AN ABSTRACT OF THESIS OF

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Title: Structural Component Composition of Pacific Northwest Grass-Derived Biomass: A Survey

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The structural component compositions of thirty nine non-commercial Pacific Northwest grasses were analyzed in order to develop a database of the grasses that may have application in bioconversion processes. The samples were chosen based on near infrared reflectance (NIR) data that suggested this group of grasses, collectively, was representative of the broad range of compositions that are likely to be encountered due to genotypic and phenotypic variability. Solvent-extracted samples were prepared by extracting the native grass sequentially with water and then 95% ethanol. Each of the grasses residues was analyzed for glycans (glucans, xylan, and arabinan), acid-insoluble lignin, acid soluble lignin, and ash.

Total glycans ranged from a low of 32% to a high of 50%. Glucan was the major glycan component, typically in the range of 60%. Xylan represented about one-third of the total glycans which is 9.5% to 18.3% while arabinan represented 1.0% to 3.3%. Total glycans seemed to increase from the younger stage to the more mature stage. The mean total glycans of the vegetative stage was 32%, while that of the seed

mature stage was 45.9%. While the amount of glycans varied between species, the ratio of glucan, xylan, and arabinan (12: 7: 1) remained relatively constant.

Acid-insoluble lignin ranged from 6.38% to 14.58%, while the acid-soluble lignin ranged from 1.57% to 4.35%. The acid-insoluble lignin of seed mature, flower, boot, and vegetative stages were 12.38%, 11.65%, 9.39%, and 8.10%, respectively. The acid-soluble lignin of seed mature, flower, boot, and vegetative stages were 2.03%, 2.61%, 3.02%, and 3.46%, respectively. Extractives represented 20.29% to 41.55% of the oven-dry grasses. The ash values of the unextracted grasses ranged from 5% to 10% while the ash values for the extracted samples were typically in the range of 0.5% to 2.5%, showing that the sequential water and ethanol extractions tended to remove 60 – 80% of the quantifiable inorganic matter from the grasses.

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Structural Component Composition of Pacific Northwest Grass-Derived Biomass: A Survey

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Structural Component Composition of Pacific Northwest
Grass-Derived Biomass: A Survey

1. INTRODUCTION

Agricultural production systems capable of producing significant amounts of plant biomass are receiving heightened attention due to their potential beneficial impact on local, national, and global energy concerns. Plant biomass is particularly relevant to the development of non-fossil, "alternative", energy options because (1) plants themselves naturally convert solar energy to store chemical energy, (2) significant amounts of plant biomass (stored chemical energy) may be produced using conventional agricultural practices on currently available land, and (3) the technical barriers currently limiting the implementation of plant biomass conversion schemes are largely being overcome as a result of an intense global research effort. A projected outcome of this research effort is the development of economically viable bio-refineries capable of converting lignocellulosic plant biomass to any of a number of value-added bio-based products, including chemicals, materials, and fuels. Grasses are among the biomass sources that are being considered as primary feedstocks for such applications.

Lignocellulose bioconversion processes are typically conducted by a "sugar platform" route in which the key intermediates in the process are the sugars derived from the structural polysaccharides inherent in the feedstock. The nature of the sugars present in the sugar platform are dependent on the starting feedstock and the conditions employed in upstream operations, but the predominant sugars from

grass-based feedstocks will likely be glucose and xylose. These two sugars may be readily converted, biologically and/or chemically, to a host of products, fuel ethanol being the one most commonly touted. Lignocellulose processing schemes typically focus on the conversion and/or modification of the structural components of feedstocks. The three major structural components are cellulose, hemicellulose, and lignin. The cellulose fraction represents the majority of the potentially fermentable glucan available in the feedstock. The hemicellulose fraction represents the majority of the potentially fermentable xylan available in the feedstock. Lignin is the non-carbohydrate component that is typically correlated with the recalcitrance of these feedstocks (Jung *et al.*, 1992).

Clearly, the utility of these grasses for biorefinery use is dependent upon the composition of the cellulose and hemicellulose components of the cell walls because these components determine the quantity of fermentable sugars that can be harvested from the feedstock. There is potential to utilize conventional plant breeding to improve the utility of grasses for biorefinery applications, but the success of these efforts is dependent on the existence of genetic variability in cell wall composition. While data exist regarding genotypic variation in the cell wall composition of cultivated grasses like switchgrass (Cassida *et al.*, 2005), little is known about native grasses that comprise many of the buffer strips, pastures and rangelands of the west.

The objective of the presented study was to develop a database of the structural component composition of non-commercial grasses, all of which are relevant to the Pacific Northwest region of the United States, which may have

application in bioconversion processes. The thirty nine grasses included in the study were chosen based on near infrared reflectance (NIR) data that suggested this group of grasses, collectively, was representative of the broad range of compositions that are likely to be encountered due to genotypic and phenotypic variability.

2. LITERATURE REVIEW

The Family Poaceae, also known as Gramineae, consist of about 600 genera and perhaps 10,000 species of grasses. They provide man with foodstuffs both directly, in the form of grains from cereals, and of sucrose from the pith of the sugar-cane, and indirectly, as herbage grasses converted into animal tissues (Moore, 1966). Grass plants ranges from those that are tiny to bamboos which have woody-textured stems over a hundred feet high (Arber, 1934). The Gramineae may be divided into 19 families, including the Hordeae (barley, couch grass, bread wheat, and hard wheat), Festuceae (cocksfoot, darnel, Italian ryegrass, and perennial ryegrass), Aveneae (oats and sweet vernal), Agrostideae (timothy), and Paniceae (millets) (Hubbard, 1968). Cereals constitute the major source of food energy for humans and perhaps the major source of protein, and include rice in South and Southeast Asia, maize in Central and South America, and wheat and barley in the Americas and North Eurasia. Many other grasses are also grown for forage and fodder for animal food, particularly for sheep and cattle.

The growth stages of grasses are classified as follows:

1. Vegetative (immature): Leaves only; no stems
2. Boot: Inflorescence (seed head) enclosed in leaf sheath of last leaf and not showing
3. Flowering (anthesis): Anthers in flowers shedding pollen
4. Seed mature: Seeds ripe

2.1 COMPOSITION OF LIGNOCELLULOSIC BIOMASS

Biomass can be defined as the mass of materials produced by the growth of living organisms, including plants, animals, and microorganisms. The term biomass can also refer to agricultural byproducts such as corn cob and wheat straw. Lignocellulosic biomass or cellulosic biomass is a more specific term referring to biomass generated from plant materials, such as agricultural crops and crop residues and energy plantations of fast growing woody species (Wayman and Parekh, 1990). Lignocellulosic biomass is composed of three major components: cellulose, hemicelluloses, and lignin. Lignocellulosic biomass includes wood, straw, corn cobs, yard waste, and even municipal solid waste. The relative amount of each of the three major components varies greatly according to biomass source (Wayman and Parekh, 1990). The typical composition of lignified cell walls is about 35-40% cellulose, 20-25% hemicelluloses, and 20-25% lignin (Lewin and Goldstein, 1991).

Extraneous substances, called extractives, are compounds not involved in the structural function of the cell wall (Browning, 1967). They consist of minor, non-structural components such as waxes, fats, gums, resins, oils, starches, alkaloids, tannins, and soluble sugars (Tsoumis, 1968). Mineral substances are also considered as extraneous substances. Important mineral elements in cell walls are Ca, P, and Si. Protein in plants exist in small amounts as glycoproteins rich in hydroxyproline, arabinose, and galactose (Jung *et al.*, 1993). Herbaceous crops contain more minerals, proteins, soluble sugars, and other water soluble extractives than do hardwoods (Torget *et al.*, 1990). A low content of pectic substances seems

also to be present in grasses. This group of amorphous polysaccharides consists of polygalacturonic acids, rhamnose, arabinose, and galactose. Starch content in lignified cell wall is negligible.

Plant cell walls consist of two phases, a microfibrillar phase and a matrix phase. The microfibrills are made up of cellulose, the cellulose being paracrystalline and having a degree of polymerization of from 5,000 to 10,000 (Brett and Waldron, 1990). The matrix of the cell wall is amorphous. It consists of a variety of polymers, including hemicelluloses, protein, and lignin.

2.1.1 Cellulose

Cellulose is a linear polymer composed of anhydro-β-D-glucopyranose units linked by 1,4 glycosidic bonds. The degree of polymerization is 5,000 to 10,000 (Brett and Waldron, 1990). These long molecules are hydrogen bonded to their neighboring linear polymers forming a crystalline lattice resulting in a structure with very high tensile strength. However, cellulose is paracrystalline, having both crystalline and amorphous regions. The amorphous regions are primarily on the surface of the microfibrils, only occasionally interrupting the central crystalline core (Sjostrom, 1993). Because of the high degree of H-bonding and crystalline structure, cellulose is quite unreactive. It is insoluble in water or in common solvents. It dissolves in strong acids (72% H_2SO_4, 85% H_3PO_4, and 41% HCl) or in alkaline copper solutions. Cellulose swells but does not dissolve in NaOH or KOH (Wayman and Parekh, 1990).

The glucose subunits obtained from cellulose saccharification (enzyme or acid hydrolysis of the cellulose) can be converted to ethanol by yeast fermentation. Saccharification of cellulose to glucose can be achieved by the use of cellulase enzyme systems, such as that produced by the fungus *Trichoderma reesei* and *Aspergillus niger*. Fermentation with *Saccharomyces cerevisiae*, a common brewer's yeast, converts glucose to ethanol. The saccharification and fermentation can take place separately in a separate hydrolysis and fermentation process (SHF) or simultaneously in a process called simultaneous saccharification and fermentation (SSF) (Wayman and Parekh, 1990).

2.1.2 Hemicelluloses

Hemicelluloses are branched and heterogeneous polysaccharides with a lower degree of polymerization than cellulose, approximately 150 to 200 molecules. Like cellulose, most hemicelluloses function as supporting material in the cell walls. In contrast to cellulose, hemicelluloses are branched with glucose, arabinose, mannose, galactose, and uronic acid side chains and do not form crystalline regions (Coughlan and Hazlewood, 1993). The hemicelluloses of the Gramineae appear to be xylans with a backbone of 1,4-linked β, D-xylopyranose residues to which L-arabinofuranose residues are attached as single-unit side-chains, usually to position 3 of the xylose residue. Additionally, single residues of D-glucuronic acid or its 4-*O*-methyl derivative are characteristically attached at postion 2 (Aspinall, 1959).

Some hemicelluloses are readily hydrolyzed to their monomeric sugars, acetic acid, and other compounds at 185°C under steam or at lower temperature

under mild acidic conditions (Wayman and Parekh, 1990). Saccharification and subsequent fermentation of the xylan backbone can be achieved using xylanase enzymes and the xylose fermenting yeast *Pichia stipitis* (Wayman *et al.*, 1987). Other uses of hemicellulose include the conversion of xylose component to xylitol, a sweetener that is found to suppress tooth decay and used largely in chewing gum. Solutions of hemicellulose can also be evaporated to form wood molasses, which may be used as a flavoring agent and binder in animal feeds. Furfural from the degradation of xylose can be used as a solvent in oil-refining and a base for furane resins (Wayman and Parekh, 1990).

2.1.3 Lignin

Lignin is the major non-carbohydrate portion of lignified cell walls. It is located mostly in the middle lamella as well as primary and secondary cell walls, acting like a glue to hold the cellulose fibers together. Lignification marks the last stage of cell wall development. Only living cells produce lignin and completion of lignification almost always coincides with the disappearance of the protoplasm or cell death (Toumis, 1968). Lignin is a very complex molecule made up of phenyl-propane like units, with a degree of polymerization of 450 to 550. The precursors of lignin are the three aromatic alcohols, coumaryl, coniferyl and sinapyl alcohols. These precursors are linked together through a variety of covalent carbon-carbon and carbon-oxygen bonds (Wayman and Parekh, 1990). Because of these structural features, lignin is difficult to measure quantitatively (Kirk and Obst, 1988).

Lignins are generally classified according to the chemical structure of their monomer units into three major groups: softwood lignin, hardwood lignin, and grass lignin. Softwood lignins are mostly composed of guiacyl units, while hardwood lignins are composed of guaiacyl and syringyl units. Grass lignins are composed of guaiacyl, syringyl, and *p*-hydroxyphenyl units. Grasses which synthesize a guaiacyl-syringyl-*p*-hydroxyphenyl lignin contain enzymes catalyzing the formation of both guaiacyl and syringyl intermediates. The formation of *p*-hydroxyphenyl lignin and esterified *p*-coumarate characteristically present in grass lignin seems to derive from *p*-coumaric acid additionally supplied from L-tyrosine by tyrosine ammonia-lyase activity of the bi-functional grass PAL (Phenylalanine ammonia-lyase) (Higuchi, 1998).

2.1.4 Extractives

Extractives or extraneous components are those substances which are removed from lignocellulose cell walls by extraction with neutral solvents. These materials are deposited after cell wall formation and are not considered essential structural components of the cell wall (Soltes, 1983).

Extractives can be classified as follows (Lewin and Goldstein, 1991):

1. Volatile materials, e.g., terpenoids and related compounds.
2. Non-volatile resinous extractives, e.g., resin acids, fatty acids, and unsaponifiable substances.
3. Phenolic extractives, e.g., stilbenes, lignans, tannins, and flavonoids.

4. Soluble carbohydrates and other polar extractives, e.g., monosaccharides, sucrose, arabinogalactans, pectins, cyclitols, and low molecular weight carboxylic acids.

Extractives are often removed from biomass for quantification, for characterization and to improve the accuracy of subsequent analyses (Browning, 1967; TAPPI, 1988). Extraction of nonvolatile extractives can be accomplished using a Soxhlet extractor. It should be noted that no single solvent is capable of solubilizing all extractives. Often successive extractions or extraction using a mixture of solvents is required. Selection of the solvent must be based on the aim of the work. For example, water extraction can remove compounds such as inorganic salts, sugars, polysaccharides, cyclitols, and some phenolic substances. Organic solvents can be used to extract resins, fatty acids, waxes, unsaponifiable substances, pigments, etc. Soxhlet extraction using 95% ethanol has been found to be effective as well as being non-toxic and is the standard method used by the National Renewable Energy Laboratory (NREL).

2.1.5 Mineral Content (Ash)

The mineral content of grasses varies and depends primarily on the species. For example, tall fescue has calcium, potassium, and phosphorus, but lack of magnesium. Among all elements found in plants, silica showed the greatest variation between plant parts, plants, and species of plants. Richardson (1920) reported its abundance in the aerial parts of plants of the Equisetum genus and many Gramineae, constituting 50 to 70 % of the ash. The Gramineae is the best

known group depositing silica. The deposition takes place in cell walls and cell lumina (Lanning *et al.*, 1958).

Members of the grass family accumulate large amount of silicon in the form of silica gel ($SiO_2.nH_2O$) that is localized in specific cell types. The function of silicon in plants has been proposed as support for cell walls, deterrence to pest and pathogens, reduction in water loss by evapotranspiration, reduction in certain heavy metal toxicities, and an essential element for normal development in some species (Savant *et al.*, 1999).

2.2 CHEMICAL ANALYSIS OF LIGNOCELLULOSIC BIOMASS

The chemical heterogeneity of lignified cell walls causes the main difficulty in their chemical analysis. The different components of the cell wall interfere with each others respective analyses. The typical characterization of a biomass involves the macrocomponent analysis which includes polysaccharides, lignin, acid soluble lignin, protein, ash, and organic acids. The primary sugars in biomass are glucose, xylose, galactose, arabinose, and some mannose. In the field of biomass conversion, the approach commonly used for carbohydrate analysis involves complete acid hydrolysis of the polysaccharides. The method discovered by Peter Klason (NREL CAT No. 003, 1992c) involves solubilization of the polysaccharides in strong mineral acid, often 72% sulfuric acid, followed by hydrolysis in a more dilute, often 4% acid, at high temperature. The "two stage" hydrolysis results in a dark insoluble material called Klason lignin (acid-insoluble lignin) and a hydrolysate liquid containing monosaccharides. The monosaccharides are a result of cellulose

and hemicellulose hydrolysis. Some of the lignin is degraded during the hydrolysis; this lignin is measured as acid soluble lignin (Tandjo, 1996).

The method most commonly used for carbohydrate quantification is High Pressure Liquid Chromatrography (HPLC). A common HPLC system used for lignocellulosic biomass carbohydrate analysis includes an Aminex HPX-87P column coupled to a refractive index detector. Aminex carbohydrate columns separate compounds using a combination of size exclusion and ligand exchange mechanisms. For monosaccharide separations, ligand exchange is the primary mechanism. It involves the binding of hydroxyl groups of the sugars with the counter-ion (Pb^{++}) of the resins. The binding ability is affected by the spatial orientation of the carbohydrate's hydroxyl groups. Stronger binding, which results in longer retention times, will occur in sugars which can favorably complex three adjacent hydroxyls to the Pb^{++} counter-ion compare to those sugars which complex the Pb^{++} with only two hydroxyls (Bio Rad, Bulletin 1928).

2.2.1 Interference of Extractives on the Chemical Analysis of Lignocellulosic biomass

The high content of extractives in herbaceous biomass complicates lignin analyses (Torget *et al.*, 1990); as has been reported by several groups (Norman, 1937, Ritter and Barbour, 1935, Smelstorius, 1971, and Thammasouk *et al.*, 1997). It was found that some components of the extractives could polymerize during acid treatment and were erroneously measured as lignin (Browning, 1967). Fats, resins, lignans, and low molecular weight lignin would remain wholly or partially in lignin

if they were not removed before lignin analysis. Tannins, which are polyphenolic compounds, will condense with lignin in the harsh acid environment used for this assay (Smelstorius, 1971). Biomass that contains significant amounts of starch and soluble sugars will also interfere in the determination of cellulose and hemicellulose. These compounds will be hydrolyzed along with cellulose and will contribute to the calculation of cellulose content since this calculation is based on the amount of glucose in the hydrolysate.

2.2.2 Solvent Extraction

The choice of extraction methods and solvents is dependent upon the biomass and the purpose of the investigation. It must be kept in mind that extractives are a diverse group of chemical compounds. Successive extraction with neutral organic solvents has been shown to improve the solubilization of most extractives (Saddler, 1993).

2.2.2.1 Water Soluble Extractives

Water extraction can remove inorganic salts, sugars, polysaccharides, gums, cyclitols, and some phenolic substances. Cold water removes tannins, gums, sugars, and coloring matter in lignocellulosic materials, while hot water may also remove starches (TAPPI T12m, 1979).

2.2.2.2 Organic Solvents Soluble Extractives

Organic solvents can be used to extract resins, fatty acids, waxes, unsaponifiable substances, and pigments. The extraction solvents commonly used are petroleum ether, ethyl ether, acetone, carbontetrachloride, 95% ethanol, and

benzene-ethanol. Petroleum ether or benzene removes fats, waxes, resinous materials, and sterols. The mixture of ethanol-benzene appears to provide complete removal of extractives in pulp, such as waxes, fats, resins, catechol tannins, sterols, nonvolatile hydrocarbons, salts, and low molecular weight sugars (TAPPI T 204 os-76, 1979). However, use of benzene as an extraction solvent has been avoided because of its toxicity.

2.3 PROCESSING OF HERBACEOUS BIOMASS FOR POWER, FUELS, AND PRODUCTS

The world shortage of fossilized organic materials for the production of industrial chemicals has increased interest in the development of technologies for the exploitation of photosynthetically renewable resources, including cereal straws and grasses (Wilkie, 1979). Environmental, long-term economic and national security concerns have motivated research over the last 25 years into renewable, domestic sources of fuels and chemicals now mostly derived from petroleum. Among the various types of biomass fuels that can be used for energy production, agroresidues resulting as by-products of agricultural or agro-industrial activities, e.g. straws, pits, hulls, pods, cobs, etc., are thought to be the most important, especially in the underdeveloped areas of the planet where the use of these biofuels for energy production could cover a substantial gap in the energy requirement of the local communities (Arvelakis *et al.*, 1999).

The US fuel ethanol industry represents an on-going success story for the production of renewable fuels. Demand of fuel ethanol is expected to increase. The

current ethanol industry (based on starch conversion) forms an infrastructure from which the future growth in cellulosic substrate utilization may occur (Mosier *et al.*, 2005). Various studies have shown that ethanol or ethanol-blended fuels produce less harmful emissions while the production of ethanol from biomass has the advantage of displacing a transportation fuel derived from petroleum with a fuel from a renewable resource. Consequently there should be little net contribution of global warming as the carbon dioxide liberated during the ethanol combustion is utilized by the growing plant material (Gregg and Saddler, 1996). Processing of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/purification (Mosier *et al.*, 2005).

Besides the fuel ethanol or biofuel, biomass has also been used to generate electricity. Biomass is the single largest source of non-hydro renewable electricity. Nowadays, hundreds of US power plants use biomass resources to produce 65 billion kilowatt-hours of electricity each year. Although biopower production results in increased particulate emissions, they are cleaner than coal fired power plants because they do not release sulfur dioxide. Additionally, bioenergy systems are carbon dioxide neutral because the plant material absorbs as much carbon dioxide during its life as released when it is burned to produce electricity. There are three different types of biopower systems: direct-fire, co-firing, and gasification. Gasification involves using high temperatures in an oxygen starved environment to convert biomass into a gas. This gas can then be used to fuel a combined-cycle gas turbine. Gasification is the preferred method given that combined gas-turbines are the most efficient of all power conversion technologies.

2.4 NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

NIRS is a rapid, cheap, and non-destructive technique offering the potential for accurate and repeatable measurements of chemical constituents in organic materials (Norris *et al.*, 1976; Williams, 1975; Suehara and Yano, 2004). It has been used to calibrate phenolic substances in some food materials, such as tea leaves (Schulz *et al.*, 1999), or forage species (Windham *et al.*, 1988), to measure concentrations of important substances in bioprocesses (Suehara and Yano, 2004) and to predict the water-soluble and total extractable polyphenolics of plant material (Coûteaux *et al.*, 2005). The major advantages of NIRS are speed, multiplicity of analyses, small sample size, non-consumption of sample, and reduced cost (Park *et al.*, 1998).

The near-infrared region ranges from 780 to 2500 nm (Sheppard *et al.*, 1985). It was discovered by Fredrick William Herschel in 1800 (Herschel, 1800). NIR spectra are produced when light is absorbed by organic molecule bonds such as C-N, C-H, N-H, C=O, S-H, and O-H. Most of the absorption bands are caused by overtones or combinations of overtones of the bonds originating in the infrared region of the spectrum. To obtain a calibration equation, relationships between absorption value and conventional analysis values of the sample are examined using multiple linear regression (MLR) analysis. However, analysis of the NIR spectrum to obtain a calibration equation is very difficult. Statistical methods are used to examine the relationship between spectral data values and concentration values obtained by conventional analytical methods (Suehara and Yano, 2004).

3. EXPERIMENTAL METHODS

Northwest native grass samples were provided by United States Department of Agriculture (USDA). All samples were milled to pass a 20 mesh screen and stored at room temperature prior to analyses. Sugar standards; glucose, xylose, and arabinose, were obtained from Sigma.

Analytical methods for the determination of moisture, carbohydrate, lignin, and ash are described in the 'Chemical Analysis and Testing (CAT) Standard Procedure' provided by the National Renewable Energy Laboratory (NREL).

3.1 SAMPLE SELECTION

Sample selection was done by Drs. Hossien El-Nashaar, Stephen M. Griffith, and Gary Banowetz (USDA-ARS-National Forage Seed Production Research Center).

Aboveground plant biomass from twelve temperate grass species was harvested from eight locations; Aberdeen, ID; Rockford and Pullman, WA; Corvallis, Shedd, and Silverton, OR; Winters and Lockeford, CA. The grass species included nine U.S. native species, *Bromus marginatus* (mountain brome), *Elymus glaucus* (blue wildrye), *Poa secunda* (Sandberg bluegrass), *Pseudoroegneria spicata* (bluebunch wheatgrass), *Elymus lanceolatus* (streambank wheatgrass), *Elymus trachycaulus* (slender wheatgrass), *Leymus cinereus* (basin wildrye), *Leymus triticoides* (beardless wildrye), and *Pseudoroegneria spicata* ssp. *inermis* (beardless wheatgrass) and four cultivated species *Festuca rubra* (red

fescue), *Lolium perenne* (perennial ryegrass), *Poa pratensis* (Kentucky bluegrass), and *Schedonorus phoenix* (tall fescue).

Biomass was collected from 30-cm^2 randomly selected sites at each location and kept in paper sacks. Plants were collected at three developmental stages: vegetative (just prior to stem elongation), anthesis (mid-anthesis), and maturity.

Biomass was air dried in a forced air oven at 80°C for 24 hours. Plant material was milled to pass a 20 mesh screen using a Tecator Cyclotec 1093 sample mill (Eden Prairie, MN). Dried biomass was placed in sample holder which was placed on the holder tract of Spinning Model configured for NIR reflectance measurement, model 6500 (Eden Prairie, MN). Samples were scanned from 1108-2492 nm using the ISIscan program and data were collected utilizing the WinISI III Software package IS-1480 (Eden Prairie, MN).

There were a total of 740 spectra obtained representing all of the native grass species evaluated in this study. Applying the option of making and using scores files of WinISI software to the population at hand, a sample of 142 observations representing the spectral boundaries of the population of the spectra were identified. Thirty eight forage grasses and one cultivated species (*Dactylis glomerata*) were selected for analysis on the basis of broad variability of their NIR spectra which encompassed the range of compositions among these contrasting genotypes and covering all constituents required for calibration.

3.2 TOTAL SOLIDS/ MOISTURE

Moisture contents were determined in each analysis in order to express results on a dry weight basis. Approximately 0.75 g of sample, weighed to the nearest 0.1 mg, was placed in a pre-weighed aluminum container and dried to constant weight in a 105°C convection oven. The sample was placed in a desiccator to cool to room temperature and was reweighed (NREL CAT No. 001, 1992a).

CALCULATION:

$$\%\text{Moisture at } 105°C = \left[1 - \frac{(\text{wt. dried sample plus container} - \text{wt. container})}{\text{wt. undried sample}}\right] \times 100\%$$

$$\%\text{Total solids} = \frac{(\text{wt. dried sample plus container} - \text{wt. container})}{\text{wt. undried sample}} \times 100\ \%$$

3.3 EXTRACTION

Prior to the carbohydrate and lignin analyses, a portion of biomass was extracted sequentially with hot water and then 95% ethanol. The procedure was adapted from the NREL procedure No. 010 using Soxhlet extraction (NREL, 1994b).

3.3.1 Hot Water Extraction

Approximately 5 grams of native, unextracted biomass, weighed to the nearest 0.1 mg, was transferred to a 33 x 80 mm cellulose medium porosity thimble (Whatman CAT No. 2800338) and extracted with water using conventional Soxhlet

glassware. A plug of glass wool was placed on top of the sample to prevent sample loss during extraction. Approximately 200 ml of water was poured in a weighed round bottom flask containing boiling chips. The total extraction time was approximately 24 hours with approximately 5 cycles/h reflux rate of water, giving a total of 110-130 water exchanges over the complete extraction period. After completion of the extraction, the contents were cooled to room temperature. The water extractive was transferred to a pre-weighed crucible and dried to constant weight in a 105°C convection oven in order to get the amount of extractable solids. The solid content that was left in the thimble was then transferred to pre-weighed dish and dried at 45°C. This sample was kept in a sealed bottle at room temperature for further analyses.

3.3.2 Ethanol Extraction

Five grams of sample, weighed to the nearest 0.1 mg, was extracted with 95% ethanol using the same procedure as described in 3.3.1 (Hot Water Extraction). The total extraction time for ethanol was also 24 hours with approximately 5 cycles/h reflux rate of solvent. After 24 hours, the extracted residue was transferred to pre-weighed dish and dried at 45°C. This sample was also kept in a sealed bottle at room temperature for further analyses. Ethanol extractive in the flask was poured into a pre-weighed dish and evaporated to dryness by air dry in hood overnight. The dried sample and dish was then reweighed.

3.3.3 Hot Water and Ethanol Extraction

A known amount of sample, weighed to the nearest 0.1 mg, was extracted with hot water followed by 95% Ethanol extraction procedure described above.

CALCULATION:

$$\%\text{Water extractive} = \frac{(\%\text{Solids}*(\text{wt. flask plus extractive} - \text{wt. flask}))}{\text{Original sample dried wt.}} \times 100\%$$

$$\%\text{Ethanol extractive} = \frac{(\text{wt. crucible plus dried extractive} - \text{wt. crucible})}{\text{Original sample dried wt.}} \times 100\%$$

%Total extractives = % ethanol extractives + % water extractives

3.4 CARBOHYDRATE

The NREL Standard procedure for carbohydrate analysis (NREL CAT No. 002, 1992b) was modified in this study based on previous findings in our laboratory. The modified protocol was found to be more reproducible (Fenske, 1994). Wet samples were dried at 45°C prior to analysis.

Approximately 0.3 g of sample, weighed to the nearest 0.1 mg, was placed in a glass test tube. Three ml of 72% H_2SO_4 were added and the tube was placed in a 30°C water bath for an hour with periodic stirring. After one hour, the samples were then removed from the water bath and quantitatively transferred to 250 ml Pyrex™ screw cap bottles. The acid slurry was diluted to 4 % acid by addition of 84 ml of deionized distilled water. The sugar mixture which included 0.12 g

glucose, 0.09 g xylose, and 0.06 g arabinose was placed in another Pyrex™ screw cap bottles and 87 mls of 4 % H_2SO_4 were added. The sample and sugars were then autoclaved for one hour at 121°C. The samples were cooled before transferring to 100 ml volumetric flasks and the liquid was brought to volume with deionized distilled water. The purpose of cooling is to prevent loss of volatile compounds. Approximately 20 ml of each sample was transferred to a 50 ml Erlenmeyer flask and neutralized with calcium carbonate to pH 6, measured by pH meter. Following neutralization, samples stood for an hour to allow complete precipitation and were then passed through 0.22 μm filter membranes (Millipore) into HPLC autosample vials. A mixture of sugar standards consisting of 0.12 g glucose, 0.09 g xylose, and 0.06 g arabinose was prepared in Milli-Q grade water. Samples were analyzed using HPLC with a Biorad Aminex HPX-87P column and the conditions used for the analysis were:

Column Temperature:	85°C
Detector:	refractive index
Mobile Phase:	Milli-Q grade water
Flow Rate:	0.6 ml/min
Injection Volume:	20 μL
Run Time:	30 minutes

CALCULATION:

$$\% \text{ Sugar recovered} = \frac{\text{Conc. Detected by HPLC, mg/ml}}{\text{known conc. of sugar before hydrolysis, mg/ml}} \times 100\ \%$$

Percentage of Sugar recovered presents the amount of each sugar standard recovered after being subjected to the hydrolysis procedure. This will give an estimate of the amount of each individual sugar destroyed as a result of the hydrolysis procedure.

$$\text{Corrected sugar conc., mg/ml} = \frac{\text{sugar conc. obtained by HPLC, mg/ml}}{\%\ \text{sugar recovered} / 100}$$

Corrected sugar concentration presents the correct sugar concentration for each hydrolyzed samples.

$$\%\ \text{Glycan} = \frac{\text{corrected sugar conc. x 100 x 1g/1000 mg}}{\text{sample dry weight, g}} \times \frac{MW_{sugar} - MW_{water}}{MW_{sugar}}$$

Where: MW of glucose = 180.00
MW of xylose = 150.13
MW of arabinose = 150.13
MW of water = 18.00

Percentage of Glycan represents the percentage of each sugar presents in the hydrolyzed samples, on a 105° C dry weight basis.

3.5 ACID-INSOLUBLE LIGNIN

Acid-insoluble lignin (AIL), also known as Klason lignin (KL), was defined as the ash-free insoluble residue resulting from the two-stage acid hydrolysis procedure in 3.4 (Carbohydrate).

The hydrolyzed solution (after 25 ml taken out for carbohydrate and acid-soluble lignin analysis) was vacuum filtered through a pre-dried and weighed 50 ml medium porosity, sintered glass. The sample was dried overnight at 105°C and weighed. In order to correct for acid-insoluble ash, the crucible containing the dried residue was ashed at 525°C. The ashed crucible with residue was cooled in a desiccator and weighed to the nearest 0.1 mg (NREL CAT No. 003, 1992c).

CALCULATION:

$$\% \text{ AIL} = \frac{\text{Wt. crucible plus acid insoluble residue} - \text{wt. crucible plus ash}}{\text{initial sample weight}} \times 100\%$$

3.6 ACID-SOLUBLE LIGNIN

Acid-soluble lignin (ASL) was determined by measuring the absorbance of the hydrolyzed solution from the hydrolysis procedure at 205 nm (NREL CAT No. 004, 1992d). A 4% (w/w) sulfuric acid solution was used as the reference blank. All samples were diluted with 4% sulfuric acid to make the absorbance value fall in the linear range. A low wavelength was chosen for this analysis in order to avoid interference from furfural and non-lignin polyphenols, both of which absorb at higher wavelengths (Smelstorius, 1974).

CALCULATION:

$$\%ASL = \frac{\frac{A}{b \times a} \times df \times V}{\frac{1000\ ml}{1\ L} \times W} \times 100$$

Where:		
	A	= absorbance at 205 nm
	df	= dilution factor
	b	= cell path length of 1 cm
	a	= absorptivity value of 110 L/g cm
	V	= Volume of filtrate
	W	= initial sample weight in grams

3.7 ASH

Approximately one gram of a 105°C dried sample was weighed to the nearest 0.1 mg in a pre-weighed ashing crucible. The sample was ashed in a muffle furnace at 525°C overnight until the residue turned gray or white. The sample was then placed in a desiccator to cool to room temperature and was weighed to the nearest 0.1 mg (NREL CAT No. 005, 1994a).

CALCULATION:

$$\%\ Ash = \frac{\text{wt. crucible plus ash} - \text{wt. crucible}}{\text{wt. 105°C dried sample}} \times 100$$

4. RESULTS AND DISCUSSION

The grasses included in this study were chosen based on NIR data suggesting that their compositions spanned the range that is likely to be encountered, taking into account both phenotypic and genotypic variability, when dealing with perennial grasses suited for the U.S. Pacific Northwest. NIR spectral analyses were thus used qualitatively to determine the relative similarities of grasses (see "Methods" for details of sample selection). NIR could not be used to quantitatively determine the composition of the grasses in this study due to the indirect nature of the method; quantitative applications require prior calibration using a direct method of analysis. To date, no such calibration is available for the diverse group of grasses considered in this study.

The identification of samples is listed in Table 1. The data presented in Tables 2-15 and Figures 1-4 provides direct compositional measurements of the structural component composition of pertinent grasses. Four commercially relevant grass straws, Kentucky Bluegrass, Perennial Ryegrass, Tall Fescue, and Wheat Straw, each having value-added processing potential, were included in this study in order to compare their component compositions with the native grasses (Data provided by Dan Smith, Food Sciene and Technology Department, Oregon State University). The compositional data is expressed as a percentage of the original oven-dry grass (non-extracted) unless specified otherwise.

4.1 COMPOSITION ANALYSES

4.1.1 Glycans

A summary of the structural glycan content of all samples analyzed, reported as the relevant homopolymers and arranged in rank from highest to lowest "total glycan", is provided in Table 2. The uncertainties associated with the different analyses are presented as mean standard deviations (Table 2). The total glycan content ranged from a high of near 50% to a low of approximately 32%. These "total glycan" values represent structural glycan content, as analyses were performed on water- and ethanol-extracted samples. The predominant structural glycan component, accounting for approximately 60% of the total glycan in all grasses tested, was glucan (presumably the cellulose component of the plant cell wall). The percentage of the total glycan that measured as xylan was approximately 35%. Arabinan was present in small (< 5%), but significant amounts, in all grasses. The glucan:xylan:arabinan ratios (Table 3) were similar for all samples, the average being 12: 7: 1 (65: 35: 5). The diversity of the samples analyzed in this study suggests that this glucan:xylan:arabinan ratio may be generally used as a rough approximation of the neutral sugar content of the structural glycans of grasses, *i.e.* applicable without regard to species and stage of developmental.

The glycan composition of the commercial straws (Table 4), in general, fell within the range of the grasses listed in Table 2. The total glycan content of the commercial straws were similar to those of the "mature" grasses; wheat straw (53.8% total glycan) being the only one whose value was outside the range defined

by the representative grasses of Table 2. The relative amounts of glucan, xylan, and arabinan were similar for all of the feedstocks tested that the glucan:xylan:arabinan ratios were in the range of that of native grasses (Table 5).

Figure 1 presents average total glycan values (across species) for plants at different stages of maturity. The data suggests that the percentage of total glycans increases with maturity. This is likely important in that "extractives" tend to decrease with maturity (as discussed below). The average total glycan content of plants in the vegetative phase was 32%, while the average for those in the mature seed phase was 45.9%. These results are consistent with those obtained in studies focusing on specific grasses (Waite and Gorrod, 1959).

4.1.2 Lignin

The lignin content of lignocellulosic materials is generally correlated with the recalcitrance of these materials, particularly the limited enzymatic accessibility of the cellulose component (Jung *et al.*, 1992). This recalcitrance is likely to be a factor in certain industrial processes, particularly those that are bio-based. The lignin affect is thought to be largely a result of steric hindrance; presumably the lignin reduces the cellulose surface area available for enzyme association (Haug, 1993). The "total lignin" values for the grasses included in this study ranged from a low of ~10% to a high of ~16% (Table 6). Total lignin was obtained by summing the measured acid-soluble and acid-insoluble (Klason) lignins. In all cases acid-insoluble lignin accounted for greater than 70 % of the total lignin. If one considers only the mature straws, such as the commercial straws tabulated in Table

4, then acid-insoluble lignin may be expected to account for greater than 80 % of the total lignin. The data of figure 2, depicting average lignin values (across species averages) grouped according to stage of maturity, suggests that each of the lignin fractions is associated with plant maturity. Acid-soluble lignin tended to decrease and acid-insoluble lignin tended to increase with maturity – the net effect being an increase in total lignin as plants mature. The increase in total lignin as plants mature is in general agreement with published data (Wayne and Harris, 1950; Ryoei and Kawamoto, 1994).

4.1.3 Ash

The presented ash values provide estimates of the total inorganic content of the biomass. The inorganic composition of grass-based feedstocks is important with regard to their ease of processing, as for example the tendency for slag development in gasification systems. In general, ash values for the unextracted samples (analyzed prior to water/ethanol extraction, see "Methods") were in the 5 – 10% range (Table 8 and 9). The ash values for the feedstocks following sequential water and ethanol extractions (see "Methods") were typically in the range of 0.5 – 2.5 percent. Thus, the sequential Soxhlet-based water and ethanol extractions tended to remove from 60 – 80% of the quantifiable inorganic matter. This extent of extraction may be of relevance for some industrial processing applications. The predominant inorganic compound of grasses in general is expected to be silica (Dagmar *et al.*, 2002).

4.2 EXTRACTIVES

Extractives represent ~ 20.29% to ~ 41.55% of the oven-dry mass of the grasses analyzed in this study (Table 6). Figure 3 shows average extractive values (across species) for grasses grouped according to stage of maturity. The depicted data suggests a decrease in extractive levels as plants mature. Figures 4a-f shows the relationship between extractive levels and the amount of other plant components. Total glycan and acid-insoluble lignin tend to be negatively associated with extractive levels with 99% confidence level (R-square = 0.5923 and 0.4333, respectively). Acid-soluble lignin and ash (Total, unextractable, and extractable ash) appears to have no correlation with extractive levels.

4.3 EVALUATION OF MASS BALANCE

It is important in analytical work in general, and in biomass analyses in particular, to consider the mass balance properties of the analyses with respect to the sum of the determined analytes. This topic was addressed in this study by evaluating the initial step in the overall analytical procedure – the extraction. Tables 10 and 11 give summative analysis data for extractions of wheat straw and tall fescue. Three different extractions were considered: water only, 95% ethanol only, and combined water and 95% ethanol. The summative values for wheat straw, for all three extraction conditions, were somewhat low, ranging from ~ 96 to ~99%. The summative values for tall fescue, considering all three extraction conditions, tend to be somewhat high, ~101%. The values for both wheat straw and tall fescue appear reasonable, although the wheat straw values suggest that a significant loss of

mass occurs during the extraction procedure. The result also demonstrate that water was the most effective extracting solvent, but that combined water and 95% ethanol extractions removed more solids than either solvent alone. Extractives include both organic and inorganic components, the majority being organics (Table 12).

The mass balances of native and commercial grasses are listed in table 13 and 14, respectively. From table 13, there are a group of 3 samples (number 18, 21, and 31) that has the summative values between 105% and 106% which seems to be outliers. These 3 samples have been analyzed at the same time (same set of sample, a set of three), so there might be a certain condition during analysis that caused the similar results to these samples, such as the way they were handled on that particular day. However, due to limited amount of samples, the researcher could not redo the experiment in order to prove whether these numbers are real.

4.4 THEORETICAL ETHANOL YIELD

The theoretical ethanol yields of extracted native and commercial grasses are listed in table 15 and 16. The calculation has been done using the theoretical ethanol yields calculator provided by United State Department of Energy – Energy Efficiency and Renewable Energy, biomass Program (http://www.eere.energy.gov/biomass). The theoretical ethanol yield of native grasses range from 55.3 to 86.7 gallons per dry ton. For the commercial grasses, wheat straw has the highest theoretical ethanol yield at 94.1 gallons per dry ton.

Table 1. Identification of analyzed grasses

#	**Species**	**Plant Stage**	**A**	**P**	**N**	**I**
1	Elymus glaucus	Seed Mature		X	X	
2	Dactylis glomerata	Seed Mature		X		X
3	Leymus triticoides	Flowering		X	X	
4	Beckmannia syzigachne	flowering	X		X	
5	Festuca idahoensis	mature		X	X	
6	Poa secunda	anthesis		X	X	
7	Leymus triticoides	Flower		X	X	
8	Deschampsia caespitosa	Flowering		X	X	
9	Pseudoroegneria spicata	anthesis		X	X	
10	Leymus triticoides	Boot		X	X	
11	Bromus marginatus	flower		X	X	
12	Leymus triticoides	Flower		X	X	
13	Bromus carinatus	Flowering		X	X	
14	Alopecurus	mature		X	X	
15	Glyceria elata	Flowering		X	X	
16	Elymus glaucus	mature stalk		X	X	
17	Beckmannia syzigachne	Boot emerg	X		X	
18	Pseudoroegneria spicata ssp inerme	boot		X	X	
19	Hordeum brachyantherum	Flowering		X	X	
20	Phalaris aquatica	flowering		X		X
21	Poa secunda	Flowering		X	X	
22	Elymus lanceolatus ssp lanceolatus	boot		X	X	
23	Elymus trachycaulus	boot		X	X	
24	Pseudoroegneria spicata ssp inerme	boot		X	X	
25	Elymus glaucus	Boot		X	X	
26	Elymus lanceolatus ssp lanceolatus	flower		X	X	
27	Dactylis glomerata	Flower		X		X
28	Deschampsia caespitosa	Boot		X	X	
29	Leymus triticoides	Boot		X	X	
30	Danthonia californica	Boot		X	X	
31	Bromus marginatus	boot		X	X	
32	Glyceria elata	flowering		X	X	
33	Calamagrostis canadensis	Boot		X	X	
34	Festuca roemerii	mature stalk		X	X	
35	Beckmannia syzigachne	Boot	X		X	
36	Pseudoroegneria spicata ssp inerme	boot		X	X	
37	Alopecurus	anthesis		X	X	
38	Hordeum brachyantherum	vegetative		X	X	
39	Festuca roemerii	vegetative		X	X	

A = Annual
P = Perrenial
N = Native
I = Introduced

Table 2. Glycans of water/ethanol extracted grasses
(All results are expressed as percentage of unextracted oven-dry grass)

#	Species	Total Glycans	Glucan	Xylan	Arabinan
1	*Elymus glaucus*	49.7	29.7	18.3	1.7
2	*Dactylis glomerata*	48.8	31.2	15.8	1.9
3	*Leymus triticoides*	47.7	28.7	17.5	1.5
4	*Beckmannia syzigachne*	47.6	28.5	17.8	1.3
5	*Festuca idahoensis*	47.3	29.3	16.6	1.3
6	*Poa secunda*	47.1	29.5	16.4	1.2
7	*Leymus triticoides*	47.1	28.8	16.8	1.4
8	*Deschampsia caespitosa*	46.6	27.3	16.4	2.9
9	*Pseudoroegneria spicata*	46.3	28.1	16.9	1.3
10	*Leymus triticoides*	45.8	27.2	17.0	1.6
11	*Bromus marginatus*	45.8	26.9	17.6	1.4
12	*Leymus triticoides*	45.8	27.7	16.8	1.4
13	*Bromus carinatus*	45.7	28.1	15.9	1.6
14	*Alopecurus* sp.	45.4	28.6	15.6	1.2
15	*Glyceria elata*	45.0	27.6	14.5	2.9
16	*Elymus glaucus*	44.9	27.0	16.4	1.5
17	*Beckmannia syzigachne*	44.8	26.8	15.8	2.1
18	*Pseudoroegneria spicata* ssp inerme	44.6	25.6	17.1	1.8
19	*Hordeum brachyantherum*	44.5	26.6	15.7	2.2
20	*Phalaris aquatica*	44.2	27.7	15.5	1.0
21	*Poa secunda*	43.8	25.3	16.6	1.9
22	*Elymus lanceolatus* ssp lanceolatus	43.6	25.9	16.1	1.7
23	*Elymus trachycaulus*	43.1	25.4	15.5	2.3
24	*Pseudoroegneria spicata* ssp inerme	42.9	24.2	17.2	1.5
25	*Elymus glaucus*	42.6	26.9	13.9	1.8
26	*Elymus lanceolatus* ssp lanceolatus	42.6	27.3	13.7	1.6
27	*Dactylis glomerata*	42.5	27.0	13.3	2.2
28	*Deschampsia caespitosa*	42.0	24.8	14.2	3.1
29	*Leymus triticoides*	42.0	25.0	15.0	2.1
30	*Danthonia californica*	41.4	24.1	14.7	2.6
31	*Bromus marginatus*	40.6	25.0	13.3	2.4
32	*Glyceria elata*	40.5	23.9	14.3	2.2
33	*Calamagrostis canadensis*	40.2	25.4	12.8	2.0
34	*Festuca roemerii*	39.5	22.5	14.4	2.6
35	*Beckmannia syzigachne*	38.7	25.0	11.1	2.6
36	*Pseudoroegneria spicata* ssp inerme	36.6	21.2	13.7	1.7
37	*Alopecurus* sp.	35.1	20.9	10.9	3.3
38	*Hordeum brachyantherum*	32.2	20.3	9.5	2.5
39	*Festuca roemerii*	31.7	18.7	10.5	2.5
	Mean Standard Deviation	1.35	0.78	1.08	0.23
	Mean Coefficient of Variation	3.12	2.99	7.13	11.76

Table 3. Percentages of glucan, xylan, and arabinan from total glycan of extracted grasses

#	Species	Glucan/glycan	Xylan/Glycan	Arabinan/Glycan
1	Elymus glaucus	60	37	3
2	Dactylis glomerata	64	32	4
3	Leymus triticoides	60	37	3
4	Beckmannia syzigachne	60	37	3
5	Festuca idahoensis	62	35	3
6	Poa secunda	63	35	3
7	Leymus triticoides	61	36	3
8	Deschampsia caespitosa	59	35	6
9	Pseudoroegneria spicata	61	37	3
10	Leymus triticoides	59	37	3
11	Bromus marginatus	59	38	3
12	Leymus triticoides	60	37	3
13	Bromus carinatus	61	35	4
14	Alopecurus	63	34	3
15	Glyceria elata	61	32	6
16	Elymus glaucus	60	37	3
17	Beckmannia syzigachne	60	35	5
18	Pseudoroegneria spicata ssp inerme	57	38	4
19	Hordeum brachyantherum	60	35	5
20	Phalaris aquatica	63	35	2
21	Poa secunda	58	38	4
22	Elymus lanceolatus ssp lanceolatus	59	37	4
23	Elymus trachycaulus	59	36	5
24	Pseudoroegneria spicata ssp inerme	56	40	3
25	Elymus glaucus	63	33	4
26	Elymus lanceolatus ssp lanceolatus	64	32	4
27	Dactylis glomerata	64	31	5
28	Deschampsia caespitosa	59	34	7
29	Leymus triticoides	60	36	5
30	Danthonia californica	58	36	6
31	Bromus marginatus	62	33	6
32	Glyceria elata	59	35	5
33	Calamagrostis canadensis	63	32	5
34	Festuca roemerii	57	36	7
35	Beckmannia syzigachne	65	29	7
36	Pseudoroegneria spicata ssp inerme	58	37	5
37	Alopecurus	60	31	9
38	Hordeum brachyantherum	63	30	8
39	Festuca roemerii	59	33	8
	Mean	60.46	34.94	4.62
	Standard Deviation	2.13	2.53	1.74
	Range	56-65	29-40	2-9

Table 4. Glycans of Extractive Free Residue following Sequential Soxhlet (H2O and EtOH) Extractions of Commercial Grass Species. All values are percent of original (unextracted) solids.

Name	Total Glycans	Glucan	Xylan	Arabinan
Kentucky Bluegrass	45.4	26.8	17.4	1.23
Perennial Ryegrass	45.8	29.5	14.4	1.84
Tall Fescue	41.0	26.0	13.0	1.95
Wheat	53.8	34.1	18.7	1.21

Table 5. Percentages of glucan, xylan, and arabinan from total glycan of commercial grasses

Name	Glucan/glycan	Xylan/Glycan	Arabinan/Glycan
Kentucky Bluegrass	59	38	3
Perennial Ryegrass	64	31	4
Tall Fescue	63	32	5
Wheat	63	35	2
Mean	62.56	34.06	3.43
Standard Deviation	2.40	3.22	1.16

Table 6. Composition analysis of water/extracted grasses
(All results are expressed as percentage of unextracted oven-dry grass)

#	Species	Glycans	AIL	ASL	Ash	Extractives	SUM
1	*Elymus glaucus*	49.7	13.76	1.74	1.03	22.48	88.69
2	*Dactylis glomerata*	48.8	12.56	1.86	1.96	21.72	86.91
3	*Leymus triticoides*	47.7	14.58	1.98	1.40	27.08	92.78
4	*Beckmannia syzigachne*	47.6	12.30	3.20	2.16	22.30	87.60
5	*Festuca idahoensis*	47.3	12.84	2.14	5.48	23.40	91.13
6	*Poa secunda*	47.1	11.61	2.31	2.53	25.02	88.58
7	*Leymus triticoides*	47.1	11.92	1.97	1.08	20.29	82.33
8	*Deschampsia caespitosa*	46.6	11.91	2.88	0.90	25.40	87.73
9	*Pseudoroegneria spicata*	46.3	12.98	2.07	2.18	25.16	88.68
10	*Leymus triticoides*	45.8	10.38	2.06	1.30	30.51	90.04
11	*Bromus marginatus*	45.8	10.76	3.26	1.93	31.18	92.92
12	*Leymus triticoides*	45.8	14.45	1.98	2.29	27.64	92.17
13	*Bromus carinatus*	45.7	10.40	2.25	0.52	31.11	89.98
14	*Alopecurus* sp.	45.4	13.54	2.27	2.67	25.75	89.59
15	*Glyceria elata*	45.0	9.97	3.48	1.00	28.28	87.72
16	*Elymus glaucus*	44.9	11.60	1.61	0.72	30.00	88.83
17	*Beckmannia syzigachne*	44.8	12.24	3.02	1.01	24.60	85.65
18	*Pseudoroegneria spicata* ssp inerme	44.6	11.62	2.89	2.13	29.29	90.49
19	*Hordeum brachyantherum*	44.5	9.39	2.64	0.59	24.50	81.65
20	*Phalaris aquatica*	44.2	13.31	2.31	1.14	25.83	86.79
21	*Poa secunda*	43.8	10.96	2.65	1.79	31.18	90.36
22	*Elymus lanceolatus* ssp lanceolatus	43.6	8.66	2.03	1.90	33.46	89.70
23	*Elymus trachycaulus*	43.1	7.53	3.11	1.74	33.42	88.92
24	*Pseudoroegneria spicata* ssp inerme	42.9	10.17	3.43	1.86	27.15	85.48
25	*Elymus glaucus*	42.6	7.94	2.30	2.89	32.89	88.58
26	*Elymus lanceolatus* ssp lanceolatus	42.6	11.17	2.47	1.82	29.11	87.18
27	*Dactylis glomerata*	42.5	12.25	1.57	1.16	28.98	86.50
28	*Deschampsia caespitosa*	42.0	9.47	4.17	1.47	27.46	84.61
29	*Leymus triticoides*	42.0	11.64	2.12	2.15	32.01	89.96
30	*Danthonia californica*	41.4	8.98	4.35	0.96	25.87	81.59
31	*Bromus marginatus*	40.6	8.27	3.32	1.95	34.85	88.99
32	*Glyceria elata*	40.5	11.00	3.40	0.80	28.41	84.07
33	*Calamagrostis canadensis*	40.2	10.75	3.00	1.06	29.43	84.47
34	*Festuca roemerii*	39.5	10.00	2.56	2.16	30.50	84.72
35	*Beckmannia syzigachne*	38.7	6.38	2.92	3.52	30.29	81.79
36	*Pseudoroegneria spicata* ssp inerme	36.6	7.42	3.55	2.02	35.19	84.82
37	*Alopecurus* sp.	35.1	9.05	3.92	1.60	29.59	79.27
38	*Hordeum brachyantherum*	32.2	7.58	3.55	1.23	39.47	84.07
39	*Festuca roemerii*	31.7	8.61	3.36	2.41	41.55	87.65
	Mean Standard Deviation	1.35	0.39	0.35	0.12	0.09	1.46
	Mean Coefficient of Variation	3.12	3.29	4.34	5.05	0.31	1.67

AIL = Acid-Insoluble Lignin
ASL = Acid-Soluble Lignin

Table 7. Composition of Extractive Free Residue following Sequential Soxhlet (H_2O and EtOH) Extractions of Commercial Grass Species. All values are percent of original (unextracted) solids.

Name	Glycans	AIL	ASL	Ash	Extractives
Kentucky Bluegrass	45.4	11.1	1.77	1.76	29.2
Perennial Ryegrass	45.8	11.8	1.76	1.31	28.1
Tall Fescue	41.0	10.7	1.95	0.84	29.4
Wheat	53.8	14.0	1.62	2.19	16.3

Table 8. Change in ash content with extraction.
Percent loss is calculated as [(ash in original sample – ash in extraction residue)/ ash in original sample] x 100.

#	Species	unextracted	extracted	% Loss of Ash
1	*Elymus glaucus*	6.47	1.03	84.1
2	*Dactylis glomerata*	6.19	1.96	68.3
3	*Leymus triticoides*	7.00	1.39	80.1
4	*Beckmannia syzigachne*	7.88	2.16	72.6
5	*Festuca idahoensis*	10.92	5.48	49.8
6	*Poa secunda*	6.97	2.53	63.6
7	*Leymus triticoides*	5.30	1.08	79.6
8	*Deschampsia caespitosa*	5.91	0.90	84.8
9	*Pseudoroegneria spicata*	6.82	2.18	68.0
10	*Leymus triticoides*	6.63	1.30	80.4
11	*Bromus marginatus*	9.4	1.93	79.5
12	*Leymus triticoides*	7.19	2.29	68.1
13	*Bromus carinatus*	4.36	0.52	88.1
14	*Alopecurus* sp.	7.52	2.67	64.5
15	*Glyceria elata*	5.64	1.00	82.2
16	*Elymus glaucus*	2.64	0.72	72.7
17	*Beckmannia syzigachne*	9.64	1.01	89.5
18	*Pseudoroegneria spicata* ssp inerme	8.23	2.13	74.1
19	*Hordeum brachyantherum*	4.24	0.59	86.1
20	*Phalaris aquatica*	5.73	1.14	80.1
21	*Poa secunda*	7.04	1.79	74.6
22	*Elymus lanceolatus* ssp lanceolatus	7.97	1.90	76.1
23	*Elymus trachycaulus*	7.00	1.74	75.2
24	*Pseudoroegneria spicata* ssp inerme	7.52	1.86	75.3
25	*Elymus glaucus*	10.21	2.89	71.7
26	*Elymus lanceolatus* ssp lanceolatus	7.79	1.82	76.6
27	*Dactylis glomerata*	6.54	1.16	82.2
28	*Deschampsia caespitosa*	7.08	1.47	79.3
29	*Leymus triticoides*	7.82	2.15	72.6
30	*Danthonia californica*	5.32	0.96	82.0
31	*Bromus marginatus*	10.23	1.95	81.0
32	*Glyceria elata*	5.28	0.80	84.8
33	*Calamagrostis canadensis*	5.21	1.06	79.6
34	*Festuca roemerii*	5.94	2.16	63.6
35	*Beckmannia syzigachne*	9.75	3.52	63.9
36	*Pseudoroegneria spicata* ssp inerme	7.41	2.02	72.7
37	*Alopecurus* sp.	7.49	1.60	78.6
38	*Hordeum brachyantherum*	6.42	1.23	80.9
39	*Festuca roemerii*	7.37	2.41	67.3

Table 9. Change in ash content with extraction of commercial grasses
Percent loss is calculated as [(ash in original sample – ash in extraction residue)/ ash in original sample] x 100.

Name	Unextracted	Extracted	% Loss of Ash
Kentucky Bluegrass	5.27	1.76	66.6
Perennial Ryegrass	5.92	1.31	77.8
Tall Fescue	6.48	0.84	87.0
Wheat	7.24	2.19	69.8

Table 10. Mass Balance of water extracted, water/ethanol extracted, and ethanol extracted Wheat Straw

	Water Extraction			Water/EtOH Extraction			EtOH Extraction		
Rep	Ext. Solid	Unext. Solid	Total Solids	Ext. Solid	Unext. Solid	Total Solids	Ext. Solid	Unext. Solid	Total Solids
1	13.75	80.99	94.74	16.59	82.46	99.05	7.33	86.49	93.83
2	12.80	82.04	94.84	16.04	82.37	98.40	6.84	90.52	97.36
3	14.50	84.14	98.64	15.37	84.70	100.07	6.41	89.21	95.62
4	14.26	81.32	95.58	17.78	82.18	99.96	7.27	88.98	96.25
5	14.36	82.32	96.68	17.99	82.31	100.30	6.99	83.08	90.07
6	15.15	81.27	96.42	17.61	81.25	98.86	7.17	95.82	102.99
Average (SD)	14.14 (0.80)	82.01 (1.16)	96.15 (1.46)	16.90 (1.06)	82.54 (1.14)	99.44 (0.77)	7.00 (0.34)	89.02 (4.25)	96.02 (4.26)

Values in parentheses are standard deviations of the mean.
Ext. Solid = Extractable Solid
Unext. Solid = Unextractable Solid

Table 11. Mass Balance of water extracted, water/ethanol extracted, and ethanol extracted Tall Fescue

Rep	Water Extraction			Water/EtOH Extraction			EtOH Extraction		
	Ext. Solid	Unext. Solid	Total Solids	Ext. Solid	Unext. Solid	Total Solids	Ext. Solid	Unext. Solid	Total Solids
1	26.79	74.21	100.99	28.58	72.72	101.30	14.83	87.01	101.84
2	26.67	74.59	101.26	29.41	72.04	101.45	14.75	87.24	101.99
3	25.43	75.58	101.02	28.24	73.07	101.31	14.39	87.67	102.07
4	26.43	74.70	101.12	28.85	71.94	100.78	15.36	86.52	101.88
5	27.22	73.75	100.96	29.45	71.90	101.35	14.95	87.08	102.03
6	27.79	73.08	100.88	29.28	71.26	100.54	14.88	85.72	100.60
Average (SD)	26.72 (0.79)	74.32 (0.86)	101.04 (0.13)	28.97 (0.49)	72.15 (0.65)	101.12 (0.37)	14.86 (0.31)	86.87 (0.67)	101.74 (0.56)

Values in parentheses are standard deviations of the mean.
Ext. Solid = Extractable Solid
Unext. Solid = Unextractable Solid

Table 12. The total solids of water extractives of wheat straw and tall fescue

Rep	Wheat Straw			Tall Fescue		
	Organic	Inorganic	Total Solids	Organic	Inorganic	Total Solids
1	9.74	4.01	13.75	22.23	4.55	26.79
2	8.50	4.29	12.80	21.98	4.69	26.67
3	10.36	4.15	14.50	21.03	4.40	25.43
4	10.20	4.05	14.26	21.67	4.75	26.43
5	10.22	4.14	14.36	22.56	4.66	27.22
6	10.96	4.19	15.15	23.06	4.73	27.79
Average (SD)	10.00 (0.83)	4.14 (0.10)	14.14 (0.80)	22.09 (0.71)	4.63 (0.13)	26.72 (0.79)

Values in parentheses are standard deviations of the mean.

Table 13. Mass Balance of water/ethanol extracted Poaceae grasses
(All results are expressed as percentage of unextracted oven-dry grasses)

#	Species	% WES	%EES	%NES	Total
1	*Elymus glaucus*	20.87	1.61	75.56	98.04
2	*Dactylis glomerata*	19.64	2.08	75.09	96.81
3	*Leymus triticoides*	25.09	2.00	74.33	101.42
4	*Beckmannia syzigachne*	21.03	1.27	76.32	98.62
5	*Festuca idahoensis*	21.32	2.08	76.99	100.39
6	*Poa secunda*	22.76	2.26	74.12	99.14
7	*Leymus triticoides*	18.13	2.16	71.98	92.27
8	*Deschampsia caespitosa*	23.06	2.35	76.97	102.38
9	*Pseudoroegneria spicata*	22.88	2.28	74.26	99.42
10	*Leymus triticoides*	28.29	2.22	71.06	101.57
11	*Bromus marginatus*	28.49	2.69	69.27	100.45
12	*Leymus triticoides*	25.44	2.20	74.70	102.34
13	*Bromus carinatus*	29.01	2.10	71.97	103.08
14	*Alopecurus* sp.	22.82	2.93	74.21	99.96
15	*Glyceria elata*	25.14	3.14	73.17	101.45
16	*Elymus glaucus*	28.60	1.46	67.5	97.60
17	*Beckmannia syzigachne*	21.57	3.03	72.67	97.27
18	*Pseudoroegneria spicata* ssp inerme	26.03	3.26	76.17	105.46
19	*Hordeum brachyantherum*	21.21	3.29	67.67	92.17
20	*Phalaris aquatica*	23.54	2.29	71.89	97.72
21	*Poa secunda*	28.13	3.05	73.96	105.14
22	*Elymus lanceolatus* ssp lanceolatus	30.76	2.70	66.29	99.75
23	*Elymus trachycaulus*	30.38	3.04	68.62	102.04
24	*Pseudoroegneria spicata* ssp inerme	24.13	3.02	72.53	99.68
25	*Elymus glaucus*	29.79	3.10	69.37	102.26
26	*Elymus lanceolatus* ssp lanceolatus	26.52	2.59	71.94	101.05
27	*Dactylis glomerata*	26.52	2.46	68.93	97.91
28	*Deschampsia caespitosa*	24.37	3.09	73.25	100.71
29	*Leymus triticoides*	25.35	6.66	66.63	98.64
30	*Danthonia californica*	23.71	2.16	70.23	96.10
31	*Bromus marginatus*	31.38	3.47	71.25	106.10
32	*Glyceria elata*	27.37	1.04	68.26	96.67
33	*Calamagrostis canadensis*	26.99	2.44	68.90	98.33
34	*Festuca roemerii*	28.00	2.49	66.4	96.90
35	*Beckmannia syzigachne*	27.45	2.84	64.56	94.85
36	*Pseudoroegneria spicata* ssp inerme	31.98	3.21	64.95	100.14
37	*Alopecurus* sp.	25.78	3.81	67.77	97.36
38	*Hordeum brachyantherum*	32.35	7.12	59.27	98.74
39	*Festuca roemerii*	36.77	4.78	58.72	100.27
	Mean Standard Deviation	1.00	0.33	1.38	1.74
	Mean Coefficient of Variation	3.85	11.72	1.95	1.75

% WES = % Water Extractable Solids
% EES = % EtOH Extractable Solids
% NES = % Non Extractable Solids

Table 14. Extractives of commercial grasses. Extractions were performed by Soxhlet (24 hours) in sequence. Water extraction was first followed by ethanol. All values are percent of original (unextracted) solids.

Name	% WES	% EES	% NES	Recovery
Kentucky Bluegrass	27.4	1.82	71.7	100.9
Perennial Ryegrass	25.0	3.11	71.0	99.2
Tall Fescue	25.2	4.20	71.6	100.3
Wheat	14.2	2.12	85.4	101.8

% WES = % Water Extractable Solids
% EES = % EtOH Extractable Solids
% NES = % Non Extractable Solids

Table 15. Theoretical ethanol yields per dry ton of grasses

#	Species	EtOH (gallons/dry ton)		
		Glucan	Xylan & Arabinan	Total
1	*Elymus glaucus*	51.3	35.4	86.7
2	*Dactylis glomerata*	53.9	31.3	85.2
3	*Leymus triticoides*	49.6	33.6	83.2
4	*Beckmannia syzigachne*	49.3	33.8	83.1
5	*Festuca idahoensis*	50.6	31.7	82.3
6	*Poa secunda*	51.0	31.1	82.1
7	*Leymus triticoides*	49.8	32.2	82.0
8	*Deschampsia caespitosa*	47.2	34.1	81.3
9	*Pseudoroegneria spicata*	48.6	32.2	80.8
10	*Leymus triticoides*	47.0	32.9	79.9
11	*Bromus marginatus*	46.5	33.6	80.1
12	*Leymus triticoides*	47.7	32.2	79.9
13	*Bromus carinatus*	48.6	31.0	79.6
14	*Alopecurus* sp.	49.4	29.7	79.1
15	*Glyceria elata*	47.7	30.8	78.5
16	*Elymus glaucus*	46.7	31.7	78.4
17	*Beckmannia syzigachne*	46.3	31.7	78.0
18	*Pseudoroegneria spicata* ssp inerme	44.2	33.4	77.6
19	*Hordeum brachyantherum*	46.0	31.7	77.7
20	*Phalaris aquatica*	47.9	29.2	77.1
21	*Poa secunda*	43.7	32.7	76.4
22	*Elymus lanceolatus* ssp lanceolatus	44.8	31.5	76.3
23	*Elymus trachycaulus*	43.9	31.5	75.4
24	*Pseudoroegneria spicata* ssp inerme	41.8	33.1	74.9
25	*Elymus glaucus*	46.5	27.8	74.3
26	*Elymus lanceolatus* ssp lanceolatus	47.2	27.1	74.3
27	*Dactylis glomerata*	46.7	27.4	74.1
28	*Deschampsia caespitosa*	42.9	30.6	73.5
29	*Leymus triticoides*	43.2	30.2	73.4
30	*Danthonia californica*	41.6	30.6	72.2
31	*Bromus marginatus*	43.2	27.8	71.0
32	*Glyceria elata*	41.3	29.2	70.5
33	*Calamagrostis canadensis*	43.9	26.2	70.1
34	*Festuca roemerii*	38.9	30.1	69.0
35	*Beckmannia syzigachne*	43.2	24.2	67.4
36	*Pseudoroegneria spicata* ssp inerme	36.6	27.2	63.8
37	*Alopecurus* sp.	36.1	25.1	61.2
38	*Hordeum brachyantherum*	35.1	21.2	56.3
39	*Festuca roemerii*	32.3	23.0	55.3

Table 16. Theoretical ethanol yields per dry ton of commercial grasses (gallons/dry ton)

Name	EtOH (gallons/dry ton)		
	Glucan	Xylan & Arabinan	Total
Kentucky Bluegrass	46.3	33.0	79.3
Perennial Ryegrass	51.0	28.7	79.7
Tall Fescue	44.9	26.4	71.3
Wheat	58.9	35.2	94.1

Figure 1. The average of glycans values of grasses based on plant stage

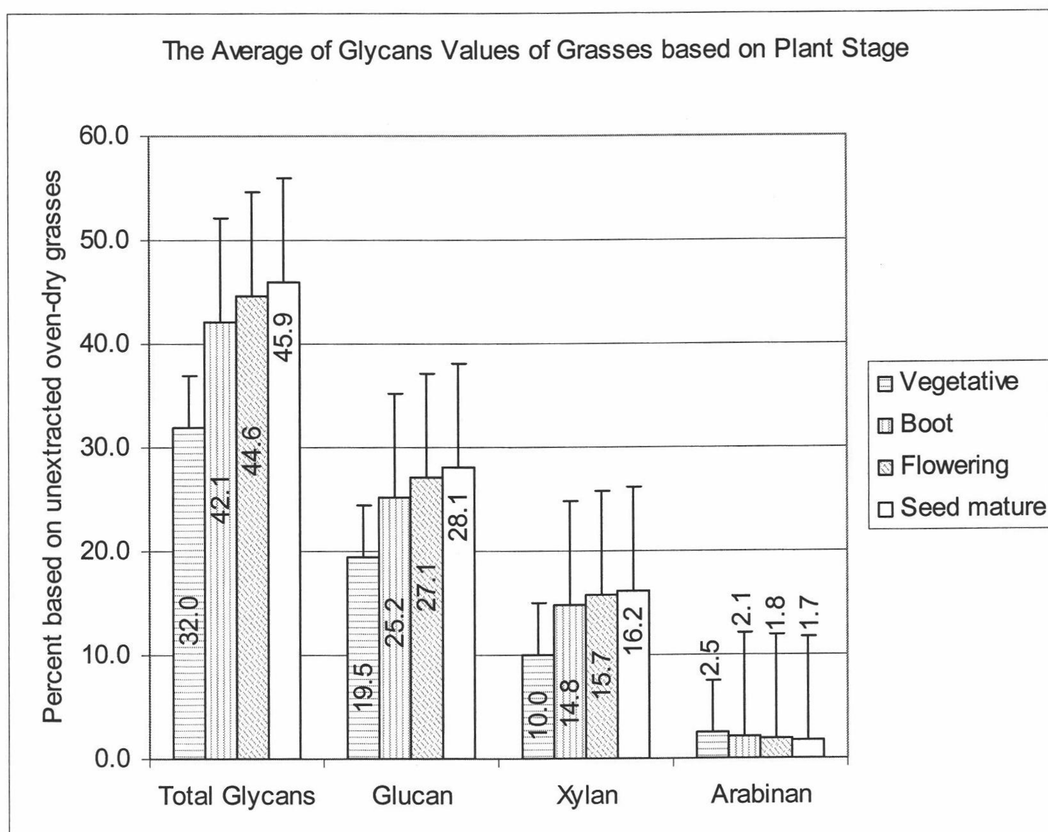


Figure 2. The average of lignin values of grasses based on plant stage

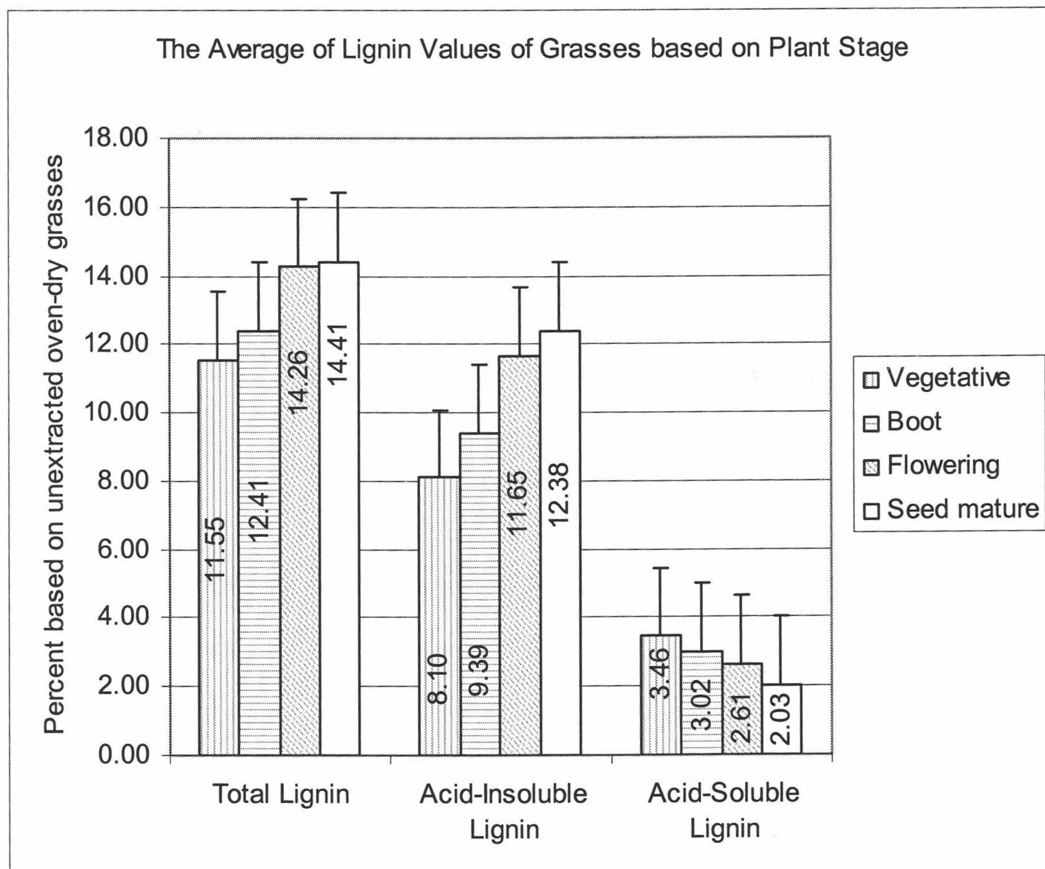


Figure 3. The average of extractives values of grasses based on plant stage

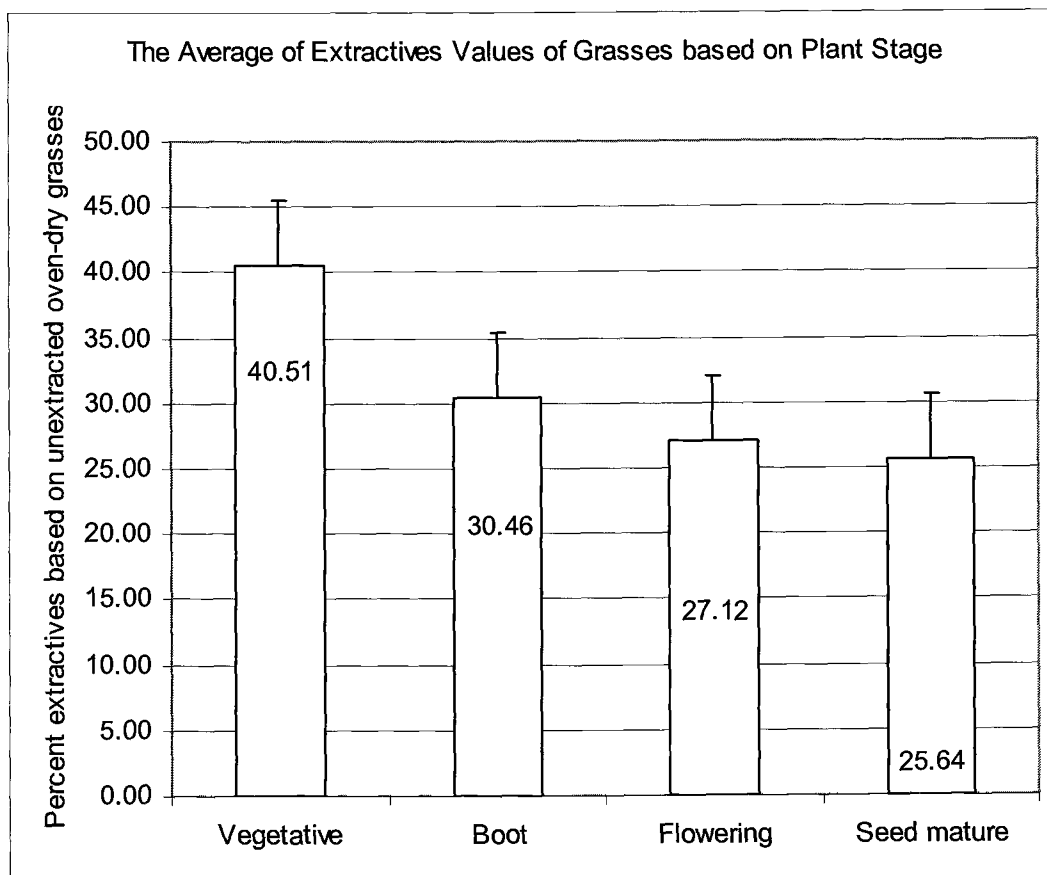


Figure 4a. The relationship of total glycans versus extractives

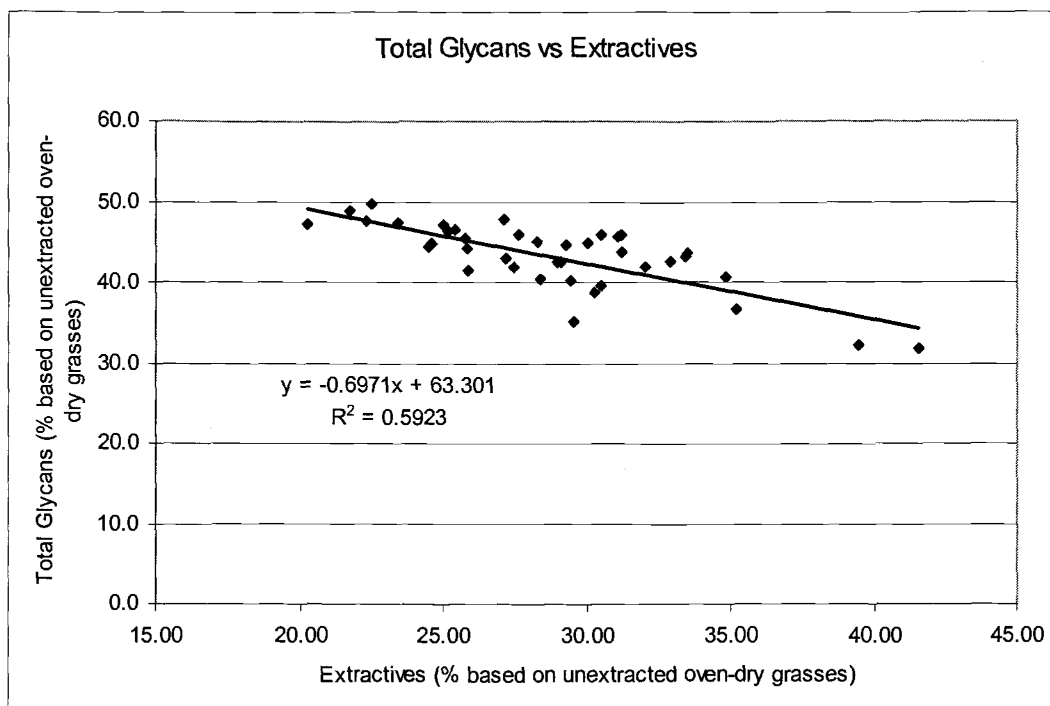


Figure 4b. The relationship of acid-insoluble lignin versus extractives

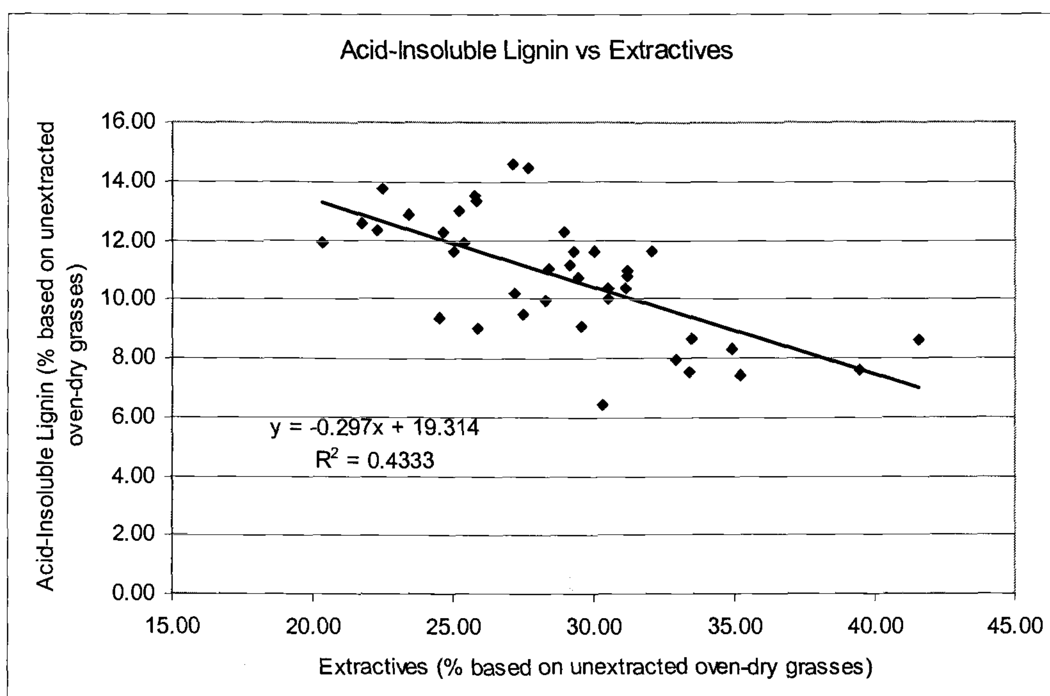


Figure 4c. The relationship of acid-soluble lignin and extractives

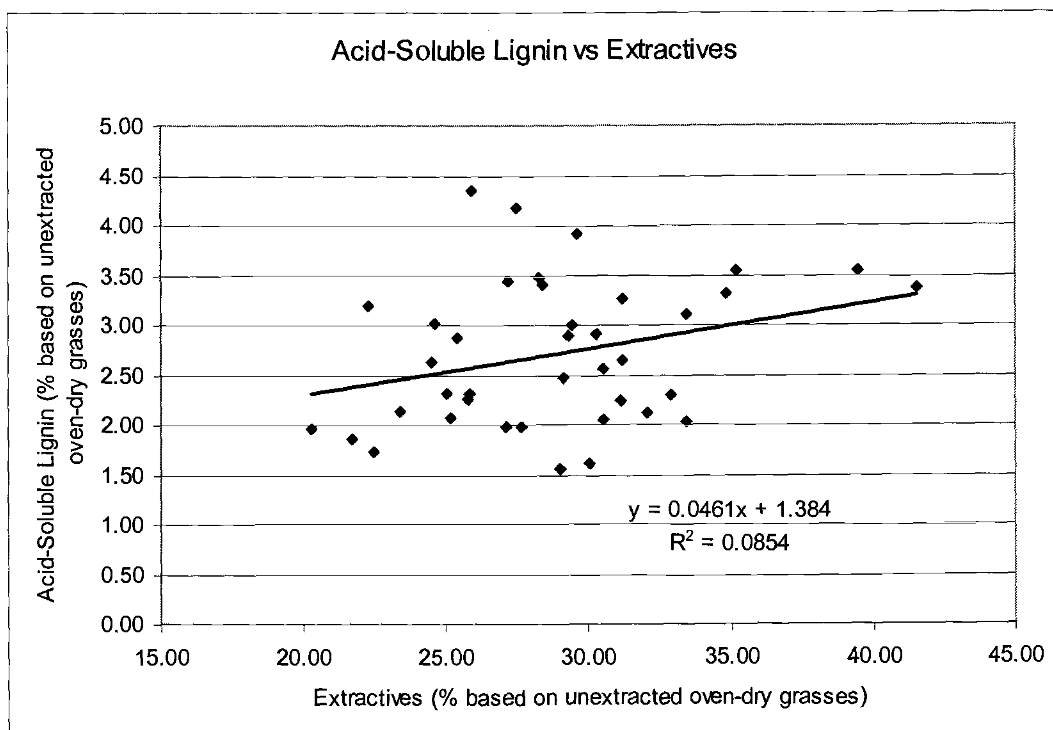


Figure 4d. The relationship of total ash and extractives

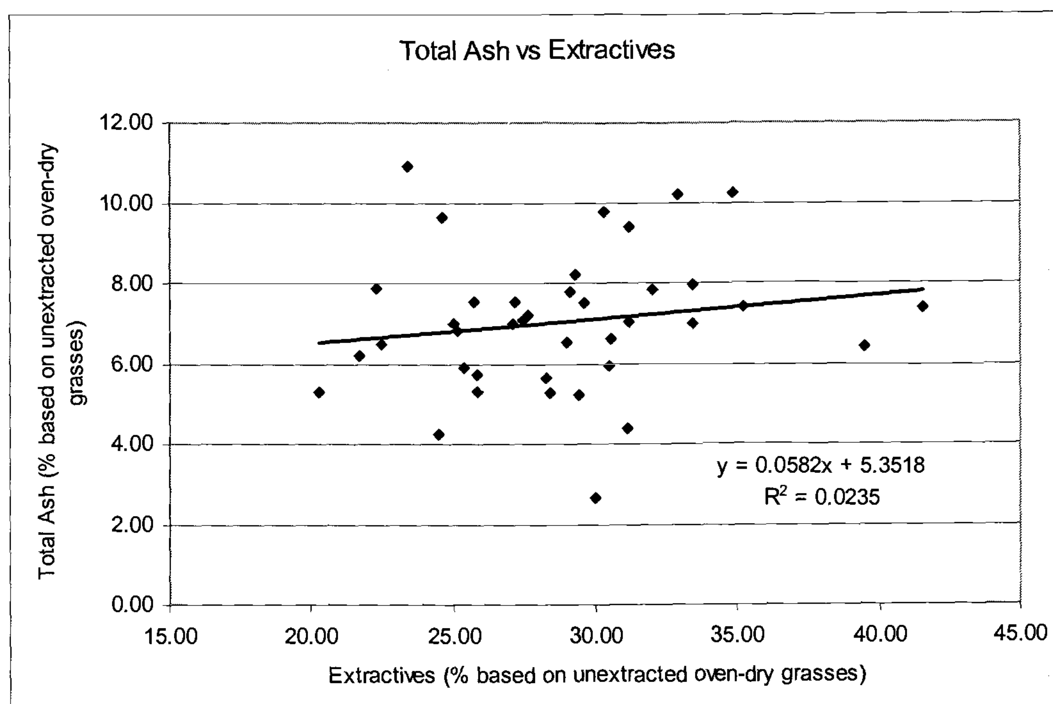


Figure 4e. The relationship of extractable ash and extractives

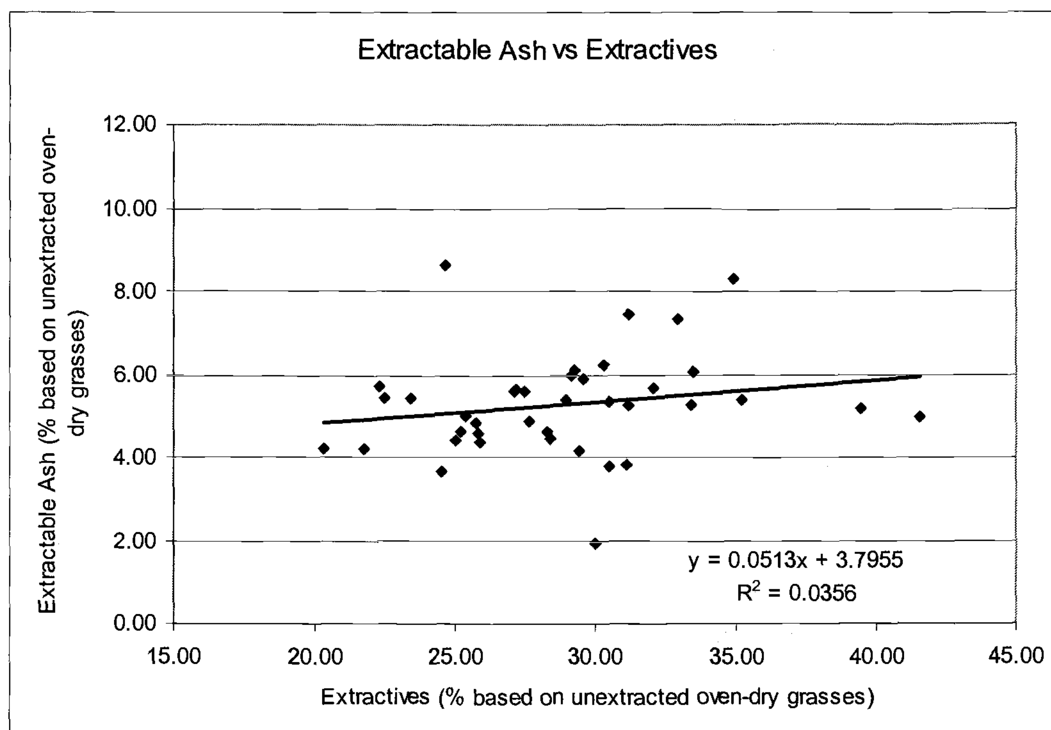
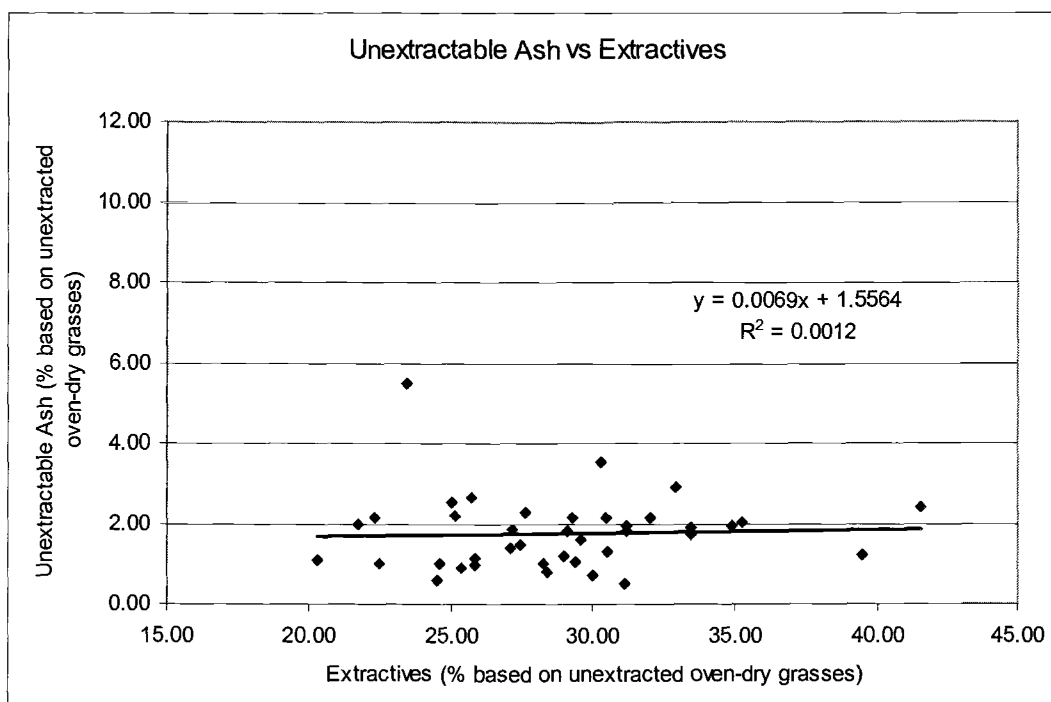


Figure 4f. The relationship of unextractable ash and extractives



5. CONCLUSION

The aim of this work was to generate an initial database that contains the structural component composition of a range of grasses having potential bio-refinery applications in the Pacific Northwest region of the United States. The grasses included in the study were chosen based on NIR data indicating that these samples were representative of the broad compositional range that may be expected for such applications. Four commercial grass straws were included in the study for comparative purposes. The structural glycan content of the samples ranged from 32 to 50 percent. On average, glucans made up ~ 60 percent of the total structural glycans. The ratio of glucans:xylans:arabinans (12:7:1) was surprisingly constant between species and stages of maturity. Acid-Insoluble lignin ranged from 6.38 to 14.58 percent, the higher values tending to be associated with the more mature plants. Acid-soluble lignin values ranged from 1.57 to 4.35 percent. The ash (inorganic) content of the oven-dried samples ranged from 5 to 10 percent. Those components that were readily extracted by sequential water and 95% ethanol treatments, termed "extractives", collectively accounted for between 20.29 to 41.55 percent of the total solids. The extractives contained from 60-80% of the total inorganic matter originally associated with the grasses. The presented data provides quantitative ranges for which the structural components of the majority of comparative grasses may be expected to fall when grown in the Pacific Northwest area.

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