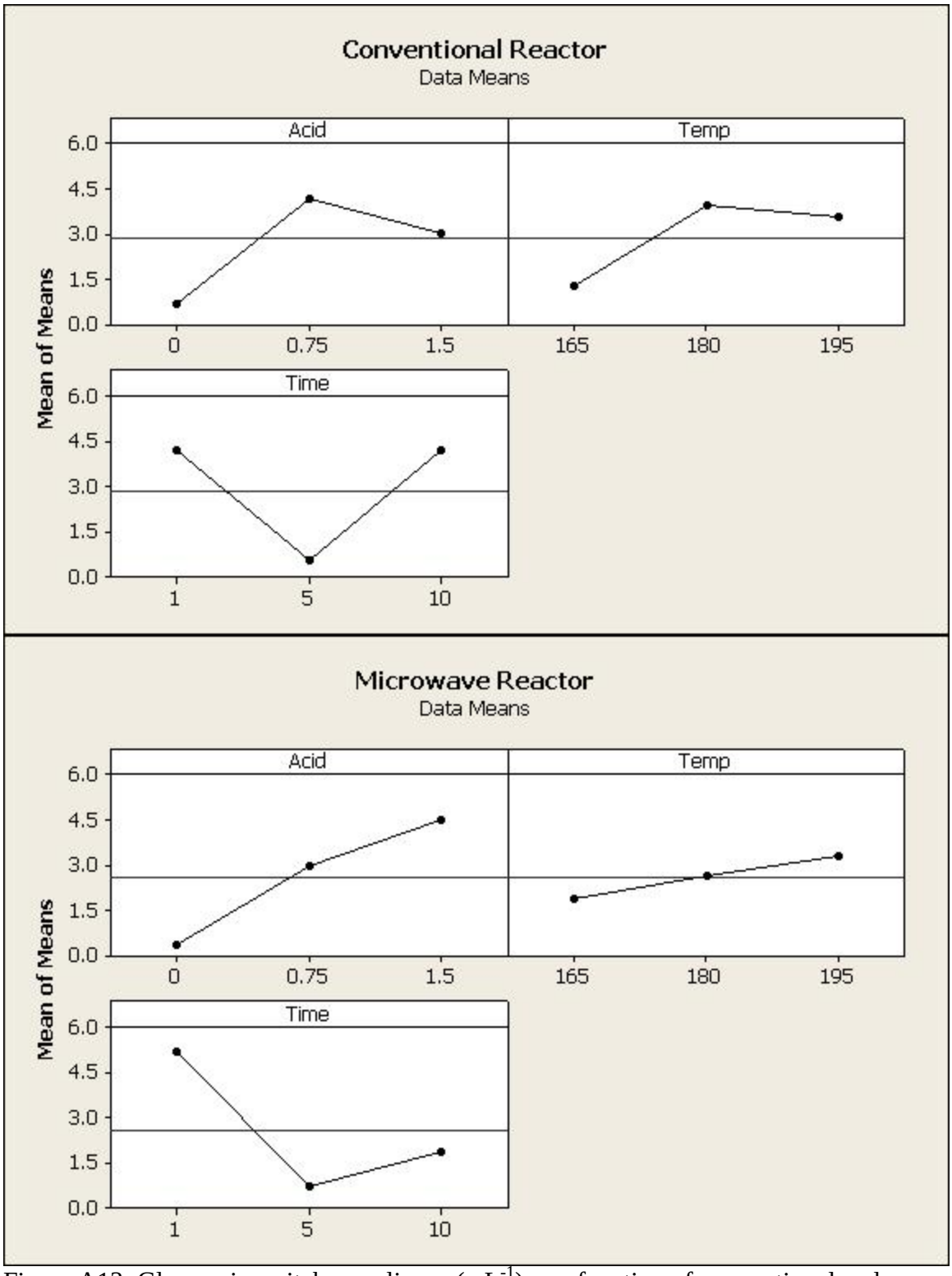

Figure A13: Glucose in switchgrass liquor (g L^{-1}) as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

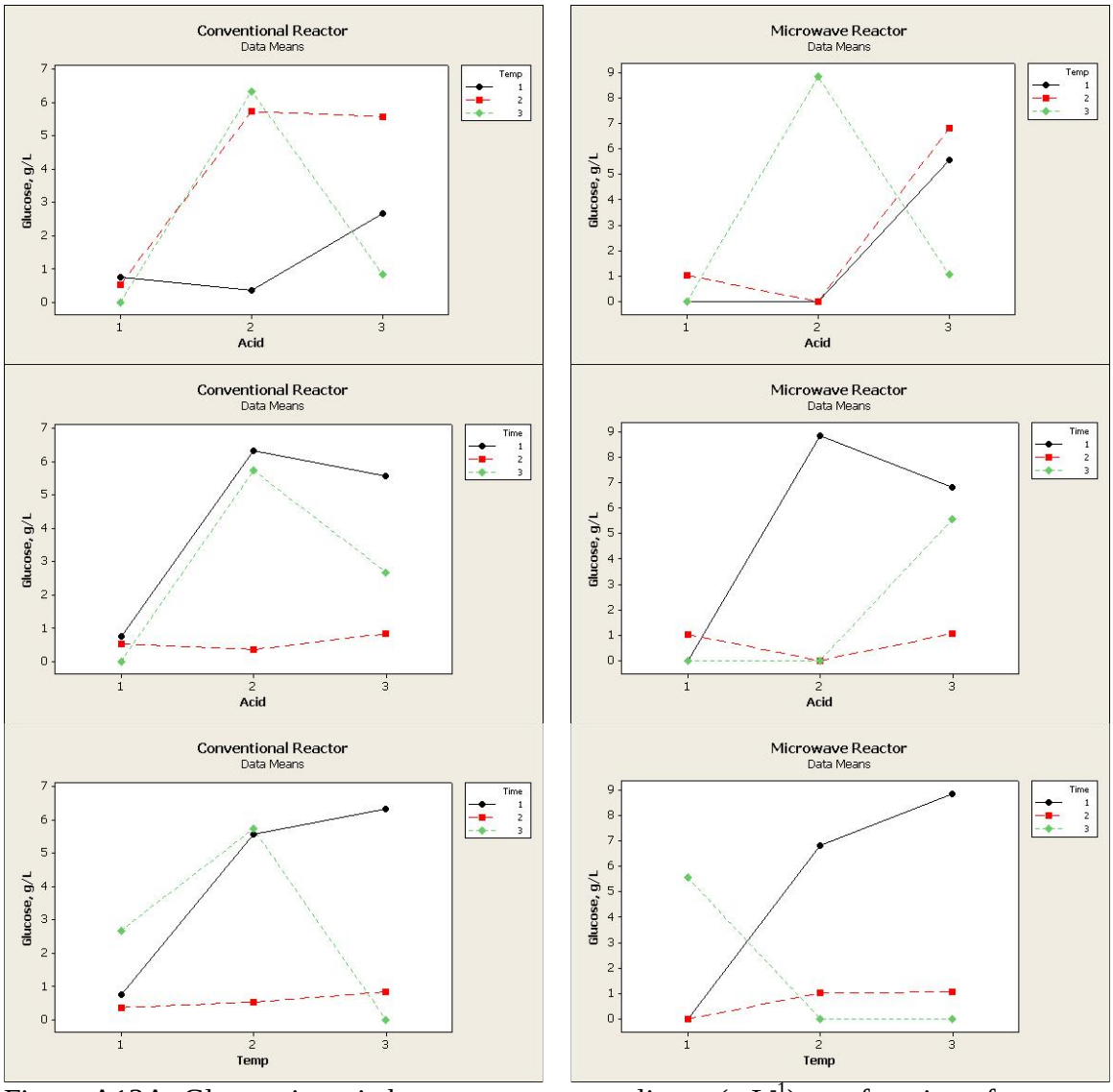

Figure A13A: Glucose in switchgrass pretreatment liquor (g L^{-1}) as a function of conventional and microwave combination pretreatment conditions- Acid (vol%), Temp (^{o}C), Time (min).

	Acid, vol%	Temp, ^{o}C	Time, min
1	0	165	1
2	0.75	180	5
3	1.5	195	10

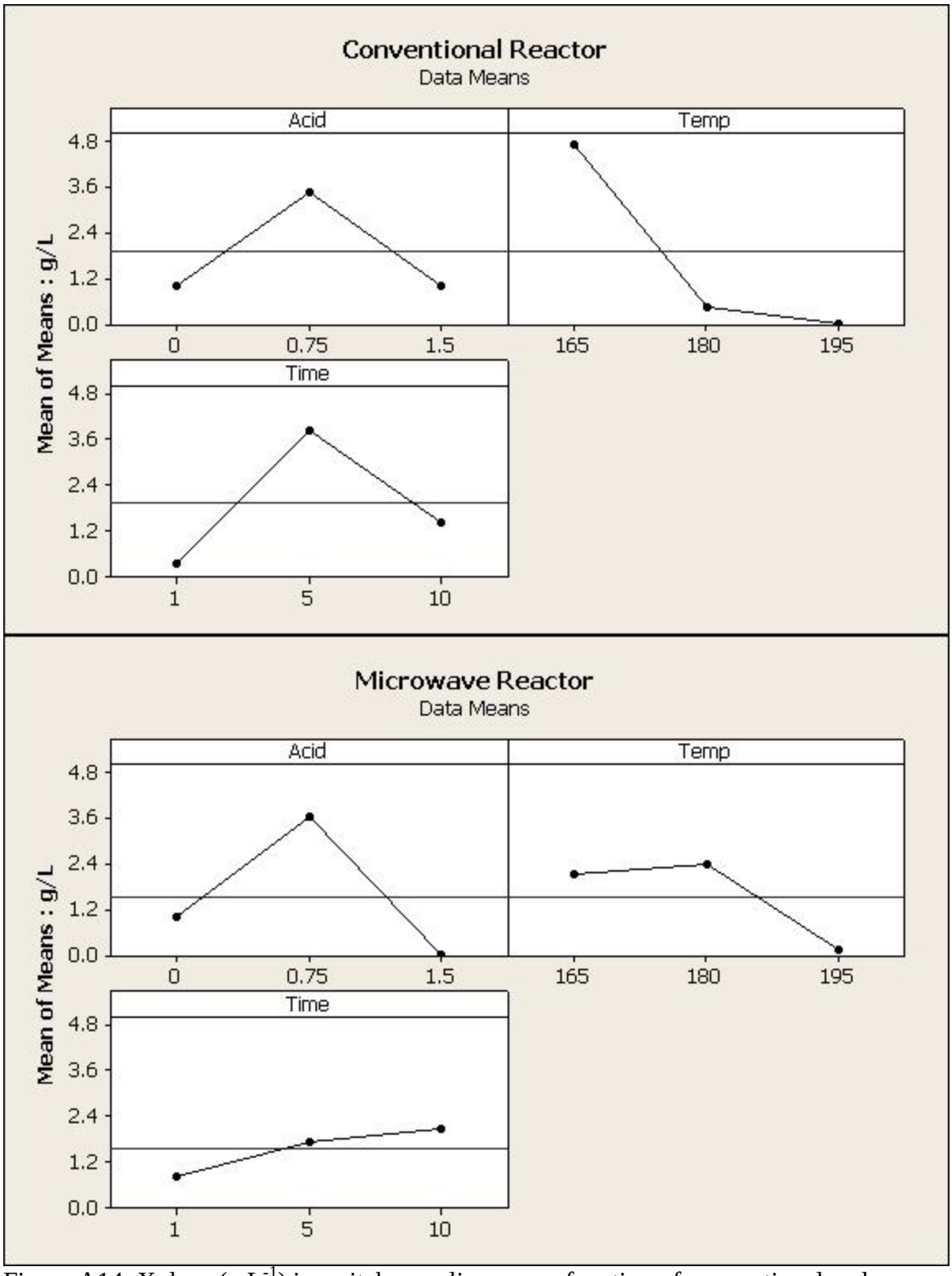

Figure A14: Xylose (g L^{-1}) in switchgrass liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

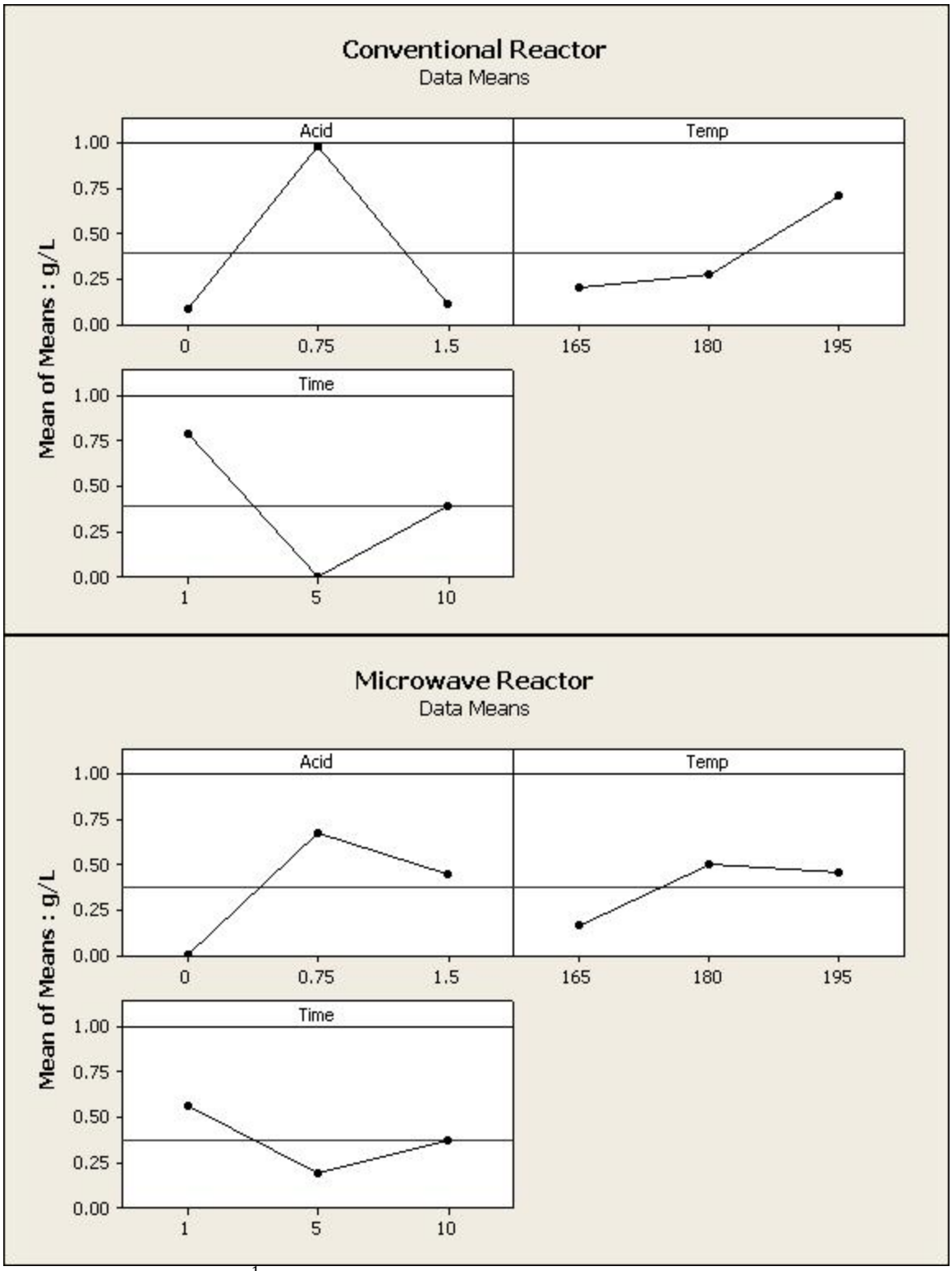

Figure A15: HMF (g L^{-1}) in Avicel® liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

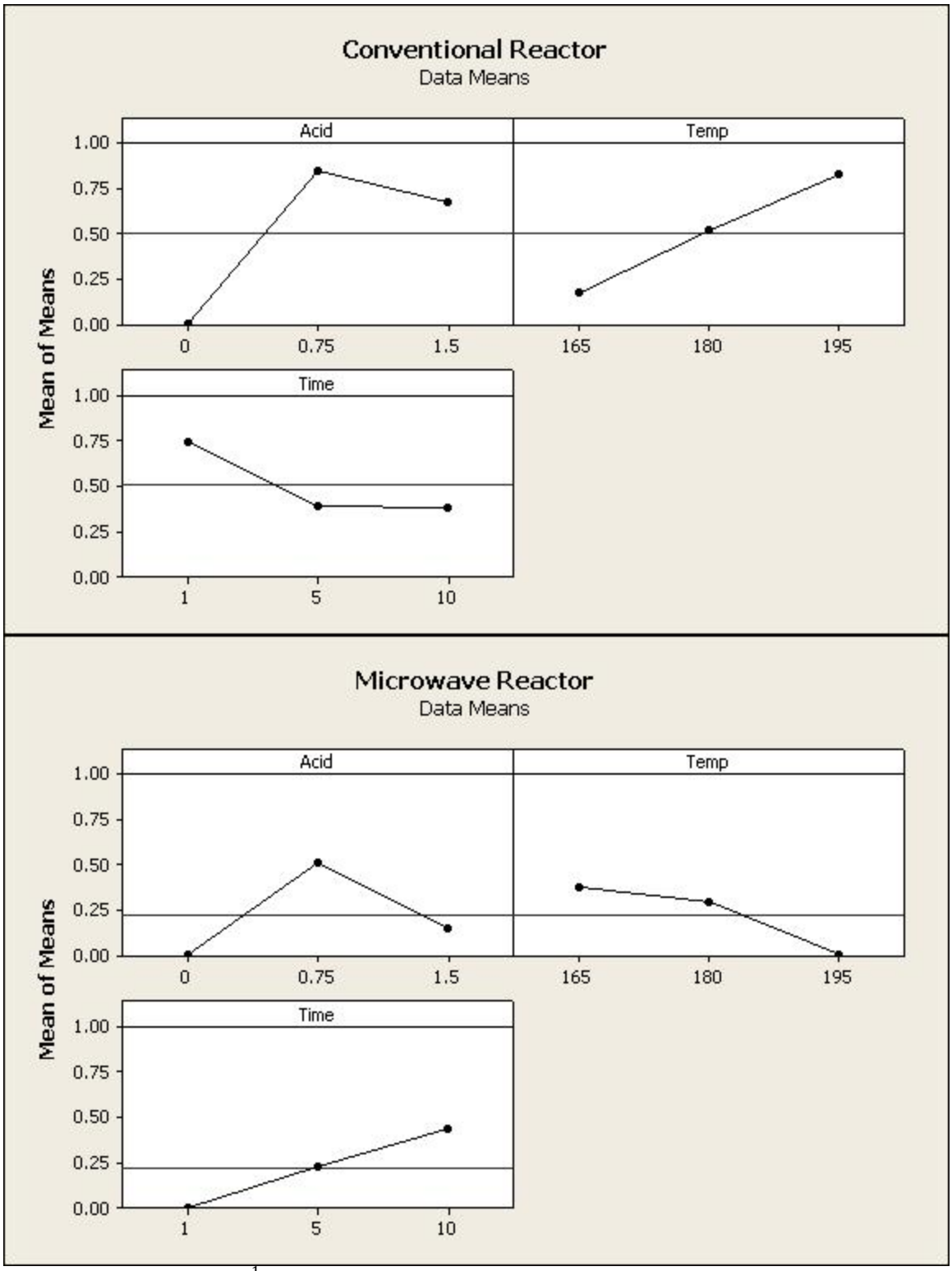

Figure A16: HMF (g L^{-1}) in Whatman paper liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

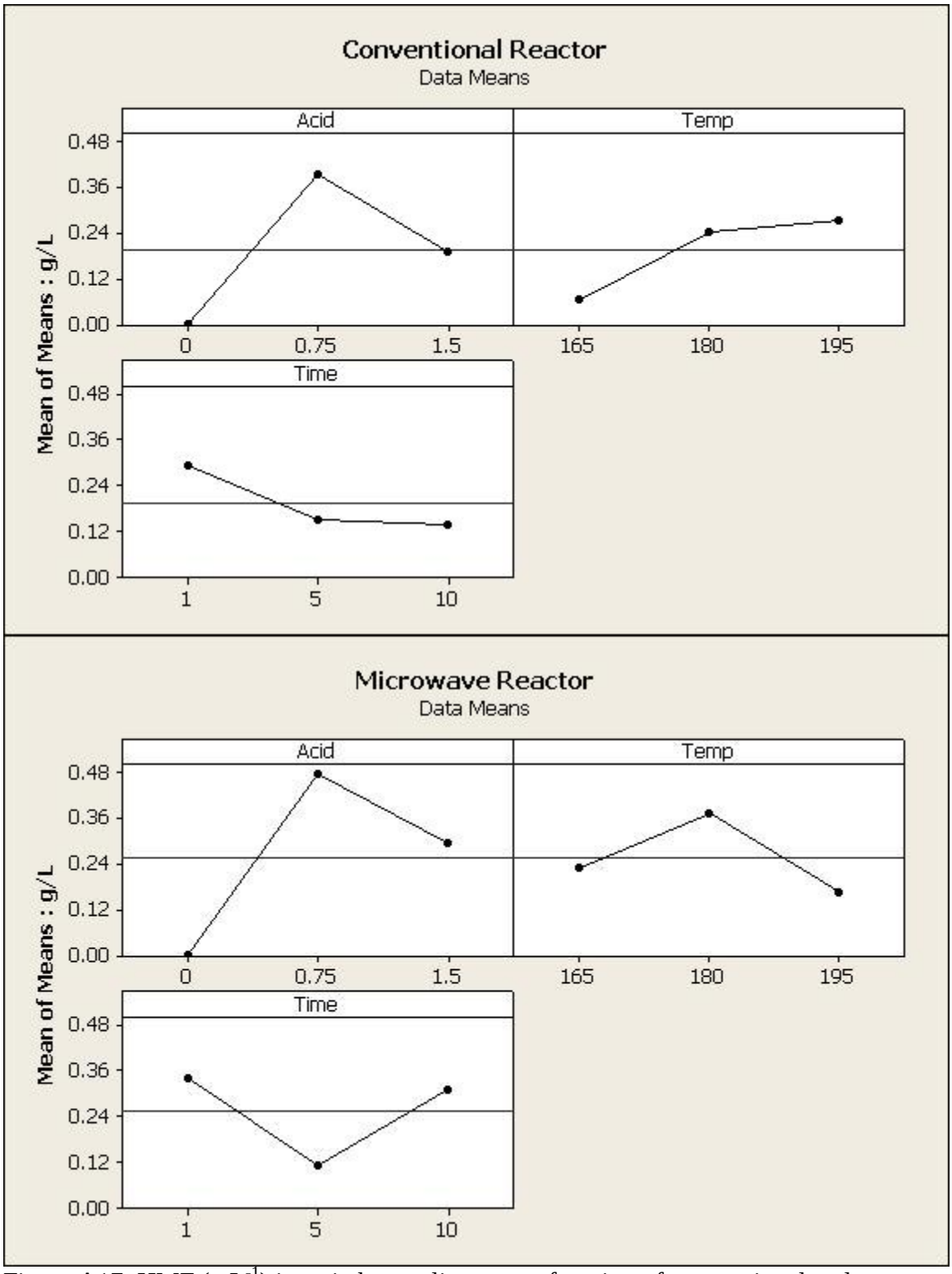

Figure A17: HMF (g L^{-1}) in switchgrass liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

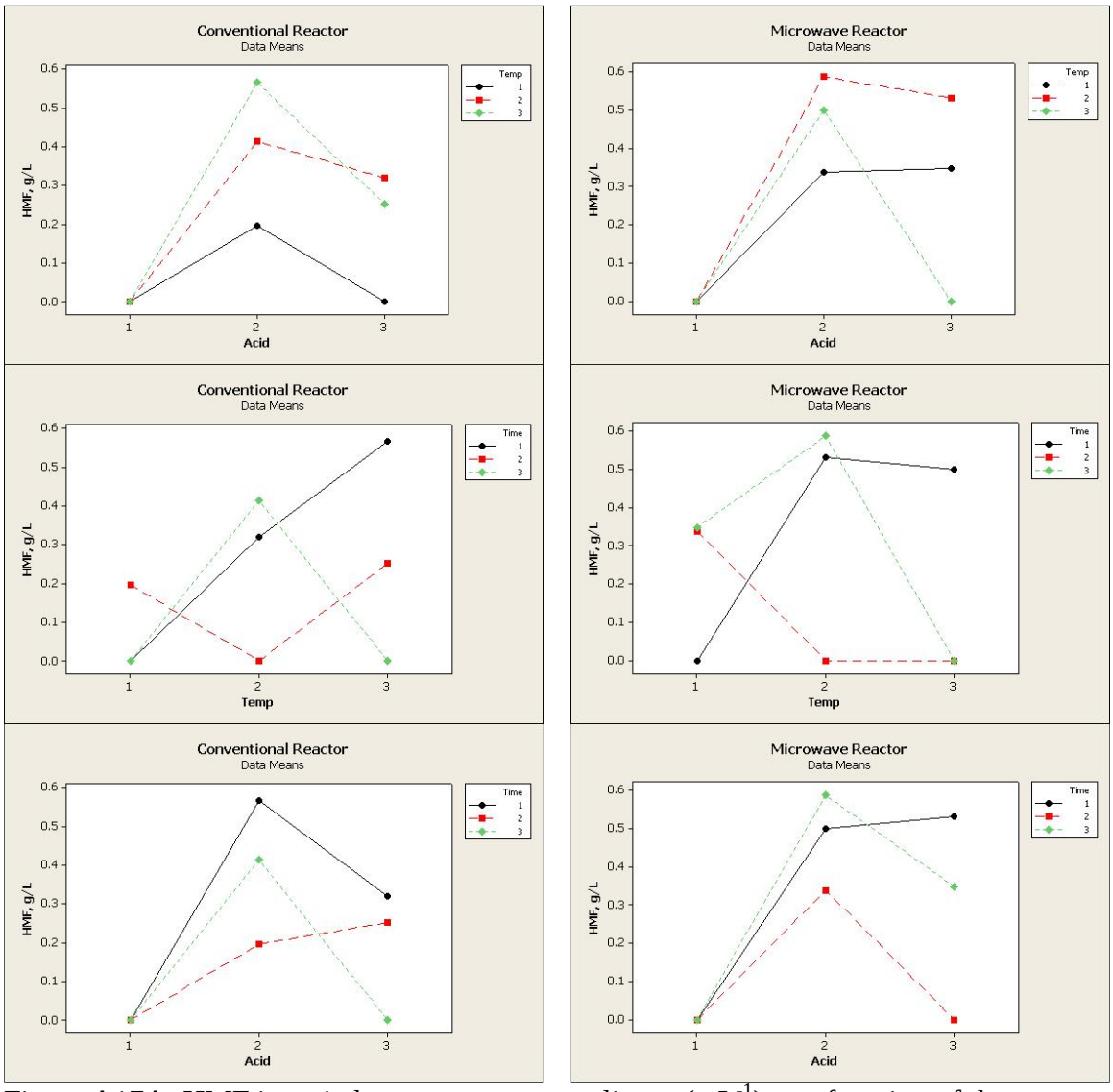

Figure A17A: HMF in switchgrass pretreatment liquor (g L^{-1}) as a function of the conventional and microwave combination pretreatment conditions- Acid (vol%), Temp (^{o}C), Time (min).

	Acid, vol%	Temp, ^{o}C	Time, min
1	0	165	1
2	0.75	180	5
3	1.5	195	10

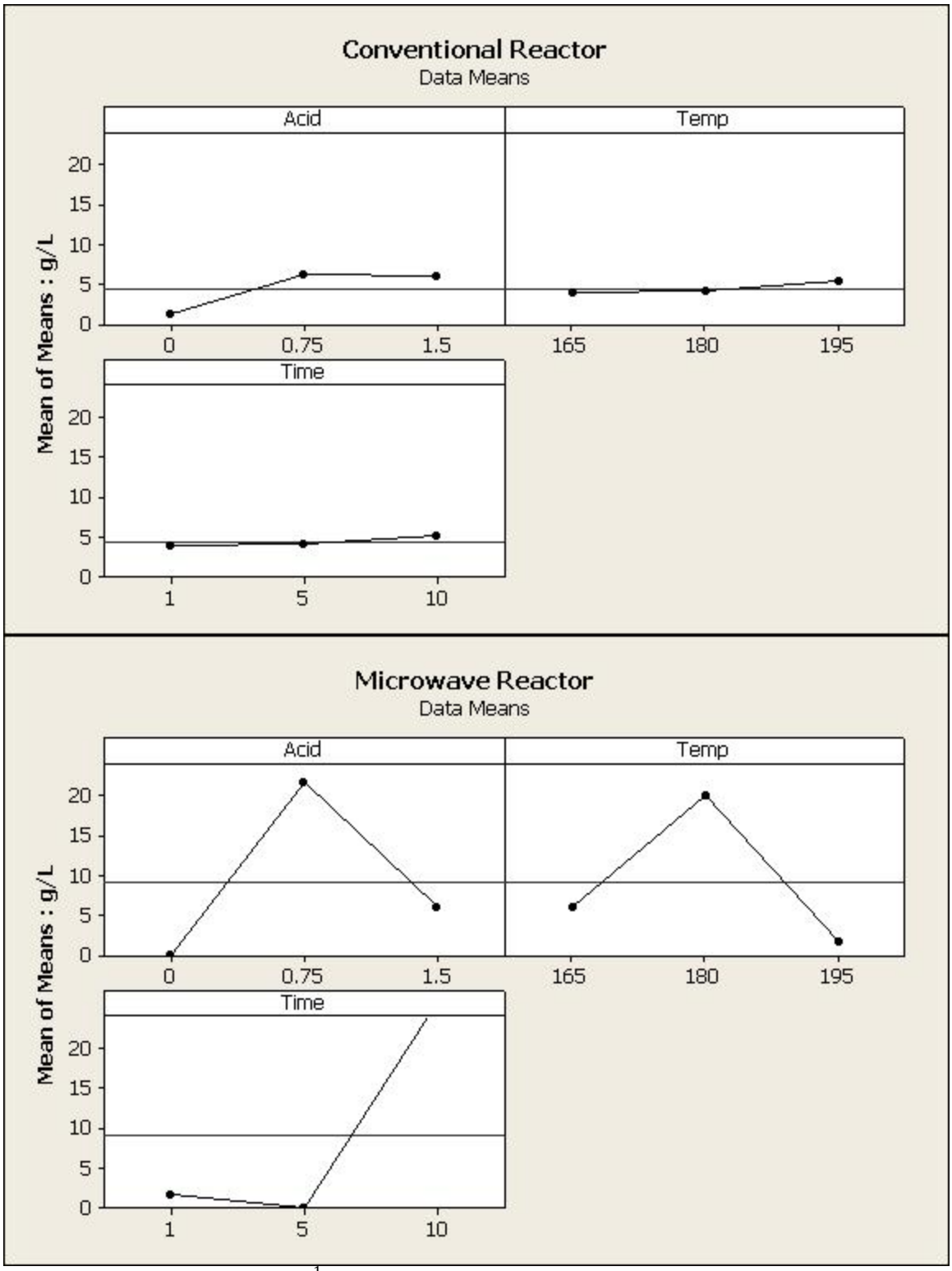

Figure A18: Acetic acid (g L^{-1}) in switchgrass liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

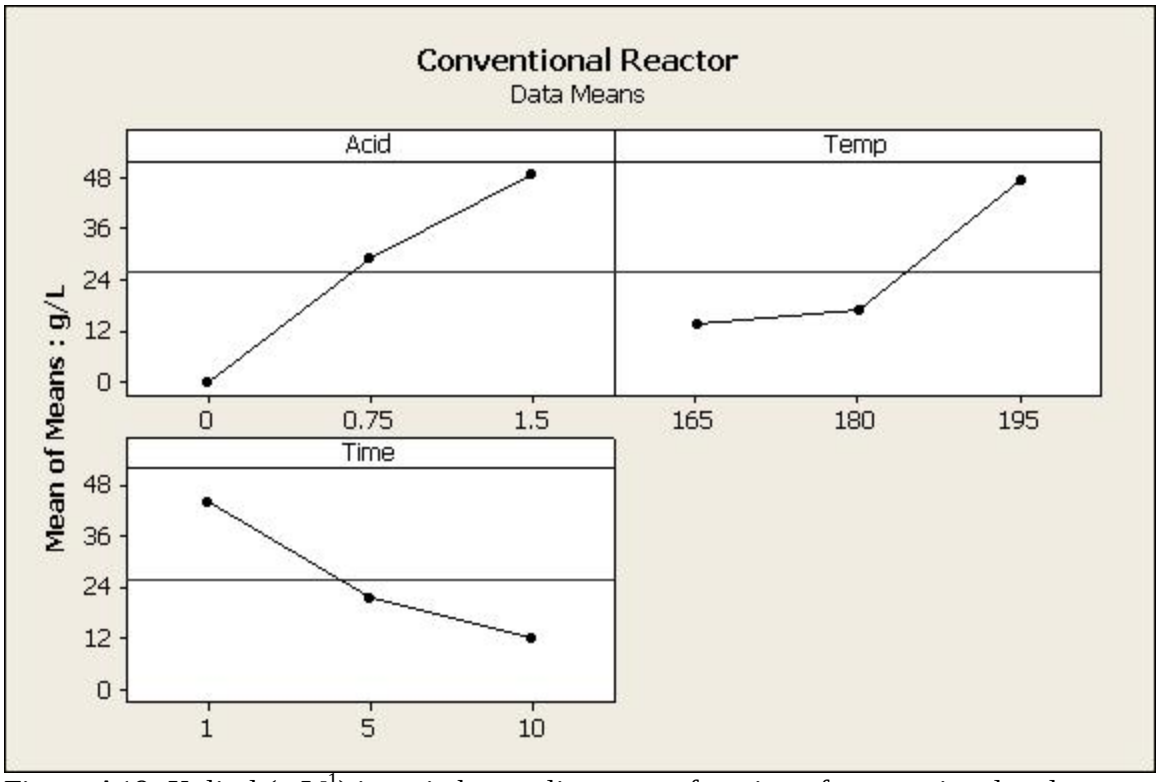

Figure A19: Xylitol (g L^{-1}) in switchgrass liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

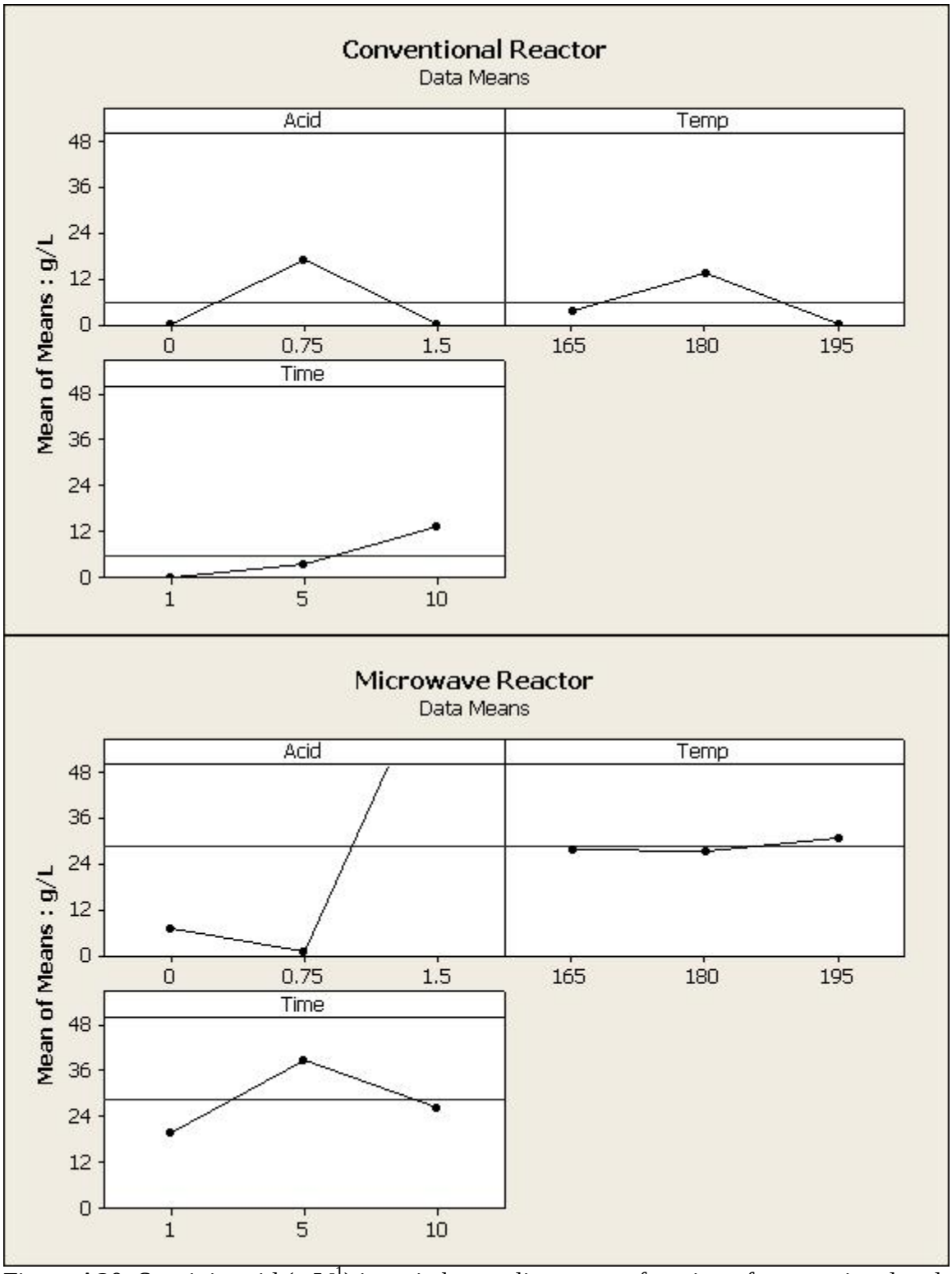

Figure A20: Succinic acid (g L^{-1}) in switchgrass liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

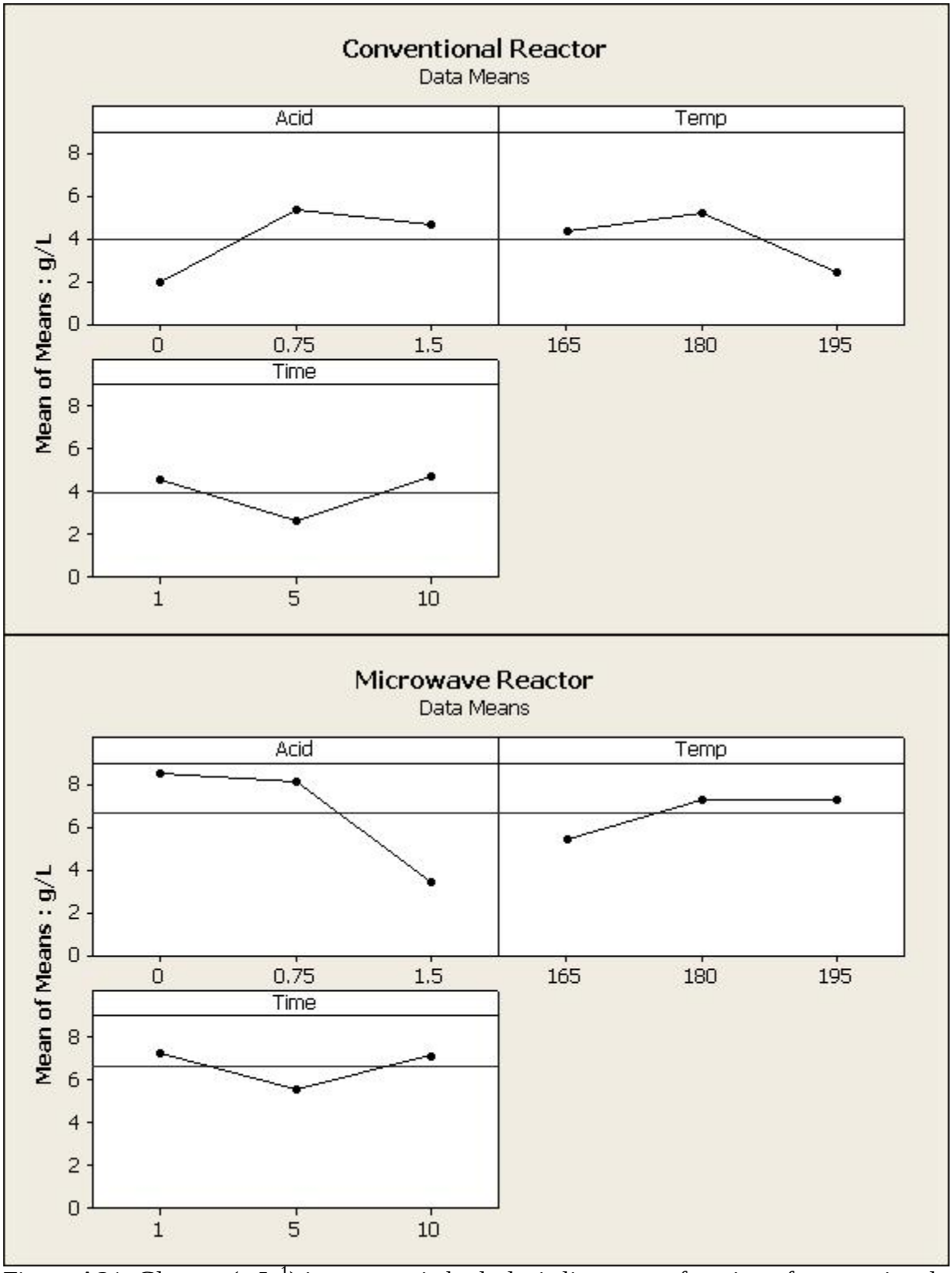

Figure A21: Glucose (g L^{-1}) in enzymatic hydrolysis liquor as a function of conventional and microwave pretreatment conditions- acid (vol%), temperature (^{o}C), residence time (min)

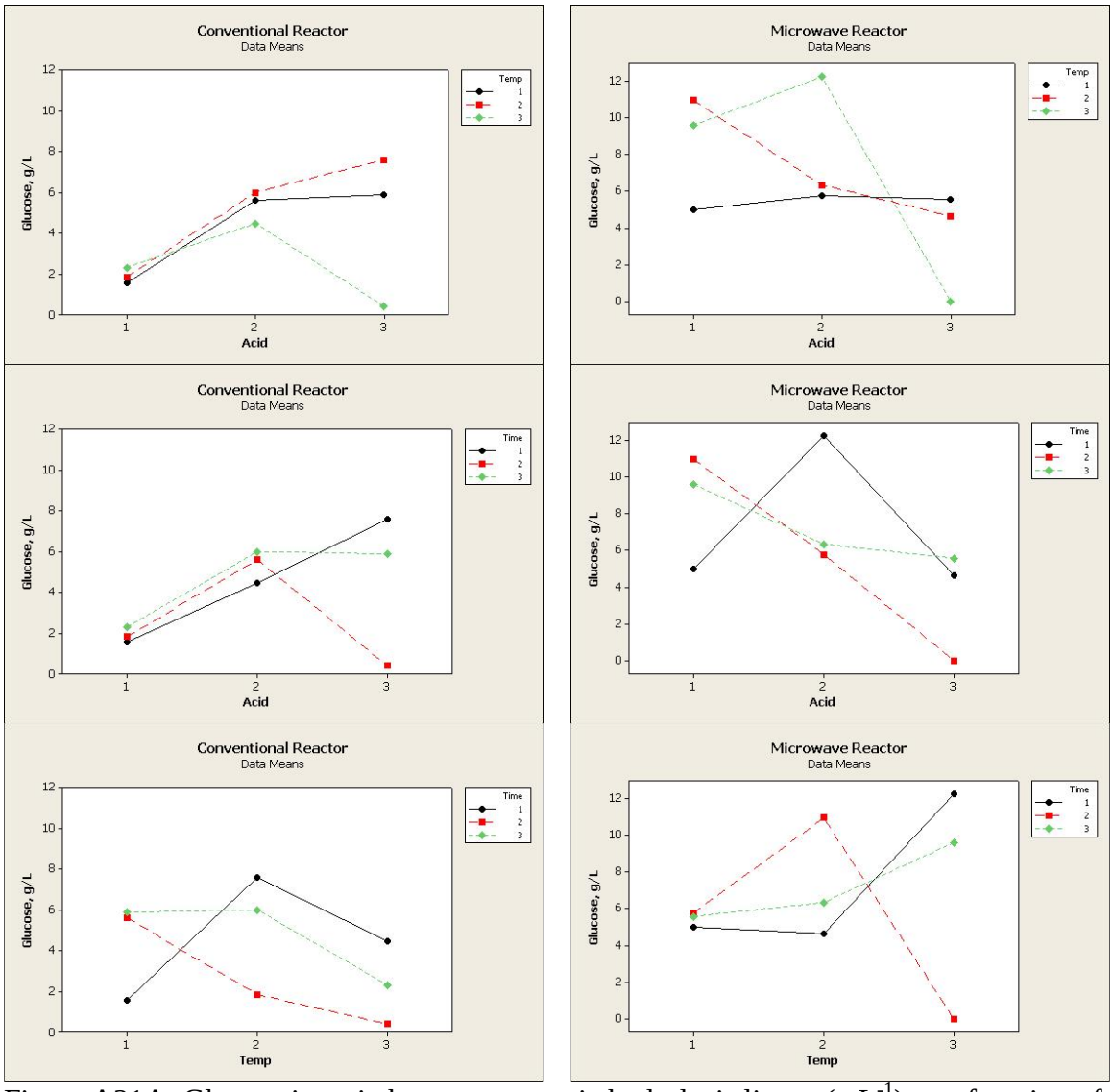

Figure A21A: Glucose in switchgrass enzymatic hydrolysis liquor (g L^{-1}) as a function of the conventional and microwave combination pretreatment conditions- Acid (vol%), Temp (^{o}C), Time (min).

	Acid, vol%	Temp, ^{o}C	Time, min
1	0	165	1
2	0.75	180	5
3	1.5	195	10

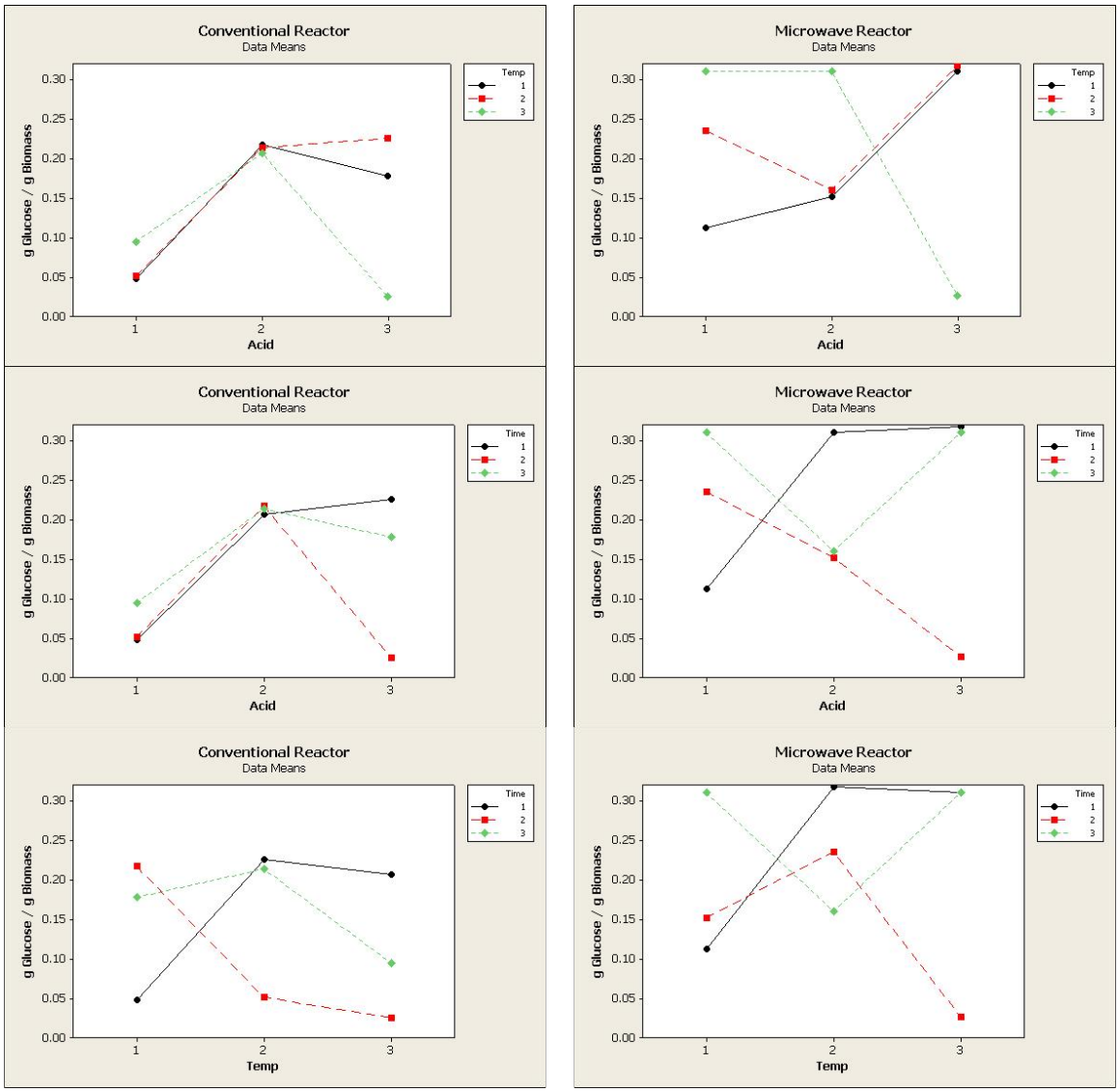

Figure A22A: Normalized glucose yield (g Glucose g Biomass) as a function of the combined conventional and microwave combination pretreatment conditions- Acid (vol%), Temp (^{o}C), Time (min).

	Acid, vol%	Temp, ^{o}C	Time, min
1	0	165	1
2	0.75	180	5
3	1.5	195	10

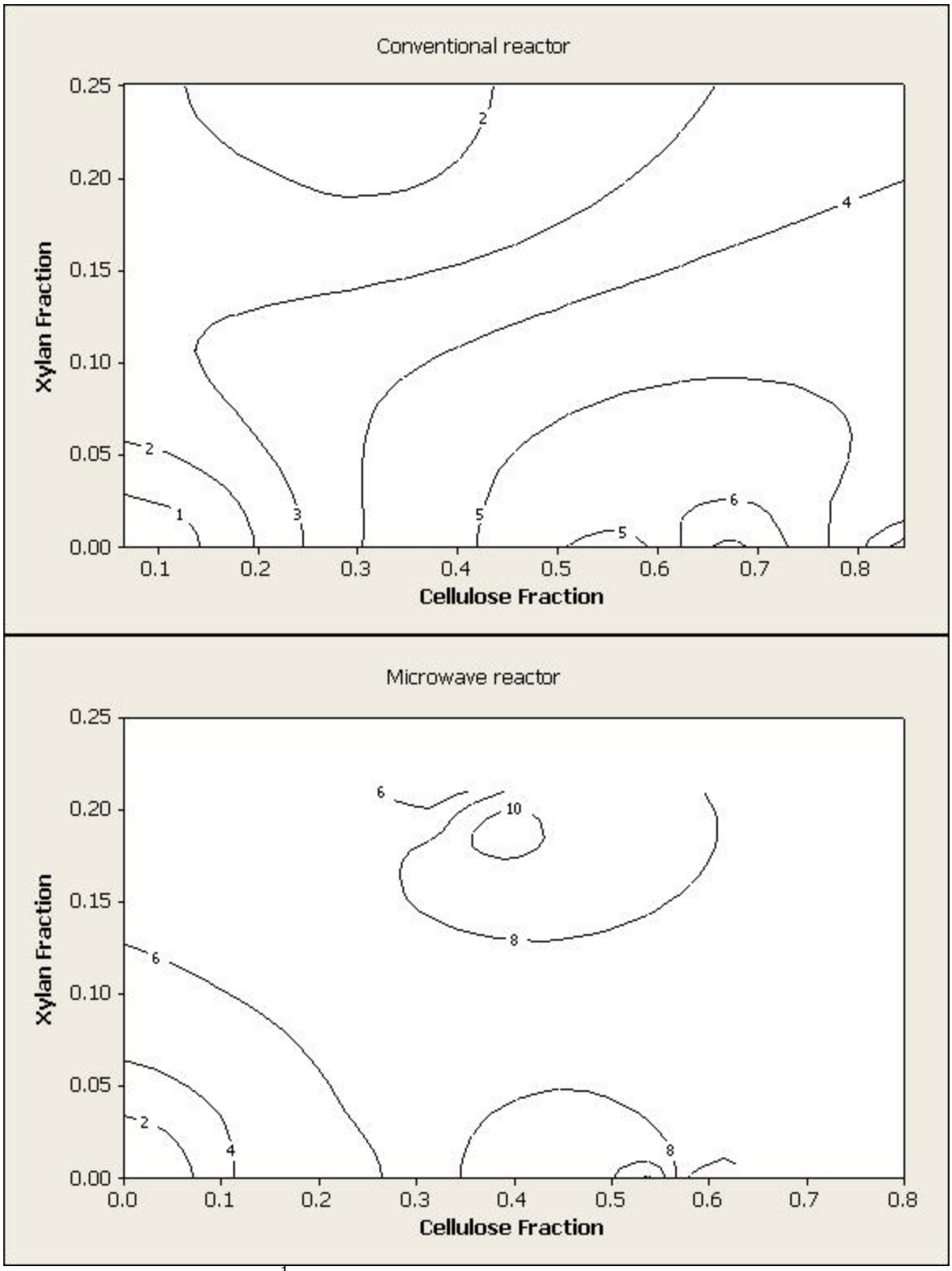

Figure A22: Glucose (g L^{-1}) in enzymatic hydrolysis liquor as a function of pretreated biomass cellulose and xylan fraction for conventional and microwave reactors

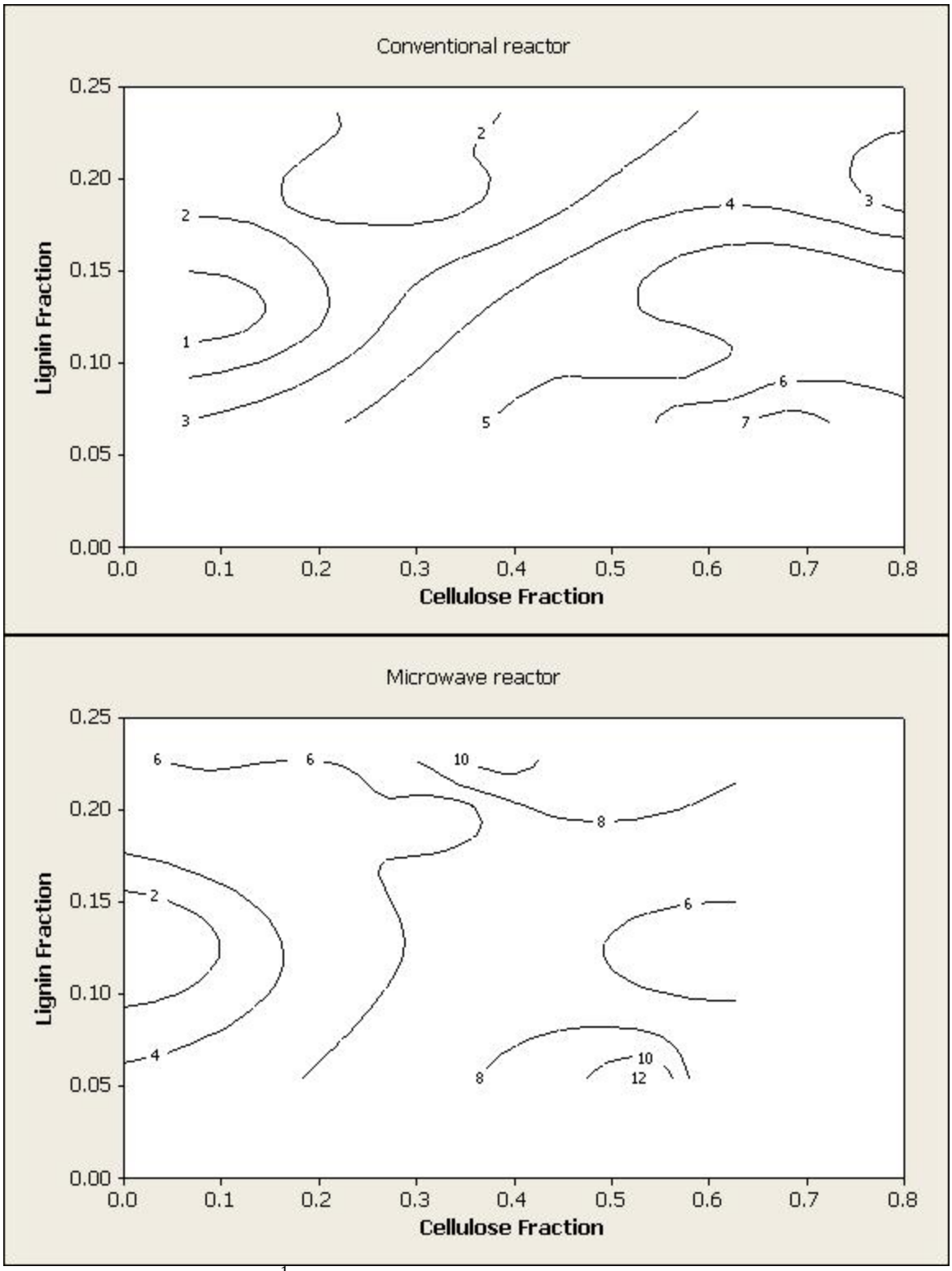

Figure A23: Glucose (g L^{-1}) in enzymatic hydrolysis liquor as a function of pretreated biomass cellulose and lignin fraction for conventional and microwave reactors

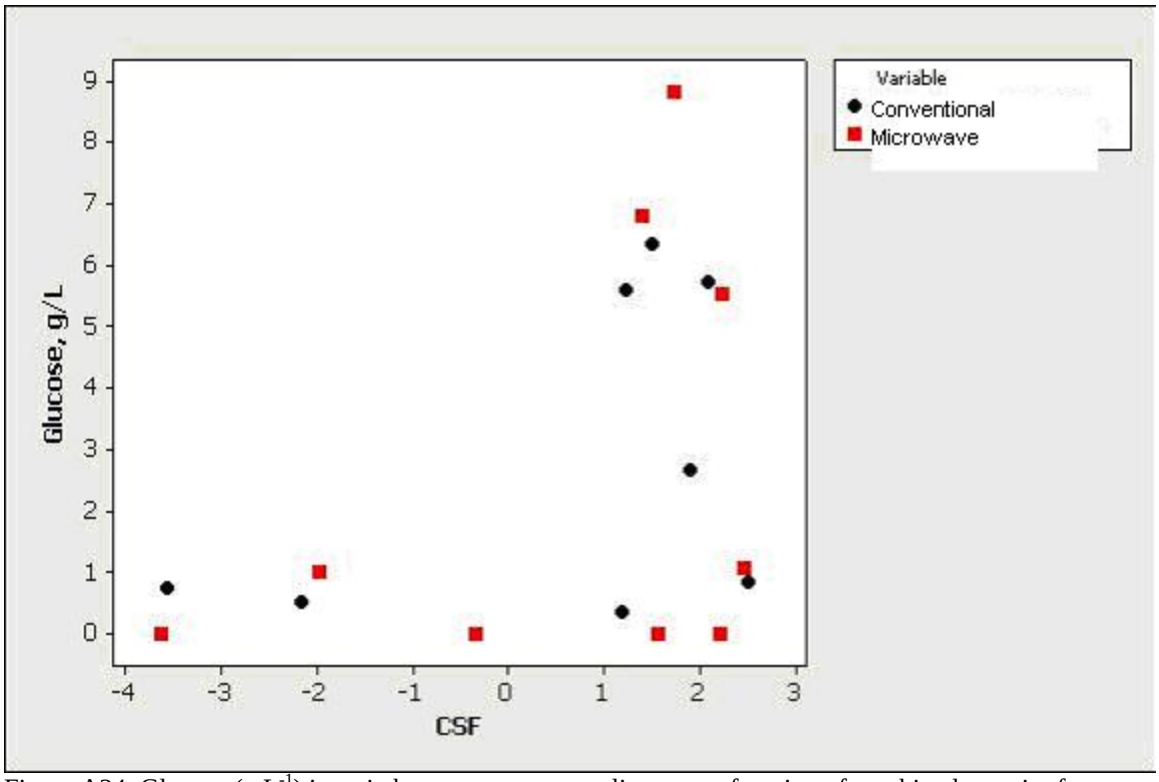

Figure A24: Glucose (g L^{-1}) in switchgrass-pretreatment liquor as a function of combined severity factor (CSF) for conventional and microwave reactors.

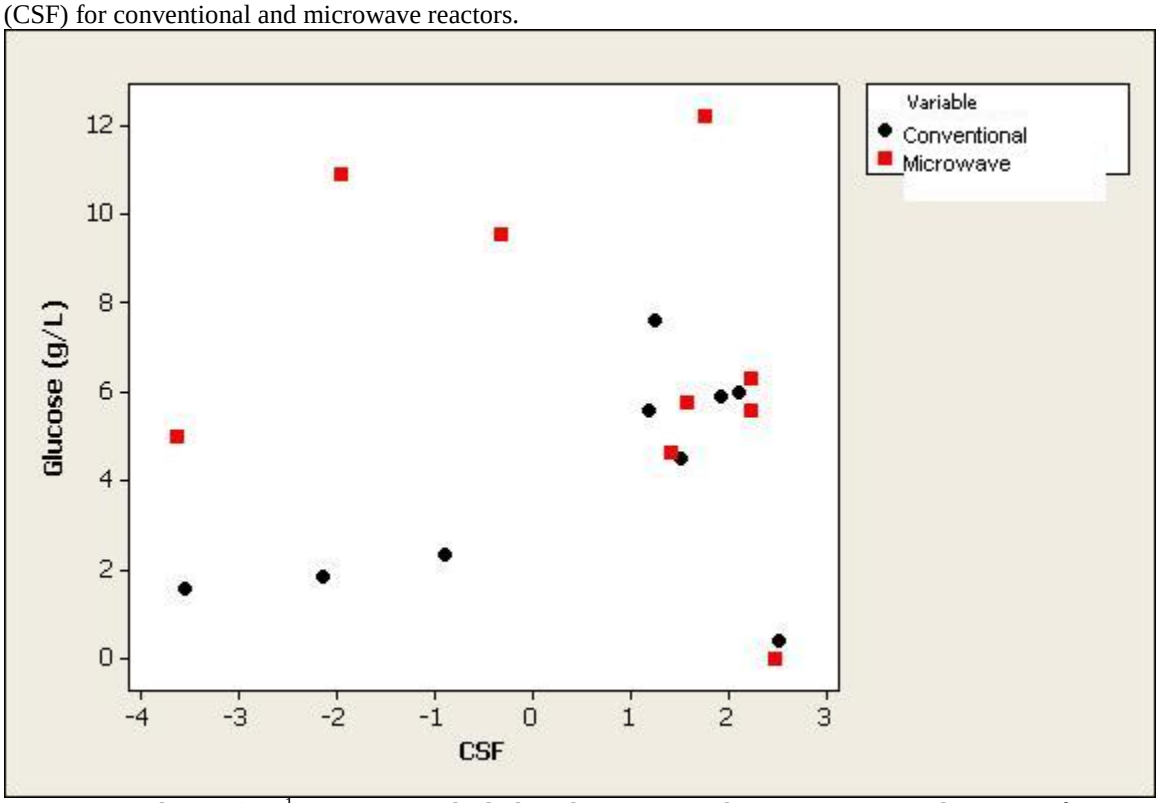

Figure A25: Glucose (g L^{-1}) in enzymatic hydrolysis liquor in switchgrass pretreatment liquor as a function of combined severity factor (CSF) for conventional and microwave reactors.

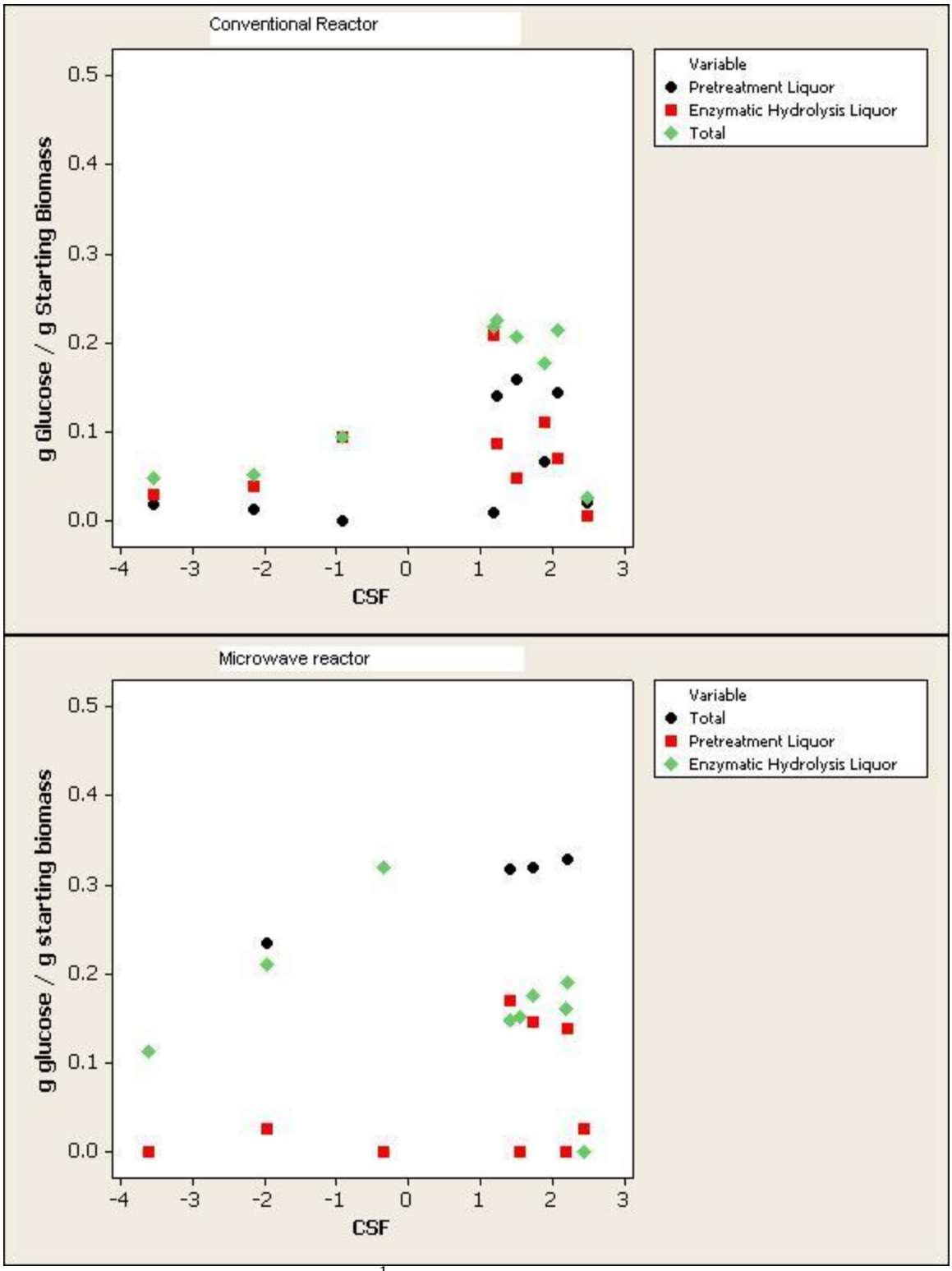

Figure A26: Combined glucose g g^{-1} (pretreatment and enzymatic hydrolysis liquors) as a function of combined severity factor (CSF) for conventional and microwave reactors

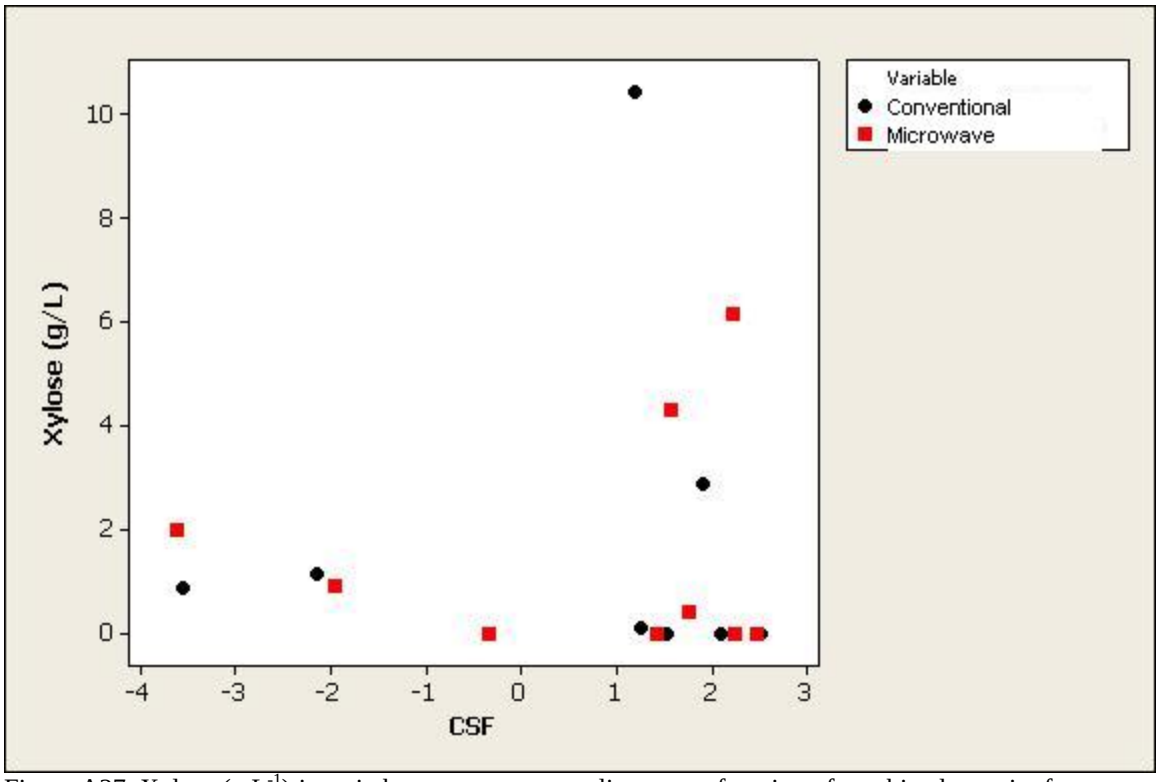

Figure A27: Xylose (g L^{-1}) in switchgrass pretreatment liquor as a function of combined severity factor (CSF) for conventional and microwave reactors.

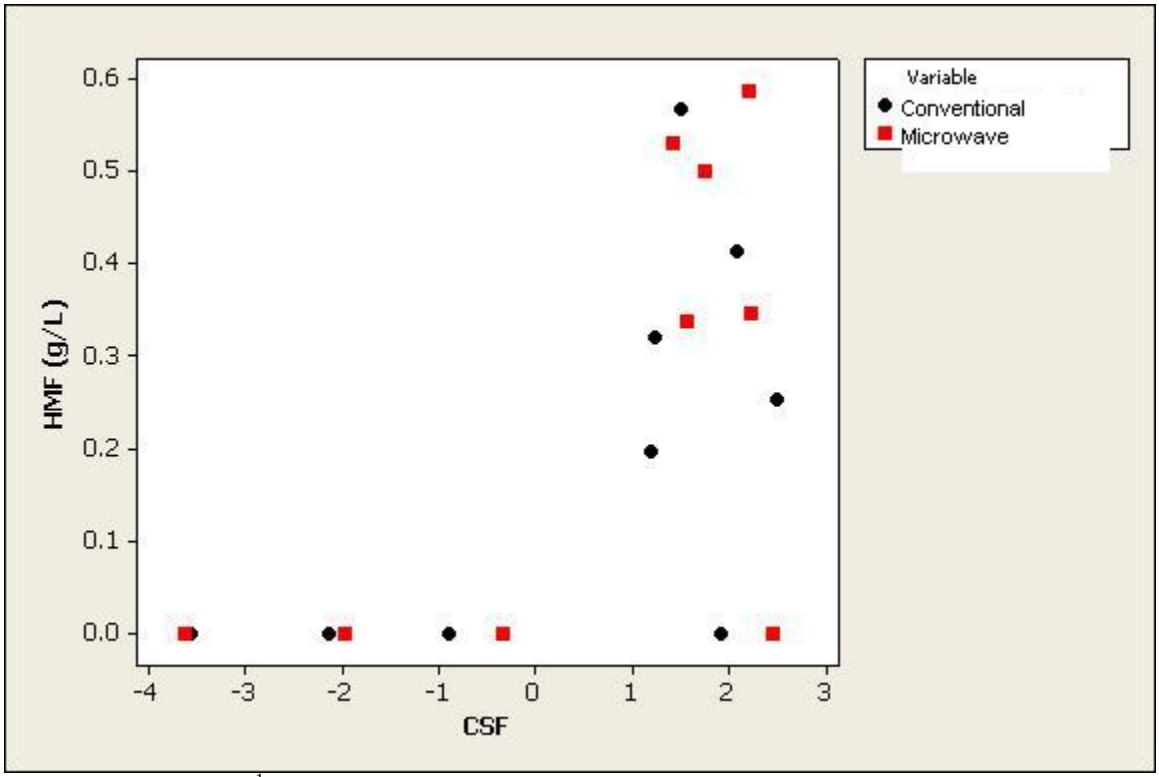

Figure A28: HMF (g L^{-1}) in switchgrass pretreatment liquors as a function of combined severity factor (CSF) for conventional and microwave reactors.

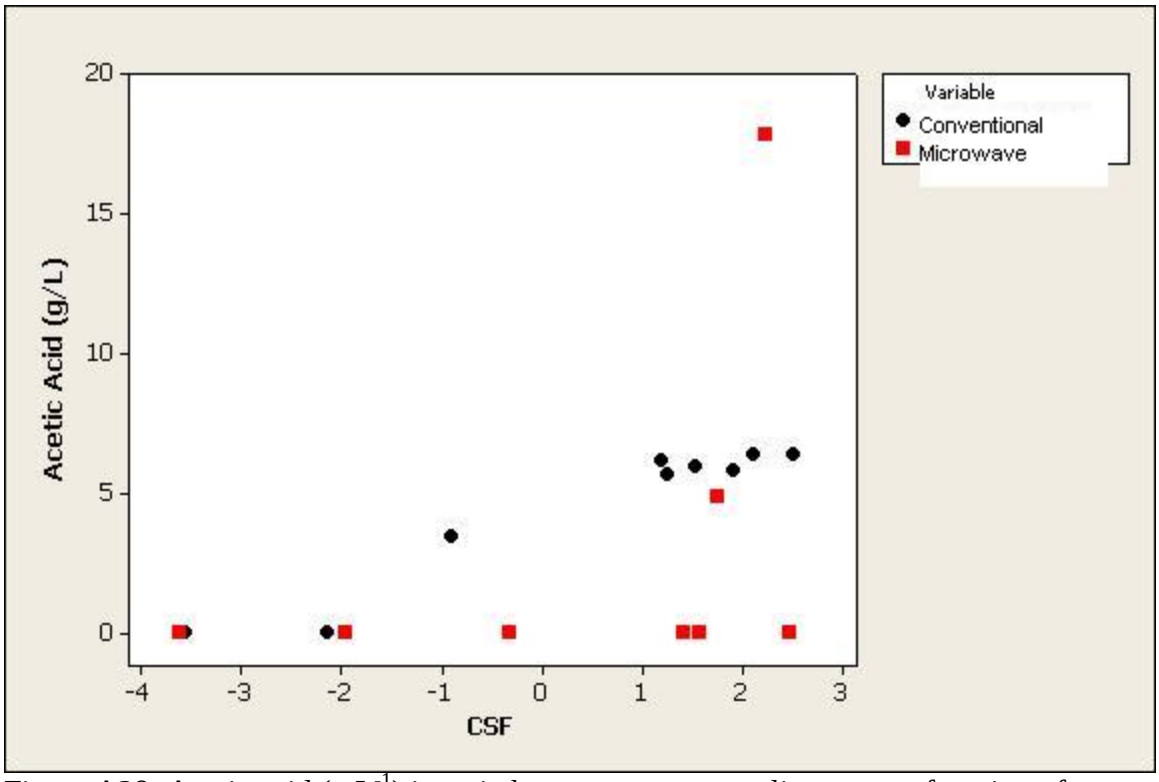

Figure A29: Acetic acid (g L^{-1}) in switchgrass pretreatment liquors as a function of combined severity factor (CSF) for conventional and microwave reactors.

		Milling				4	% solids		Pretreatment			Heat				
100	kg total					100	kg		Pressure	254	psi	1,810,924	kJ			
30	kg glucan		4	kWh		30	kg glucan		T	195	C					
29	kg xylan	T	25	C		29	kg xylan		pH	1.3						
24	kg lignin					24	kg lignin		60	% mass loss						
17	kg water					17	kg water									
									2,611	kg total						
									16	kg glucan						
									0	kg xylan						
									24	kg lignin						
									13.10	kg glucose						
									5.14	kg xylose						
									0	kg xylitol						
									0	kg succinic acid						
									15.55	kg acetic acid						
									1.19	kg HMF						
					6	kg Tetracycline			2,509	kg water						
					5	kg Cycloheximide			67	kg gypsum						
					16	kg cellulase										
					10	kg b glucosidase										
					27	kg total glucose										
Enzyme Yield		0.14			14	kg glucose hydrolysis			Enzymatic			Heat				
Pretreatment Yield		0.13			13	kg glucose pretreatment			Hydrolysis			-1,500,239	kJ			
Total Yield		0.27			2,621	kg balance			T	50	C					
									Digestion	0.9						
												Total Heat				
												310,685	kJ			

Figure A30: Mass and energy balance

APPENDIX 2

Methods

Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography

Laboratory Analytical Procedure #002

1. Introduction

1.1 The carbohydrates making up a major portion of biomass samples are polysaccharides composed primarily of glucose, xylose, arabinose, galactose, and mannose subunits. The polysaccharides present in a biomass sample can be hydrolyzed to their component sugar monomers by sulfuric acid in a two-stage hydrolysis process. The sample can then be quantified by ion-moderated partition HPLC.

1.2 This procedure has been adopted by ASTM as the Standard Test Method for Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography, E1758-95.

2. Scope

2.1 This method covers the determination of carbohydrates, expressed as the percent of each sugar present in a hydrolyzed biomass sample. The sample is taken through a primary 72% sulfuric acid hydrolysis, followed by a secondary dilute-acid hydrolysis.

2.2 Sample material suitable for this procedure include hard and soft woods, herbaceous materials (such as switchgrass and sericea), agricultural residues (such as corn stover, wheat straw, and bagasse), waste-paper (such as office waste, boxboard, and newsprint), washed acid- and alkaline-pretreated biomass, and the solid fraction of fermentation

residues. All results are reported relative to the 105°C oven-dried weight of the sample. In the case of extracted materials, the results may also be reported on an extractives-free basis.

2.3 All analyses shall be performed according to the guidelines established in the Ethanol Project Quality Assurance Plan (QAP).

3. References

3.1 Moore, W.E., and D.B. Johnson. 1967. *Procedures for the Chemical Analysis of Wood and Wood Products.* Madison, WI: U.S. Forest Products Laboratory, U.S. Department of Agriculture.

3.2 Ethanol Project Laboratory Analytical Procedure #001, "Standard Method for the Determination of Total Solids in Biomass".

3.3 Ethanol Project Laboratory Analytical Procedure #003, "Determination of Acid-Insoluble Lignin in Biomass".

3.4 NREL Ethanol Project Laboratory Analytical Procedure #004, "Determination of Acid-Soluble Lignin in Biomass".

3.5 NREL Ethanol Project Laboratory Analytical Procedure #010, "Standard Method for the Determination of Extractives in Biomass".

3.6 TAPPI Test Method T264 om-88, "Preparation of Wood For Chemical Analysis." *In Tappi Test Methods.* Atlanta, GA: Technical Association of the Pulp and Paper Industry.

3.7 Vinzant, T.B., L. Ponfick, N.J. Nagle, C.I. Ehrman, J.B. Reynolds, and M.E. Himmel. 1994.

"SSF Comparison of Selected Woods From Southern Sawmills." Appl. Biochem. Biotechnol., 45/46:611-626.

4. Terminology

4.1 Prepared Biomass - Biomass that has been prepared by lyophilization, oven drying, air drying, and in some instances by extraction, to reduce the moisture content of the sample so it is suitable for carbohydrate analysis.

4.2 Oven-Dried Weight - The moisture-free weight of a biomass sample as determined by LAP-001, "Standard Method for Determination of Total Solids in Biomass".

5. Significance and Use

5.1 The percent sugar content is used in conjunction with other assays to determine the total composition of biomass samples.

6. Interferences

6.1 Samples with high protein content may result in percent sugar values biased low, as a consequence of protein binding with some of the monosaccharides.

6.2 Test specimens not suitable for analysis by this procedure include acid- and alkaline-pretreated biomass samples that have not been washed. Unwashed pretreated biomass samples containing free acid or alkali may change visibly on heating.

7. Apparatus

7.1 Hewlett Packard Model 1090 HPLC, or equivalent, with refractive index detector.

7.2 HPLC columns, BioRad Aminex7 HPX-87C and/or Aminex7 HPX-87P (or equivalent).

7.3 Guard columns, cartridges appropriate for the column used.

Note: Deashing guard column cartridges from BioRad, of the ionic form H+/CO3%, are an option when using an HPX-87P column. These cartridges have been found to be effective in eliminating baseline ramping.

7.4 Analytical balance readable to 0.1 mg.

7.5 Convection ovens with temperature control to 45 ± 3°C and 105 ± 3°C.

7.6 Autoclave capable of maintaining 121 ± 3°C.

7.7 Water bath set at 30 ± 3°C.

7.8 Desiccator containing anhydrous calcium sulfate.

8. Reagents and Materials

8.1 Reagents

8.1.1 High purity sugars for standards (98%+) - two sets of glucose, xylose, galactose, arabinose,and mannose from different lots or manufacturers.

8.1.2 72% w/w H2SO4 (12.00 ± 0.02 M or specific gravity 1.6389 at 15.6 °C /15.6°C).

8.1.3 Calcium carbonate, ACS reagent grade.

8.1.4 Water, 18 megohm deionized.

8.2 Materials

8.2.1 Glass test tubes, 16x100 mm.

8.2.2 125 mL glass serum bottles, crimp top style, with rubber stoppers and aluminum seals to fit.

8.2.3 pH paper, suitable to cover the pH range of 4 to 7.

8.2.4 Disposable nylon syringe filters, 0.2 μm.

8.2.5 Disposable syringes, 3 mL.

8.2.6 Autosampler vials, with crimp top seals to fit.

8.2.7 Erlenmeyer flasks, 50 mL.

9. ES&H Considerations and Hazards

9.1 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

9.2 72% H2SO4 is very corrosive and must be handled carefully.

9.3 Use caution when handling hot glass bottles after the autoclave step, as they may have become pressurized.

10. Sampling, Test Specimens and Test Units

10.1 Test specimens suitable for analysis by this procedure are as follows:

- biomass feedstocks, dried and reduced in particle size, if necessary.
- pretreated biomass, washed free of any residual acid or alkali.
- the solids fraction of fermentation residues.

10.2 The sample must not contain particles larger than 1 mm in diameter. If milling is required to reduce the particle size of the test specimen, a laboratory mill equipped with a 40 mesh (or smaller) screen should be used.

10.3 The total solids content of the "as received" test specimen (prior to any drying or extraction steps) must be determined by LAP-001 in parallel with the carbohydrate analysis. Record this value as %Tas received.

10.4 Material with a total solids content less than 85%, on a 105°C dry weight basis, will require drying by lyophilization, oven drying, or air drying prior to milling or analysis. The amount of moisture lost as a result of the preparation procedure must be determined. This moisture content is used to calculate the total solids content of the sample based on its preparation and is recorded as %Tprep. This value is used to correct the weight of the prepped material used in the carbohydrate analysis, as described in the calculations section. The prepared sample should be stored in a manner to ensure its moisture content does not change prior to analysis.

Note: Preparing samples for analysis by oven drying can produce hard chunks of material. This material must then be milled to reduce the size of the large pieces to less then 1 mm in diameter. The sample is then redried prior to testing.

10.5 Some samples may require extraction prior to analysis, to remove components that may interfere with the analysis. LAP-010, "Standard Method for the Determination of Extractives in Biomass", is used to prepare an extractives-free sample with a moisture content suitable for carbohydrate analysis. As part of this procedure, the percent extractives in the prepared sample, on a 105°C dry weight basis, is determined. This value, recorded as % extractives, can be used to convert the % sugar reported on a extractives-free basis to an as received (whole sample) basis.

10.6 The test specimen shall consist of approximately 0.3 g of sample. The test specimen shall be obtained in such a manner to ensure that it is representative of the entire lot of material being tested.

11. Procedure

11.1 This procedure is suitable for air-dried, lyophilized, and extracted biomass samples, as well as for samples that have been oven dried at a temperature of 45°C or less. It is not suitable for samples that have been dried at a temperature exceeding 45°C.

Note: The total solids content of the original sample, %Tas received, must be determined using LAP-001, prior to any preparatory steps. The total solids content of the sample based on its preparation, %Tprep , must also be known.

11.2 Determine the total solids content of the prepared or extractives-free biomass sample by LAP-001 and record this value as %Tfinal .

Note: Samples for total solids determination (LAP-001) must be weighed out at the same time as the samples for the carbohydrate determination. If this is done later, it can introduce an error in the calculation because ground biomass can rapidly gain or lose moisture when exposed to the atmosphere.

11.3 Weigh 0.3 ± 0.01 g of the prepared or extractives-free sample to the nearest 0.1 mg and place in a 16x100 mm test tube. Record as *W1*, the initial sample weight in grams. Each sample must be run in duplicate, at minimum.

11.4 Add 3.00 ± 0.01 mL (4.92 ± 0.01 g) of 72% H2SO4 and use a glass stirring rod to mix for 1 minute, or until the sample is thoroughly wetted.

11.5 Place the test tube in the water bath set at 30 ± 1°C and hydrolyze for 2 hours.

11.6 Stir the sample every 15 minutes to assure complete mixing and wetting.

11.7 Weigh out 0.3 ± 0.01 g of each high purity sugar (predried at 45°C) to the nearest 0.1 mg, and place each in its own 16x100 mm glass test tube. Add acid, hydrolyze, and stir these sugars as described in the previous three steps. These sugar recovery standards

(SRS) will be taken through the remaining steps in the procedure in parallel with the samples. The calculated recovery of the SRSs will be used to correct for losses due to the destruction of sugars during the hydrolysis process. It may be useful to run selected SRSs in duplicate, particularly if specific sugars are deemed critical.

11.8 Prepare a method verification standard (MVS) by weighing out 0.3 ± 0.01 g of a well characterized standard material suitable for analysis. Add acid, hydrolyze, and stir the MVS as was done with the samples and SRSs (see 11.4-11.6 above). This MVS will be taken through the remaining steps in the procedure in parallel with the samples and the SRSs, and is used to test the reproducibility of the method as a whole.

Note: A suitable method verification standard, *Populus deltoides*, may be obtained from NIST (research material #8492).

11.9 Upon completion of the two hour hydrolysis step, transfer each hydrolyzate to its own serum bottle and dilute to a 4% acid concentration by adding 84.00 ± 0.04 mL deionized water. Be careful to transfer all residual solids along with the hydrolysis liquor. The total weight added to the tared bottle is 89.22 g (0.3 g sample, 4.92 g 72% H2SO4, and 84.00 g deionized water). Since the specific gravity of the 4% acid solution is 1.0250 g/mL, the total volume of solution, VF , is 87.0 mL.

11.10 Stopper each of the bottles and crimp aluminum seals into place.

11.11 Set the autoclave to a liquid cycle to prevent loss of sample from the bottle in the event of a loose crimp seal. Autoclave the samples in their sealed bottles for 1 hour at 121 ± 3°C.

11.12 After completion of the autoclave cycle, allow the samples to cool for about 20 minutes at room temperature before removing the seals and stoppers.

11.13 These autoclaved solutions may also be used for the determination of acid-insoluble residue and/or acid-soluble lignin, in parallel with this carbohydrate determination.

Note: If acid-insoluble lignin and/or acid-soluble lignin determinations are to be conducted on a sample, the residual solids must be collected by filtering the hydrolyzate through an ashed and weighed filtering crucible prior to proceeding with the carbohydrate determination. Refer to LAP-003, "Determination of Acid-Insoluble Lignin in Biomass", for details. If an acid-soluble lignin determination is to be conducted, a portion of the filtrate must be reserved for analysis. Acid-soluble lignin should be analyzed within 24 hours, preferably within 6 hours of hydrolysis. Refer to the procedure "Determination of Acid-Soluble Lignin in Biomass" (LAP-004) for details.

11.14 Transfer 20 mL aliquots of each hydrolyzate, or filtrate, to 50 mL Erlenmeyer flasks.

11.15 Neutralize with calcium carbonate to a pH between 5 and 6. Do not over-neutralize. Add the calcium carbonate slowly with frequent swirling to avoid problems with foaming. Monitor the pH of the solution with pH paper to avoid over-neutralization.

11.16 Filter the neutralized hydrolyzate using a 3 mL syringe with a 0.2 μm filter attached. One portion of the hydrolyzate should be filtered directly into a sealable test tube for storage. A second portion should be filtered directly into an autosampler vial if the hydrolyzate is to be analyzed without dilution. If the concentration of any of the analytes is expected to exceed the validated linear range of the analysis, dilute the hydrolyzate as required and filter into an autosampler vial for analysis.

Note: It is advisable to determine the initial glucose concentration of the sample using an alternative technique, such as a YSI glucose analyzer, in order to predict whether or not the glucose in the sample will fall within the linear range of the analysis.

11.17 The portion of the neutralized hydrolyzate filtered into the test tube should be securely sealed, labeled, placed in the refrigerator, and reserved in case a repeat analysis is required. The sample should be stored for no longer than two weeks.

11.18 Prepare a series of sugar calibration standards in deionized water at concentrations appropriate for creating a calibration curve for each sugar of interest. A suggested scheme for the HPX-87C column is to prepare a set of multi-component standards containing glucose, xylose, and arabinose in the range of 0.2 -12.0 mg/mL. For the HPX-87P column, galactose, and mannose should be included as additional components in the standards. Extending the range of the calibration curves beyond 12.0 mg/mL will require validation.

11.19 Prepare an independent calibration verification standard (CVS) for each set of calibration standards, using sugars obtained from a source other than that used in preparing the calibration standards. The CVS must contain precisely known amounts of each sugar contained in the calibration standards, at a concentration that falls in the middle of the validated range of the calibration curve. The CVS is to be analyzed after each calibration curve and at regular intervals in the HPLC sequence, bracketing groups of samples. The CVS is used to verify the quality of the calibration curve(s) throughout the HPLC run.

11.20 Analyze the calibration standards, the CVS, the samples, the SRSs, and the MVS by HPLC using a Biorad Aminex7 HPX-87C or HPX-87P column for glucose, xylose, and arabinose. If mannose and galactose are also to be determined, a Biorad Aminex7 HPX-87P column must be used instead. For many analyses, it is useful to run the same samples on both columns and compare the results. The following instrumental conditions are used for both the HPX-87C and the HPX-87P columns:

Sample volume: 50 μL.

Eluant: 0.2 μm filtered and degassed, deionized water.

Flow rate: 0.6 mL/min.

Column temperature: 85°C.

Detector: refractive index.

Run time: 20 minutes data collection plus a 15 minute post-run.

Procedure Title: Determination of Structural Carbohydrates and Lignin in Biomass

6. Apparatus

6.1 Analytical balance, accurate to 0.1 mg

6.2 Convection drying oven, with temperature control of 105 ± 3oC

6.2 Muffle furnace, equipped with a thermostat, set to 575 + 25 °C or equipped with optional ramping program

6.3 Water bath, set at 30 ± 3 °C

6.4 Autoclave, suitable for autoclaving liquids, set to 121 ± 3 °C

6.5 Filtration setup, equipped with a vacuum source and vacuum adaptors for crucibles

6.6 Desiccator containing desiccant

6.7 HPLC system equipped with refractive index detector and the following columns:

6.7.1 Shodex sugar SP0810 or Biorad Aminex HPX-87P column (or equivalent) with ionic form H+/CO3- deashing guard column

6.7.2 Biorad Aminex HPX-87H column (or equivalent) equipped with an appropriate guard column

6.8 UV-Visible spectrophotometer, diode array or single wavelength, with high purity quartz cuvettes of pathlength 1 cm

6.9 Automatic burette, capable of dispensing 84.00 mL water, optional

7. Reagents and materials

7.1 Reagents

7.1.1 Sulfuric acid, 72% w/w (specific gravity 1.6338 at 20oC)- (also commercially available as a reagent for the determination of fluorine, from Fluka #00647)

7.1.2 Calcium carbonate, ACS reagent grade

7.1.3 Water, purified, 0.2 μm filtered

7.1.4 High purity standards : D-cellobiose, D(+)glucose, D(+)xylose, D(+)galactose, L(+)arabinose, and D(+)mannose

7.1.5 Second set of high purity standards, as listed above, from a different source (manufacturer or lot), to be used to prepare calibration verification standards (CVS)

7.2 Materials

7.2.1 QA standard, well characterized, such as a National Institute of Standards and Technology (NIST) biomass standard or another well characterized sample of similar composition to the samples being analyzed

7.2.2 Pressure tubes, minimum 90 mL capacity, glass, with screw on Teflon caps and o-ring seals (Ace glass # 8648-30 tube with #5845-47 plug, or equivalent)

7.2.3 Teflon stir rods sized to fit in pressure tubes and approximately 5 cm longer than pressure tubes

7.2.4 Filtering crucibles, 25 mL, porcelain, medium porosity, Coors #60531 or equivalent

7.2.5 Bottles, wide mouth, 50 mL

7.2.6 Filtration flasks, 250 mL

7.2.7 Erlenmeyer flasks, 50 mL

7.2.8 Adjustable pipettors, covering ranges of 0.02 to 5.00 mL and 84.00 mL

7.2.9 pH paper, range 4-9

7.2.10 Disposable syringes, 3 mL, fitted with 0.2 μm syringe filters

7.2.11 Autosampler vials with crimp top seals to fit

8. ES&H Considerations and Hazards

8.1 Sulfuric acid is corrosive and should be handled with care.

8.2 Use caution when handling hot pressure tubes after removal from the autoclave, as the pressurized tubes can cause an explosion hazard.

8.3 When placing crucibles in a furnace or removing them, use appropriate personal protective equipment, including heat resistant gloves.

8.4 Operate all equipment in accordance with the manual and NREL Safe Operating Procedures

8.5 Follow all applicable NREL chemical handling procedures

9. Sampling, Test Specimens and Test Units

9.1 Care must be taken to ensure a representative sample is taken for analysis.

9.2 LAP “Preparation of Samples of Biomass Compositional Analysis” should be performed prior to this analysis. Samples must have a minimum total solids content of 85%.

9.3 LAP “Determination of Extractives in Biomass” should be performed prior to this analysis if extractives are present in the sample.

9.4 LAP “Determination of Solids in Biomass” should be performed at the same time that samples for this analysis are weighed out.

9.5 This procedure is suitable for samples that have been air dried or lyophilized. Samples dried at a temperature of 45°C or higher are not suitable for this procedure.

9.6 Steps 9.2 to 9.4 should be applied to the QA standard

10. Procedure

10.1 Prepare the sample for analysis and hydrolyze

10.1.1 Place an appropriate number of filtering crucibles in the muffle furnace at 575 +25 °C for a minimum of four hours. Remove the crucibles from the furnace directly into a desiccator and cool for a specific period of time, one hour is recommended. Weigh the crucibles to the nearest 0.1 mg and record this weight. It is important to keep the crucibles in a specified order, if they are not marked with identifiers. Permanent marking decals are available from Wale Apparatus. Do not mark the bottom of the filtering crucible with a porcelain marker, as this will impede filtration.

10.1.2 Place the crucible back into the muffle furnace at 575 ± 25 oC and ash to constant weight. Constant weight is defined as less than ± 0.3 mg change in the weight upon one hour of re-heating the crucible.

10.1.3 Weigh 300.0 + 10.0 mg of the sample or QA standard into a tared pressure tube. Record the weight to the nearest 0.1 mg. Label the pressure tube with a permanent marker. LAP “Determination of Total Solids in Biomass” should be performed at the same time, to accurately measure the percent solids for correction. Each sample should be analyzed in duplicate, at minimum. The recommended batch size is three to six samples and a QA standard, all run in duplicate.

10.1.4 Add 3.00 ± 0.01 mL (or 4.92 ± 0.01 g) of 72% sulfuric acid to each pressure tube. Use a Teflon stir rod to mix for one minute, or until the sample is thoroughly mixed.

10.1.5 Place the pressure tube in a water bath set at 30 ± 3 °C and incubate the sample for 60 + 5 minutes. Using the stir rod, stir the sample every five to ten minutes without removing the sample from the bath. Stirring is essential to ensure even acid to particle contact and uniform hydrolysis.

10.1.6 Upon completion of the 60-minute hydrolysis, remove the tubes from the water bath. Dilute the acid to a 4% concentration by adding 84.00 ± 0.04 mL deionized water using an automatic burette. Dilution can also be done by adding 84.00 ± 0.04 g of purified water using a balance accurate to 0.01 g. Screw the Teflon caps on securely. Mix the sample by inverting the tube several times to eliminate phase separation between high and low concentration acid layers.

10.1.7 Prepare a set of sugar recovery standards (SRS) that will be taken through the remaining hydrolysis and used to correct for losses due to destruction of sugars during dilute acid hydrolysis. SRS should include D-(+)glucose, D-(+)xylose, D-(+)galactose, -L(+)arabinose,and D-(+)mannose. SRS sugar concentrations should be chosen to most closely resemble the concentrations of sugars in the test sample. Weigh out the required amounts of each sugar, to the nearest 0.1 mg, and add 10.0 mL deionized water. Add 348 μL of 72% sulfuric acid. Transfer the SRS to a pressure tube and cap tightly.

10.1.7.1 A fresh SRS is not required for every analysis. A large batch of sugar recovery standards may be produced, filtered through 0.2 μm filters, dispensed in 10.0 mL aliquots into sealed containers, and labeled. They may be stored in a freezer and removed when needed. Thaw and vortex the frozen SRS prior to use. If frozen SRS are used, the

appropriate amount of acid must be added to the thawed sample and vortexed prior to transferring to a pressure tube.

10.1.8 Place the tubes in an autoclave safe rack, and place the rack in the autoclave. Autoclave the sealed samples and sugar recovery standards for one hour at 121°C, usually the liquids setting. After completion of the autoclave cycle, allow the hydrolyzates to slowly cool to near room temperature before removing the caps. (If step 10.2 is not performed, draw a 10 mL aliquot of the liquor for use in step 10.5.)

10.2 Analyze the sample for acid insoluble lignin as follows

10.2.1 Vacuum filter the autoclaved hydrolysis solution through one of the previously weighed filtering crucibles. Capture the filtrate in a filtering flask.

10.2.2 Transfer an aliquot, approximately 50 mL, into a sample storage bottle. This sample will be used to determine acid soluble lignin as well as carbohydrates, and acetyl if necessary. Acid soluble lignin determination must be done within six hours of hydrolysis. If the hydrolysis liquor must be stored, it should be stored in a refrigerator for a maximum of two weeks. It is important to collect the liquor aliquot before proceeding to step 10.2.3.

10.2.3 Use deionized water to quantatively transfer all remaining solids out of the pressure tube into the filtering crucible. Rinse the solids with a minimum of 50 mL fresh deionized water. Hot deionized water may be used in place of room temperature water to decrease the filtration time.

10.2.4 Dry the crucible and acid insoluble residue at 105 + 3 °C until a constant weight is achieved, usually a minimum of four hours.

10.2.5 Remove the samples from the oven and cool in a desiccator. Record the weight of the crucible and dry residue to the nearest 0.1 mg.

10.2.6 Place the crucibles and residue in the muffle furnace at 575 + 25 °C for 24 + 6 hours.

10.2.6.1 A furnace with temperature ramping may also be used Furnace Temperature Ramp Program: Ramp from room temperature to 105 °C Hold at 105°C for 12 minutes Ramp to 250 °C at 10°C / minute Hold at 250 °C for 30 minutes Ramp to 575 °C at 20 °C / minute Hold at 575 °C for 180 minutes Allow temperature to drop to 105 °C Hold at 105 °C until samples are removed

10.2.7 Carefully remove the crucible from the furnace directly into a desiccator and cool for a specific amount of time, equal to the initial cool time of the crucibles. Weigh the crucibles and ash to the nearest 0.1 mg and record the weight. Place the crucibles back in the furnace and ash to a constant weight. (The amount of acid insoluble ash is not equal to the total amount of ash in the biomass sample. Refer to LAP “Determination of Ash in Biomass” if total ash is to be determined.)

10.3 Analyze the sample for acid soluble lignin as follows

10.3.1 On a UV-Visible spectrophotometer, run a background of deionized water or 4% sulfuric acid.

10.3.2 Using the hydrolysis liquor aliquot obtained in step 10.2.2, measure the absorbance of the sample at an appropriate wavelength on a UV-Visible spectrophotometer. Refer to section11.3 for suggested wavelength values. Dilute the sample as necessary to bring the absorbance into the range of 0.7 – 1.0, recording the dilution. Deionized water or 4% sulfuric acid may be used to dilute the sample, but the

same solvent should be used as a blank. Record the absorbance to three decimal places. Reproducibility should be + 0.05 absorbance units. Analyze each sample in duplicate, at minimum. (This step must be done within six hours of hydrolysis.)

10.3.3 Calculate the amount of acid soluble lignin present using calculation 11.3.

Procedure Title: Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples Laboratory Analytical Procedure

1. Introduction

1.1 Carbohydrates make up a major portion of biomass samples. These carbohydrates are polysaccharides constructed primarily of glucose, xylose, arabinose, galactose, and mannose monomeric subunits. During certain pretreatments of biomass, a portion of these polysaccharides are hydrolyzed and soluble sugars are released into the liquid stream. This method is used to quantify the total amount of soluble carbohydrates released into solution as well as the amount of monomeric sugars released into solution. The soluble sugars in the liquid fraction of process samples can be quantified by HPLC with refractive index detection. If the sugars are present in oligomeric form further processing into their monomeric units is required prior to HPLC analysis.

1.2 The liquid portion may also contain carbohydrate degradation products, such as HMF and furfural, as well as other components of interest, such as organic acids and sugar alcohols. This method is used to measure the level of these degradation products and byproducts. These components are analyzed by HPLC with refractive index detection to determine optimal production process parameters or to monitor ongoing processes.

1.3 The concentrations of monomeric sugars (soluble monosaccharides) and cellobiose, total sugars (monosaccharides and oligosaccharides), as well as carbohydrate degradation products and sugar alcohols can be determined using this procedure. Monomeric sugars are quantified by HPLC with refractive index detection.

Oligomeric sugars are converted into the monomeric form using acid hydrolysis and quantified by HPLC with refractive index detection. Byproducts and degradation products are quantified by HPLC with refractive index detection.

2. Scope

2.1 This procedure is used to characterize liquid process samples, including pretreatment liquors, liquid fermentation samples, and liquid fractions of process solids.

2.2 This procedure is appropriate for biomass containing the components listed throughout the procedure. Any biomass containing other interfering components (such as co-eluting constituents) must be further investigated.

2.3 All analyses should be performed in accordance with an appropriate laboratory specific Quality Assurance Plan (QAP).

3. Terminology

3.1 None

4. Significance and Use

4.1 This procedure is used to determine the composition of liquid fraction process samples. Other optional procedures can be used in conjunction with this procedure, including a measure of acid soluble lignin in LAP “Determination of Structural Carbohydrates and Lignin in Biomass”. 4.2 This procedure is used, in conjunction with other procedures to determine the chemical composition of biomass samples, see LAP “Summative Mass Closure for Biomass Samples”.

5. Interferences

5.1 When analyzing for carbohydrate degradation products and sugar alcohols, the following interferences should be noted:

5.1.1 Arabitol coelutes with xylitol. If the sample is thought to contain arabitol, the experimentally determined xylitol concentration should be flagged as potentially being biased high due to the suspected arabitol component.

5.1.2 Some samples may contain sorbitol, which elutes about a minute earlier than xylitol on the Aminex HPX-87H column, and will appear as a peak in between the xylose and arabinose peaks.

5.1.3 Some samples may contain glycerol, which elutes at the same time as formic acid on the Aminex HPX-87H column.

5.2 Certain guard columns for carbohydrate quantification may cause artifact peaks. Individual carbohydrates should be run on new columns and guard columns to verify the absence of artifact peaks.

6. Apparatus

6.1 Analytical balance, accurate to 0.1mg

6.2 pH meter, accurate to 0.01pH unit

6.3 Autoclave, suitable for autoclaving liquids, set to 121° + 3°C

6.4 HPLC system equipped with refractive index detector and the following columns:

6.4.1 Shodex sugar SP0810 or Biorad Aminex HPX-87P column (or equivalent) with ionic form H+/CO3- deashing guard column

6.4.2 Biorad Aminex HPX-87H column (or equivalent) with corresponding guard column

7. Reagents and materials

7.1 Reagents

7.1.1 High purity standards

7.1.1.1 D-cellobiose, D-(+)glucose, D-(+)xylose, D-(+)galactose, L-(+)arabinose, and D-(+)mannose 7.1.1.2 Xylitol, succinic acid, L-lactic acid, glycerol, acetic acid, ethanol, 5-hydroxy-2-furaldehyde (HMF), and furfural

7.1.2 Second set of high purity standards, as listed above, from a different source (manufacturer or lot), to be used to prepare calibration verification standards (CVS)

7.1.3 Sulfuric acid, concentrated, ACS reagent grade

7.1.4 Sulfuric acid, 72% w/w (specific gravity 1.6338 at 20oC)- (also commercially available as a reagent for the determination of fluorine, from Fluka #00647)

7.1.5 Calcium carbonate, ACS reagent grade 7.1.6 Water, HPLC grade, 0.2 μm filtered

7.2 Materials 7.2.1 Erlenmeyer flasks, 20 mL

7.2.2 Pressure tubes, minimum 65 mL capacity, glass, with screw on Teflon caps and o-ring seals (Ace glass # 8648-30 tube with #5845-47 plug, or equivalent) or glass bottles, autoclave safe, crimp to, with rubber stoppers and aluminum seals to fit

7.2.3 pH paper (range 2-9)

7.2.4 Disposable syringes, 3 mL, fitted with 0.2 μm syringe filters

7.2.5 Autosampler vials with crimp top seals to fit

7.2.6 Volumetric pipets, class A, of appropriate sizes or corresponding pipettors

7.2.7 Volumetric flasks, class A, of appropriate sizes for standard and CVS dilution

7.2.8 Adjustable pipettors, covering ranges of 10 µl to 10 ml

8. ES&H Considerations and Hazards

8.1 Sulfuric acid is corrosive and should be handled with care

8.2 Follow all applicable NREL chemical handling procedures

9. Sampling, Test Specimens and Test Units

9.1 Vigorously shake or vortex the sample to suspend any entrained solids. Samples may be filtered prior to analysis if entrained solids are not of interest.

9.2 Care must be taken to ensure a representative sample is taken for analysis at each step. When measuring volumes for analysis, the sample should be at room temperature.

9.3 Store samples in sealed containers so the volatile component concentration remains consistent. Samples should be stored in a refrigerator until ready to use.

10. Procedure

10.1 Measure and record the pH of each sample to the nearest 0.01 pH unit

10.2 Analyze the sample for byproducts and degradation products as follows

10.2.1 Prepare 0.005 M (0.01 N) sulfuric acid for use as a HPLC mobile phase. In a 2L volumetric flask, add 2.00 mL of standardized 10 N sulfuric acid and bring to volume with HPLC grade water. Filter through a 0.2 µm filter and degas before use. If 10N sulfuric acid is not available, concentrated sulfuric acid may also be

used. 278 µl concentrated sulfuric acid brought to volume in a 1L volumetric flask with HPLC grade water will also produce 0.005 M sulfuric acid. 10.2.2 Prepare a series of calibration standards containing the compounds that are to be quantified, referring to Table 1 for suggested concentration ranges. Use a four point calibration. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated. The linear range of HMF and furfural is limited by their solubility. Add these two components to the standards after the ethanol has been added to increase the HMF and furfural solubility. Filter the standard solutions through 0.2 µm filters into autosampler vials. Seal and label the vials.

10.2.2.1 The retention times of xylitol and succinic acid are close. Test the column to verify adequate peak separation and quantification. If adequate separation is not achieved, regenerate or replace the column and confirm improved separation.

10.2.2.2 A fresh set of standards is not required for every analysis. A large batch of standards may be produced, filtered through 0.2 µm filters into autosampler vials, sealed and labeled. The standards and CVS samples may be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use. During every use, standards and CVS samples should be observed for unusual concentration behavior. Unusual concentrations may mean that the samples are compromised or volatile components have been lost. Assuming sufficient volume, standards and CVS samples should not have more than 12 injections drawn from a single vial. In a chilled autosampler chamber, the lifetime of standards and CVS samples is approximately seven days.

10.2.2.3 Table 1- Suggested concentration ranges for 10.2.2 calibration standards Component Approximate Retention time (min) Suggested concentration range (mg/ml) Xylitol 11.6 0.2 – 6.0 Succinic acid 12.0 0.2 – 10.0 L-Lactic acid 13.2 0.2 – 12.0 Glycerol 14.2 0.2 – 8.0 Acetic acid 15.5 0.2 – 12.0 Ethanol 22.7 1.0 - 15.0 HMF 29.4 0.02 – 5.0 Furfural 42.8 0.02 - 5.0 CVS - Middle of linear range 10.2.3 Prepare an independent calibration verification standard (CVS) for each set of calibration standards. Use reagents from a source or lot other than that used in preparing the calibration standards. Prepare the CVS at a concentration that falls in the middle of the validated range of the calibration curve. The CVS should be analyzed on the HPLC after each calibration set and at regular intervals throughout the sequence, bracketing groups of samples. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run. 10.2.4 Prepare the sample(s) for HPLC analysis by passing it through a 0.2 μm filter into an autosampler vial. Seal and label the vial. Prepare each sample in duplicate if desired. If an analyzed sample falls outside of the validated calibration range, dilute as needed and analyze the sample again. The concentrations should be corrected for dilution after running. See sections 11.1 and 11.2 for calculations.

10.2.5 Analyze the calibration standards, CVS, and samples by HPLC using a Biorad Aminex HPX-87H column. HPLC conditions: Sample volume: 10 - 25 μL, dependent on sample concentration and detector limits Mobile phase: 0.005 M sulfuric acid, 0.2 μm filtered and degassed Flow rate: 0.6 mL / minute Column temperature: 55 – 65 °C Detector temperature: as close to column temperature as possible Detector: refractive index Run time: 50 minutes

10.3 Analyze the sample for monomeric sugars and cellobiose as follows

10.3.1 Prepare a series of calibration standards containing the compounds that are to be quantified, referring to Table 2 for suggested concentration range. Use a four point calibration. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated.

10.3.2 Table 2- Suggested concentration ranges for 10.3.1 calibration standards Component Suggested concentration range (mg/ml) D-cellobiose 1.2 – 24.0 D(+)glucose 1.2 – 24.0 D(+)xylose 1.2 – 24.0 D(+)galactose1.2 – 24.0 L(+)arabinose1.2 – 24.0 D(+)mannose 1.2 – 24.0 CVS Middle of linear range, concentration not equal to a calibration point (12.0 suggested) Note: A larger concentration range is possible on some HPLC instruments.

10.3.3 A fresh set of standards is not required for every analysis. A large batch of standards may be produced, filtered through 0.2 μm filters into autosampler vials, sealed and labeled. The standards and CVS samples may be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use. During every use, standards and CVS samples should be observed for unusual concentration behavior. Unusual concentrations may mean that the samples are compromised or volatile components have been lost. Assuming sufficient volume, standards and CVS samples should not have more than 12 injections drawn from a single vial. In a chilled autosampler chamber, the lifetime of standards and CVS samples is approximately three to four days.

10.3.4 Prepare an independent calibration verification standard (CVS) for each set of calibration standards. Use reagents from a source or lot other than that used in preparing the calibration standards. Prepare the CVS at a concentration that falls

in the middle of the validated range of the calibration curve.. The CVS should be analyzed on the HPLC after each calibration set and at regular intervals throughout the sequence, bracketing groups of samples. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run.

10.3.5 Measure and record pH or refer to step 10.1 for pH measurement. If the pH is less than 5, use calcium carbonate to neutralize an aliquot (10 mL is recommended) of each sample in an Erlenmeyer flask. Neutralize to pH 5 – 6. Avoid neutralizing to a pH greater that 6 by monitoring with pH paper. Add the calcium carbonate slowly upon reaching a pH of 4. Swirl the sample frequently. After reaching pH 5 – 6, allow the sample to settle and decant off the clear liquid. The pH of the liquid after settling will be approximately 7. Samples with a pH greater than 9 cannot be analyzed using the HPX-87P column.

10.3.6 Prepare the sample for HPLC analysis by passing the decanted liquid through a 0.2 μm filter into an autosampler vial. Seal and label the vial.. Prepare each sample in duplicate if desired. If it is suspected that the sample concentrations may exceed the calibration range, dilute the samples as needed, recording the dilution. The concentrations should be corrected for dilution after running. If necessary, neutralized samples may be stored in the refrigerator for three or four days. After this time, the samples should be considered compromised.

10.3.7 Analyze the calibration standards, CVS, and samples by HPLC using a Shodex sugar SP0810 or Biorad Aminex HPX-87P column equipped with the appropriate guard column. HPLC conditions: Injection volume: 10 – 50 μL, dependent on concentration and detector limits Mobile phase: HPLC grade water, 0.2 μm

filtered and degassed Flow rate: 0.6 mL / minute Column temperature: 80 - 85°C Detector temperature: as close to column temperature as possible Detector: refractive index Run time: 20 minute data collection plus 15 minute post run (with possible adjustment for later eluting compounds) Note: The deashing guard column should be placed outside of the heating unit and kept at ambient temperature. This will prevent artifact peaks in the chromatogram. See sections 11.1 and 11.2 for calculations.

10.4 Analyze the sample for total sugar content (monosaccharides and oligoscaaharides)

10.4.1 Refer to steps 10.3.1 through 10.3.4 for preparation of calibration standards and CVS samples. It is often useful to combine the analyses from 10.3 and 10.4 into one HPLC sequence.

10.4.2 Pipette duplicate representative aliquots of sample into a pressure tube, or autoclave safe bottle if pressure tubes are not available. Aliquots of 5.0, 10.0, or 20.0 mL may be used, depending on available sample volume.

10.4.3 Measure and record the pH of the sample of refer to step 10.1 for pH measurement. Based on sample pH, calculate the amount of 72% w/w sulfuric acid required to bring the acid concentration of each aliquot to 4% (refer to section 11.3 for example calculations and section 15.1 for a quick reference sheet). Add the required amount of acid while swirling the sample. Stopper the bottles and crimp aluminum seals into place. Using a permanent marker, label the aluminum seals with sample identification. Record the amount of acid added so the dilution of the solution can be accounted for.

10.4.4 Prepare a set of sugar recovery standards (SRS) that will be taken through the analysis and used to correct for losses due to decomposition of sugars during dilute acid hydrolysis. Refer to Table 3 for SRS concentration suggestions. SRS concentrations should be chosen to most closely resemble the concentrations of sugars in the sample. Weigh out the required amounts of each sugar, to the nearest 0.1 mg, and transfer to a crimp top bottle. Add 10.0 mL HPLC grade water.

10.4.4 Table 3- Suggested concentrations for 10.4.4 sugar recovery standards Sugar concentrations (mg / mL) SRS type glucose xylose galactose arabinose mannose High 40 100 20 20 10 Medium 20 50 10 10 5 Low 4 10 2 2 1

10.4.5 Add the appropriate amount of 72% sulfuric acid to each sugar recovery standard (refer to section 11.3 for example calculations). For a starting pH of 7, the amount of 72% sulfuric acid needed will be 348 μL. Stopper the bottles and crimp aluminum seals into place. Using a permanent marker, clearly label the aluminum seals with sample identification.

10.4.6 A fresh SRS is not required for every analysis. A large batch of sugar recovery standards may be produced, filtered through 0.2 μm filters, dispensed in 10.0 mL aliquots into sealed containers, and labeled. They may be stored in a freezer and removed when needed. Thaw and vortex the frozen SRS prior to use. If frozen SRS are used, the appropriate amount of acid must be added to the thawed sample and vortexed prior to transferring to a glass crimp top bottle.

10.4.7 Autoclave the sealed samples and sugar recovery standards for one hour at 121°C, usually the liquids setting. After completion of the autoclave cycle, allow the

hydrolyzates to slowly cool to near room temperature before removing the seals and stoppers.

10.4.8 Use calcium carbonate to neutralize each sample to pH 5 – 6. Avoid neutralizing to a pH greater that 6 by monitoring with pH paper. Add the calcium carbonate slowly upon reaching a pH of 4. Swirl the sample frequently. After reaching pH 5 – 6, allow the sample to settle and decant off the clear liquid. The pH of the liquid after settling will be approximately 7.

10.4.10 Repeat steps 10.3.6 and 10.3.7, analyzing calibration standards, CVS, SRS, and samples. Refer to sections 11.1, 11.2, 11.4, and 11.5 for calculations. 10.5 Analyze the sample for acid soluble lignin content 10.5.1 See section 10.3 in LAP “Determination of Structural Carbohydrates and Lignin in Biomass” for a method for determining acid soluble lignin. Filter the liquor prior to this analysis if necessary.

11. Calculations

11.1 Create a calibration curve for each analyte to be quantified using linear regression. From these curves, determine the concentration in mg/mL of each component present in the samples analyzed by HPLC, correcting for dilution if required.

11.2 Calculate and record the amount of each calibration verification standard (CVS) recovered following HPLC analysis. % CVS recovery = conc. detected by HPLC,mg/mL known conc. of standard, mg/mL x 100

Enzymatic Saccharification of Lignocellulosic Biomass

Laboratory Analytical Procedure #009

1. Introduction

1.1 This procedure describes the enzymatic saccharification of cellulose from native or pretreated lignocellulosic biomass to glucose in order to determine the maximum extent of digestibility possible (a saturating level of a commercially available or in house produced cellulase preparation and hydrolysis times up to one week are used).

2. Scope

2.1 This procedure is appropriate for lignocellulosic biomass. If the biomass is suspected to have some starch content, dry weight percent cellulose calculated from total glucan (LAP-002) must be corrected to subtract the starch contribution to total dry weight percent glucose.

2.2 All analyses shall be performed according to the guidelines established in the Ethanol Project Quality Assurance Plan (QAP).

3. References

3.1 Grohmann, K., Torget, R., and Himmel, M. (1986), Biotech. Bioeng. Symp. No. 17, 135-151.

3.2 Ghose, T.K. (1987), Pure & Appl. Chem., **59,** 257-268.

3.3 Stockton, B.C., Mitchell, D.J., Grohmann, K., and Himmel, M.E. (1991), Biotech. Let.,**13,** 57-62.

3.4 Adney, B. and Baker, J. (1993), Ethanol Project Laboratory Analytical Procedures, LAP-006, National Renewable Energy Laboratory, Golden, CO, 80401.

3.5 Ehrman, C. I. (1996), Ethnaol Project Laboratory Analytical Procedures, LAP-016, National Renewable Energy Laboratory, Golden, CO, 80401.

4. Terminology

4.1 Pretreated biomass - Biomass that has been subjected to milling, chemical treatment with water or steam, strong or dilute acid or alkali, or other physical or chemical methods to render the cellulose content of the material more accessible to enzymatic action.

4.2 Cellulase enzyme - an enzyme preparation exhibiting all three synergistic cellulolytic activities: endo-1,4-β-D-glucanase, exo-1,4-β-glucosidase, or β-D-glucosidase activities, which are present to different extents in different cellulase preparations.

5. Significance and Use

5.1 The maximum extent of digestibility is used in conjunction with other assays to determine the appropriate enzyme loading for saccharification of biomass.

6. Interferences

6.1 Test specimens not suitable for analysis by this procedure include acid- and alkaline pretreated biomass samples that have not been washed. Unwashed pretreated biomass samples containing free acid or alkali may change solution pH to values outside the range of enzymatic activity.

7. Apparatus

7.1 VWR model 1540 incubator set at 50o ± 1oC.

7.2 Cole-Parmer model 7637-20 "Roto-Torque" Fixed Speed Rotator.

7.3 A 24-slot large-holed test tube rack that can be attached to the "Roto-Torque" Rotator.

7.4 Eppendorf model 5414 microcentrifuge.

7.5 pH meter.

7.6 Analytical balance, sensitive to 0.0001 g.

7.7 Yellow Springs Instrument, Inc., Model 27 Glucose Analyzer or Model 2700 Select Biochemistry Analyzer.

7.8 Drying oven adjusted to 105oC ± 2oC.

7.9 A 200 μL and a 1000 μL Eppendorf Pipetman pipet with tips.

8. Reagents and Materials

8.1 Tetracycline (10 mg/mL in 70% ethanol).

8.2 Cycloheximide (10 mg/mL in distilled water).

8.3 Sodium citrate buffer (0.1M, pH 4.80).

8.4 Cellulase enzyme of known activity, FPU/mL.

8.5 _-glucosidase enzyme of known activity, pNPGU/mL.

8.6 Solka Floc 200 NF, FCC (microcrystalline cellulose) from Brown Company with ash, moisture, and xylan contents determined (see Ethanol Project Laboratory Analytical Procedures, LAP-001, -002, and -005).

8.7 Eppendorf Safe-Lock 1.5-mL microcentrifuge tubes.

8.8 20-mL glass scintillation vials equipped with plastic-lined caps.

9. ES&H Considerations and Hazards

9.1 Cycloheximide and tetracycline are hazardous and must be handled with appropriate care.

9.2 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

10. Procedure

10.1 Total solids must be determined for all cellulose containing samples to be digested (LAP-001).

Note: all lignocellulosic materials which have undergone some aqueous pretreatment must never undergo any drying whatsoever prior to enzyme digestibility, since irreversible pore collapse can occur in the micro-structure of the biomass leading to decreased enzymatic release of glucose from the cellulose. Additionally, all frozen lignocellulosic materials which are to be subjected to digestibility tests can not have been frozen for more than one month prior to analysis, since, depending on the environment, sublimation could have occurred, leading to possible irreversible collapse of micropores in the biomass.

10.2 Weigh out a biomass sample equal to the equivalent of 0.1 g of cellulose on a 105^{o}C dry weight basis (the cellulose content of the sample is initially determined as glucose by LAP- 002, minus the contribution of any starch present, LAP-016) and add to a 20 mL glass scintillation vial. Also, weigh out 0.1 g of the Solka Floc MVS and add to another vial.

10.3 To each vial, add 5.0 mL 0.1 M, pH 4.8 sodium citrate buffer.

10.4 To each vial, add 40 μL (400 Fg) tetracycline and 30 μL (300 μg) cycloheximide to prevent the growth of organisms during the digestion.

10.5 Calculate the amount of distilled water needed to bring the total volume in each vial to 10.00 mL after addition of the enzymes specified in the following step. Add the appropriate calculated volume of water to each vial. All solutions and the biomass are assumed to have a specific gravity of 1.000 g/mL. Thus, if 0.200 g of biomass is added to the vial, it is assumed to occupy 0.200 mL and 9.733 mL of liquid is to be added.

10.6 Bring the contents of each vial to 50°C by warming in the incubator set at 50° ± 1°C. To each vial is added an appropriate volume of the cellulase enzyme preparation to equal approximately 60 FPU/g cellulose and the appropriate volume of β-glucosidase enzyme to equal 64 *p*NPGU/g cellulose.

Note: If the rate of enzymatic release of glucose is to be measured, all contents of the vial prior to the addition of the enzyme must be at 50°C. The enzymes are always added last since the reaction is initiated by the addition of enzyme.

10.7 Prepare a reaction blank for the substrate. The substrate blank contains buffer, water, and the identical amount of substrate in 10.00 mL volume.

10.8 Prepare enzyme blanks for cellulase and β-glucosidase with buffer, water, and the identical amount of the enzyme.

10.9 Close the vials tightly and place them in the "Roto-Torque" fixed speed rotator set at an approximate angle of 45°C that has been placed in the VWR incubator set at 50°C. Incubate with gentle rotation (68 RPM) for a period of 72 to 168 hours or until the

release of soluble sugars from the sample(s) becomes negligible when measured by YSI, as described in the next step.

10.10 If the progress of the reaction is to be measured, a 0.3-0.5 mL aliquot is removed at each predetermined time interval after the vial contents have been well mixed by shaking. This is accomplished by using a 1.0-mL Eppendorf Pipetman pipet with the tip of the plastic 1.0-mL tip slightly cut off (to allow solids, as well as liquid, to be withdrawn into the orifice). The sample is expelled into a 1.5-mL microcentrifuge tube and centrifuged for 1.5 minutes. The supernatant is subjected to glucose analysis using the YSI glucose analyzer.

PARR Pretreatment Protocol

Apparatus

- Reaction Vessel
- Lift
- Clamps
- Heater Assembly
- Pressure Gauge
- Motor
- Cooling Water
- Torque Wrench

Personal Protection Equipment

- Forearm length Kevlar® gloves
- Safety glasses
- Rubber apron (suggested)

Procedure

1. Ensure that the flexible gasket ring is secured in the reactor head. This will ensure that no vapors escape during reaction
2. Place the slurry in the Reaction Vessel.

   Note: The working volume is 250 mL liquid and 4% solids. Do not place exceed this level

3. Place the Reaction Vessel in the Lift
4. Raise the Reaction Vessel up to the reactor head. Ensure that the Lift snaps in place before releasing
5. Place the 2 Clamps around the Reactor Vessel-Head
6. Tighten the compression bolts in a criss-cross fashion using the torque wrench.
7. Bring the Lift down
8. Raise the Heater Assembly and secure beneath and around the Reaction Vessel
9. Turn Display on I
10. Turn Heater on II
11. Turn Motor On for stirring (optional)
12. Set the temperature read-out to the target temperature (optional)
13. Starting temperature is usually $20^{o}C$
14. Monitor the reaction. Record pressure, temperature, and time
15. When target temperature (or pressure) is reached, turn the Cooling Water valve on
16. Lower the Heater Assembly from the Reaction Vessel
17. Allow the temperature to fall below $50^{o}C$ before proceeding
18. Place the Kevlar® gloves on
19. Raise the Lift beneath and around the Reaction Vessel. Ensure that the Lift locks into place (and test)
20. Loosen the compression bolts using the torque wrench

21. Remove the clamps

22. Lower the Lift and Reaction Vessel

23. Remove the Reaction Vessel from the Lift

24. Empty the contents and collect sample and liquor

25. Evaluate the condition of the flexible gasket ring

VITA

Oscar L. Martin, Jr. was born on August 21, 1974, in Birmingham, Alabama, and is an American citizen. He graduated from Ramsay Alternative High School, Birmingham, Alabama in 1991. He received his Bachelor of Science in Chemical Engineering from The University of Alabama, Tuscaloosa in 1995, Master of Science in Chemical Engineering from The University of Tennessee, Knoxville in 1996, and Master of Business Administration from Tennessee State University, Nashville in 1999. Oscar has been employed by DuPont since 1993 serving in various capacities, including Research Intern, Research Engineer, Marketing Manager, Senior Research Scientist, New Product Development Project Manager, and Regional Supply Chain Manager. He is the recipient of three US Patents- 7,555,182, 7,536,072, and 7,522,794, and two patent applications- 20050255771 and 20020106959.

He is married to Menjiwe Martin, and has two children, Malia Celia, and Oscar, III.