Executive Summary

Purpose and Scope

Biomass conversion to fermentable sugars for the purpose of producing fuels, chemicals, and other industrial products is well understood. Most bioenergy strategies rely on low-cost fermentable sugars for sustainability and economic viability in the marketplace. Exploitation of the “whole crop”—specifically, wheat straw or other plant material currently regarded as residue or waste—is a practical approach for obtaining a reliable and low-cost source of sugars. However, industrial-scale production of sugars from wheat straw, while technically feasible, is plagued by obstacles related to capital costs, energy consumption, waste streams, production logistics, and the quality of the biomass feedstock. The collective effect of these obstacles has a negative impact on the economic viability of wheat straw and other crop residue biomass as an inexpensive source of commodity sugars.

This project focused on addressing a priority set of issues within three of the barrier areas identified by the U.S. Department of Energy (DOE) as hindering the exploitation of biomass as a source of commercial energy: plant science, crop production, and biomass processing. The objective of this research was to address these barrier areas and to develop commercially viable technologies needed to use crop residues as an inexpensive source of fermentable sugars by investigating the following concerns:

- Expanding the engineering understanding of today’s grain harvesters through high-speed imagery/visualization, computational modeling, and virtual engineering techniques, and applying this new understanding to the engineering and development of a single-pass multi-component harvester controlled by an autonomous intelligent control system.
- Studying and understanding gene regulation of lignin synthesis in wheat for the purpose of exploiting lower lignin and increased polysaccharides to make the crop residue stems more adaptable to mechanical harvesting and produce higher fermentable sugar yields.
- Assessing the benefit of modified straw stems for hydrolysis conversion, and assessing conversion-processing enhancements that could be effected using a feedstock expressly developed for acid hydrolysis conversion into low-cost fermentable sugars.

All three of these research areas include the potential for significant economic, environmental, and energy benefits.

Key Results

The following sections outline the results of our research that are related to the previously described priority issues.

Harvester

Currently available separation options with combine harvesters are not able to achieve sufficient separation of the straw/stover and chaff streams to realize the full potential of selective harvest. Studies conducted at the National Renewable Energy Laboratory (NREL) (corn stover survey studies) and Idaho National Laboratory (INL) (cereal straw fractionation and storage studies) demonstrated that glucan and xylan sugar content variability can be greater than 10% for stover and cereal straw anatomical fractions.

Lignin content variability between anatomical fractions can approach 6%. The NREL studies showed that total structural sugar content can vary between 45-88% as a function of variety and environment. Of further interest, the glucan and lignin content was least sensitive to these genetic and environmental factors; as such, the compositional variability of these constituents between anatomical fractions appears to be significant and have some degree of stable predictability that could be exploited to improve feedstock structural carbohydrate content. Since ethanol yield is a function of feedstock structural carbohydrate content, biomass anatomical fractions of higher product yield can have a significant beneficial impact on minimum ethanol selling price. However, tests conducted by INL showed that by using baffles and other machine adjustment measures, the best achievable anatomical fractionation of cereal straw stems was between 70-80% straw stem purity. This separation process also resulted in significant losses of the desirable stem material, and represented only a 2.2% increase in glucan content. However, based on analytical analysis of the discreet cereal straw anatomical fractions, a 6% glucan content increase is potentially achievable if a high-fidelity separation in excess of 80% stem purity can be achieved. Additionally, improvements in separations are needed to prevent yield losses of desirable biomass components resulting from simply losing the material during the harvest/collection. The purpose of these advanced biomass separation computation engineering models is to more effectively and efficiently engineer high-fidelity and high throughput separation systems for biomass components.

Early in the project, INL and Iowa State University (ISU) developed a computational modeling strategy for simulating multi-phase flow with an integrated solver using various computational fluid dynamics (CFD) codes. INL and ISU then began simulating the multi-phase flow with an integrated solver using various CFD codes: MFIX (Multiphase Flow with Interphase eXchanges), ANSYS CFX, and ANSYS Fluent 6. ISU set up a classic multi-phase test problem to be solved by the various CFD codes. The benchmark case was based on experimental data for bubble gas holdup and bed expansion for a gas/solid fluidized bed. Preliminary fluidization experiments identified some unexpected fluidization behavior, where rather than the bed uniformly fluidizing, a “blow out” would occur where a hole would open up in the bed through which the air would preferentially flow, resulting in erratic fluidization.

To improve understanding of this phenomena and aid in building a design tool, improved computational tools were developed. MFIX-DEM (discrete element model) functionality was improved in terms of computational efficiency and numerical accuracy. Specifically, the following tasks were completed:

- A pressure correction numerical method for the Semi-Implicit Method for Pressure Linked Equations (SIMPLE) algorithm was developed and implemented in MFIX. This numerical method improved the coupling between the particle phase and the gas phase decreased the computational time by about four folds.
- A method of calculating drag force based on individual particle velocity was implemented into MFIX. This method differentiated the drag force between particles in the same computational cell while the original method assigned the same drag force. This method gave a more physically realistic description of the drag forces on particles.
- A method of estimating particle mean field using Monaghan’s kernel function was implemented into MFIX. A fixed bandwidth, which is wider than the Eulerian grid spacing for the gas phase, was used for the kernel function. Therefore, the method eliminated the increase of statistical error with refining the Eulerian grid.

However, the method predicted lower than expected particle mean values at wall boundaries due to the symmetry of the kernel function and the wide bandwidth.

The virtual engineering techniques developed in this project were tested and utilized to design a separation baffle in a Case New Holland (CNH) combine. Selective harvest performance targets were established, based on compositional analysis of anatomical fractions, that represented the best residue fractionation current combines are capable of achieving. A computational engineering approach involving modeling, analysis, and simulation was used in the form of virtual engineering to design a baffle separator capable of accomplishing the high-fidelity residue separation established by the performance targets. Through the use of the virtual engineering model, baffle designs were simulated to (1) determine the effect of the baffle on the airflow of the combine cleaning system, and (2) predict the effectiveness of the baffle in separating the residue streams. The virtual engineering simulation of the baffle design that was selected for testing is shown in Figure 9. A side-by-side comparison with the simulation that represents normal combine operation shows the baffle to have little effect on the flow field behavior. Although a partial baffle still allows some open space between the two residue streams in which mixing could occur, it satisfies the two main design requirements in that it eliminates the potential for mixing at the discharge and it does not impede the predominant air streams that are significant to combine performance.

Accordingly, this baffle design was selected, built into the INL selective harvest test combine, and tests were performed to evaluate the separation fidelity and the resulting effect on composition and theoretical ethanol yield. The result of the baffle changes improved the crop separation capability of the CNH test combine that enabled the downstream improvement in composition and theoretical ethanol yield. In addition, the positive results from the application of the virtual engineering tools to the CNH combine design resulted in further application of these tools to other INL areas of research.

Carbon Flow in Live Wheat Plants

INL and the University of Idaho (U of I) identified, characterized, and modified a key plant biosynthetic lignin gene, cinnamoyl CoA reductase (CCR), to assess its influence on the efficiency with which the resulting biomaterials interact with and are processed by engineering systems. The characterization of CCR genes and resulting antisense gene constructs allowed us to modify and assess the impact of this cell wall biosynthetic gene in wheat. This allows the model systems needed to study the structural and functional post-harvest properties of straw to be created and assessed. Post-harvest properties and qualities associated with key genes involved in cell wall biosynthesis like CCR are important in developing engineered systems that can most effectively harvest and extract value from crop residues.

Progress was made toward identifying and using key CCR lignin biosynthesis gene sequences for controlling lignin biosynthesis with the goal of altering lignin content and composition in wheat. This was important because of the potential impact that reduced lignin could have on feedstock harvesting, transportation, storage, pretreatment, and processing.

Modified Feedstock

Initial analysis at the U of I of straw from the T_1 generation of transgenic spring wheat was done on five plants for each T_1 population. Several genetically modified individuals were identified with a lower level of acid insoluble lignin and total lignin than the non-transformed check cultivar. Greatest reduction was shown in the progeny of one specific transgenic line showing a 40% reduction in percent acid insoluble lignin and in total lignin. However, because of known transgene instability in plants (De Neve et al. 1999), lines need to be examined over a number of generations to obtain the necessary data on transgene stability and identify stable transgenic lines for agronomic applications. Therefore, second generation transgenic lines were evaluated.

T_2 generation plants derived from the T_1 spring wheat plants that had shown the greatest reduction in acid insoluble lignin content were then planted in the greenhouse. As the plants matured, stem internode tissue was collected for analysis of insoluble and total lignin. T_1 winter wheat lines were submitted to the U of I for lignin analysis. The first three populations examined did not show a significant reduction in lignin content. Seed produced by pollen-mediated transformation was harvested. Upon reaching maturity, the T_2 generation plants were harvested and processed for lignin analysis. Variations were observed among the populations for soluble and insoluble lignin content determined at the U of I. The decreases observed were greater for the soluble lignin content than insoluble Klason lignin content.

Analysis using near infrared (NIR) spectroscopy provided a rapid indirect measurement of plant composition once calibrated with suitable representatives of a specific feedstock type like corn stover or wheat straw. Over 150 NIR spectra were collected in duplicate from mature transgenic T2 and control parental spring and winter wheat plants that were greenhouse grown, dried, and ground to pass through a two-millimeter screen. These ground biomass materials were analyzed at the INL using a FOSS 6500 NIR spectrophotometer. Collected spectra were compared by principal component analysis (PCA). This rapid NIR assay allowed the down-selection of a smaller PCA-identified subset of modified wheat straw samples for further analysis of their lignin and structural sugar composition using the standard quantitative saccharification (Quan Sac) assay at the INL.

The chemical composition analysis of the PCA-identified subset of T2 modified spring wheat straw indicated that variation in the C6 structural sugars ranged from 33.6% to 38.2%, a span of about 4.5%, even though little or no change in Klason lignins was found in these specific transgenic straw samples. However, even though these specific straw samples showed no major change in Klason lignin content, some had higher observable theoretical ethanol yields then unmodified control plants as determined by simultaneous saccharification/fermentation (SSF). These results highlight the potential of capturing genetic variation in conversion efficiency among transgenic wheat plants. Ethanol conversion efficiency differed by over 16% among the genetically modified spring wheat variety and by over 13% among the genetically modified winter wheat variety. The observed variation in ethanol conversion among the altered transgenic plants indicates the potential for genetically modifying straw composition in a way that can impact feedstock quality and ethanol conversion efficiency.

Conclusions

Our proposed straw separation system harvests the large internode sections of the straw which has the greater potential as a feedstock for lignocellulosic ethanol production while leaving the chaff and nodes in the field (environmental benefit). This strategy ensures sustainable agriculture by preventing the depletion of soil minerals, and it restores organic matter to the soil (environmental benefit) in amounts and particle sizes that accommodate farmers' needs to keep tillage and fertilizer costs low (economic and energy benefits). A ton of these nutrient-rich plant tissues contains as much as $10.55

worth of fertilizer (economic and energy benefits), in terms of nitrogen, phosphorus, potassium, and other nutrients provided to the soil when incorporated by tillage instead of being burned (environmental benefits).

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Project Summary

Introduction

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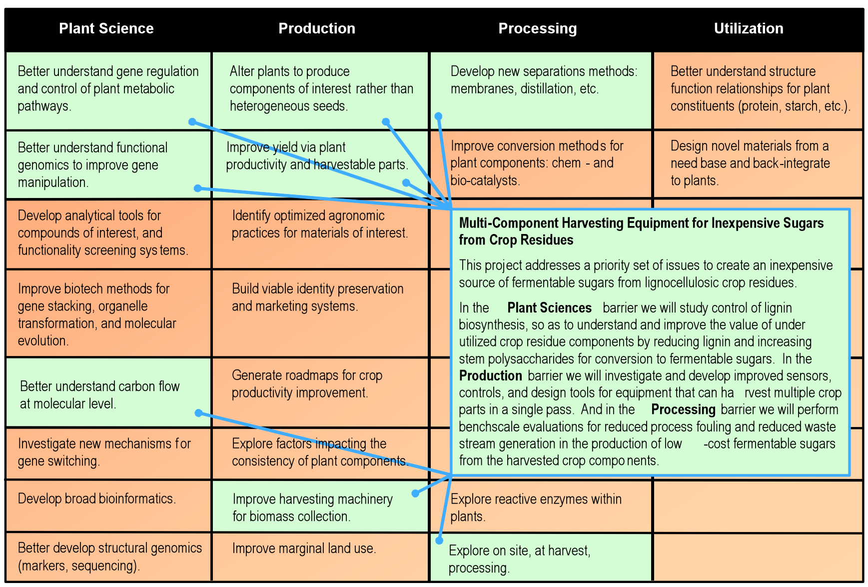


Figure 1. This project addresses three problems critical to the production of inexpensive lignocellulosic sugars for chemicals processing: (a) metabolic pathway manipulation, (b) harvesting systems, and (c) conversion methods.

The objective of this project was to develop the commercially viable technologies needed to use crop residues as an inexpensive source of fermentable sugars and to make this process economically and environmentally attractive. To accomplish this goal, the genetics of the live wheat plant needed to be optimized for biomass production, the harvesters engineered for biomass collection and separation, and the biomass processing equipment improved for reduced fouling and waste generation; and this all needed to be done in an integrated manner. Specifically, this project's goals were to:

1. Expand the engineering understanding of today's grain harvesters through high-speed imagery/visualization, computational modeling, and virtual engineering techniques, and apply this new understanding to the engineering and development of a single-pass multi-component harvester controlled by an autonomous intelligent control system.
2. Study and understand gene regulation of lignin synthesis in wheat for the purpose of exploiting lower lignin and increased polysaccharides to make the crop residue stems more adaptable to mechanical harvesting and produce higher fermentable sugar yields.

3. Assess the benefit of modified straw stems for hydrolysis conversion, and assess conversion-processing enhancements that could be effected using a feedstock expressly developed for acid hydrolysis conversion into low-cost fermentable sugars.

All three of these research areas include the potential for significant economic, environmental, and energy benefits.

This project includes four technical endpoints:

1. A prototype autonomous intelligent control system for the threshing and separation systems of a grain/biomass combine harvester.
2. A virtual simulation and engineering tool set based on physical and computational data sets for a single-pass multi-component (grain/biomass) combine harvester.
3. Wheat plants in which the lignin biosynthetic carbon flow has been altered to divert more of the carbon to the cellulosic polysaccharides.
4. An optimized acid hydrolysis processing design for conversion of modified wheat straw stems to fermentable sugars, including estimates of straw production support infrastructure.

This report presents the results of the research on efficiently producing higher fermentable sugar yields through the development of a better harvester and the ability to use the whole wheat plant.

Single-Pass Multi-Component Harvester

Background

The expression “combine harvester” is derived from the fact that field binders used to harvest the grain crop into shocks, which were taken to the stationary threshing machines to separate the grain. These two operations were “combined” in the next-generation grain harvester. Today, the capacity and efficiency of grain combine harvesters are nothing short of an engineering marvel. Modern grain harvesters combine two operations: first, the grain is threshed from the straw, using mechanical energy. Second, an air stream and a pair of sieves are employed to “winnow” the material, removing the chaff and residual straw to produce a pure grain stream. The grain is collected and the straw is discharged, either by scattering it in the field or by laying it in a harrow swath for baling. This approach has two major drawbacks for collecting biomass:

1. It is necessary to make a separate trip across the field with a baler, which requires an additional machine, additional labor, and additional energy.
2. Current baler technology is “all or nothing.” Balers are not able to harvest only the more desirable stems and leave the less desirable nodes and chaff in the field where they are valuable for soil replenishment.

Currently, commercially available combines have no sensors to identify the biomass components to be harvested. The only sensor used is a piezoelectric sensor, which attempts to measure grain being lost off the back of the sieve and out of the combine. Idaho National Laboratory (INL) field research has confirmed that the sensor does not accurately measure grain loss; instead, it measures the impact of any piece of material without distinguishing, for example, grain from stem. Therefore, the piezoelectric sensor technology as it currently exists is not only unsuitable for grain loss measurement, but also is unable to identify or measure the amount of any other part of the crop biomass. Clearly, the time has come to develop the next generation of harvesters that “combine” yet another set of processes into a single harvest machine. The result will be equipment that can selectively and separately harvest multiple crop components (i.e., grain and stems) in a single pass across the field.

A 1999 National Research Council Report on research priorities for biobased industrial products lists development of “equipment and methods to harvest, store, and fractionate biomass for subsequent conversion processes” as a key research priority. Another prominent theme of the report is development of technologies for the effective and economic utilization of lignocellulosic biomass, particularly waste sources such as crop residues.

Not all parts of the crop residue are equally valuable. For cost-efficient utilization of straw and other crop residues, separation processes must remove the undesirable components while simultaneously capturing all valued components. The current paradigm for crop residue utilization involves making multiple harvest passes across the field and transporting all the components of the crop residue to the point of utilization. There is no cost-efficient way to recover desirable and remove undesirable biomass components before transporting the whole crop. This problem represents a burdensome expense not only because of the low bulk density of crop residues, but also because of the economic and environmental liabilities created by bringing the less valuable components to the manufacturer’s gate. The goal of this area of research was to address the “equipment” challenge for separating and harvesting the valuable components of the wheat straw in a single pass (Figure 2). Specifically, we:

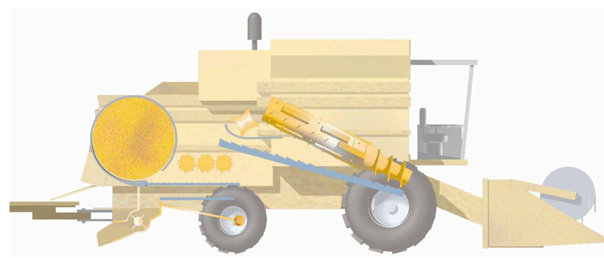


Figure 2. Conceptual illustration of how the harvesting operations for both grain and straw sub-components can be integrated into a single-pass harvester.

- Experimentally and computationally investigated the various unit operations of the grain harvester to gain additional insight into the threshing and separation mechanisms. The purpose of these investigations was to provide the technical foundation and design specifications for employing advanced engineering tools to design a single-pass multi-component harvester.
- Investigated and developed a combination of advanced tools (e.g., sensor systems, control systems, advanced learning/decision making controls) for autonomous intelligent control of multi-component harvesters.

A conceptual depiction of the single-pass multi-component harvester is shown in Figure 2. To develop the engineering understanding necessary to develop this concept, we:

- Created visual images of the flow within the combine using high-speed motion-picture cameras and quantified the flow for visualization and analysis within a fully immersive virtual environment.
- Quantified the anatomical structure of straw and determine how this structure relates to the material properties of the straw. These data were collected to provide the necessary inputs to the stress/strain models to preliminarily assess various straw threshing methods as well as providing the necessary inputs to the computational fluid dynamics (CFD) models.
- Performed experimental analysis and CFD analysis of the air fractionation stream to assess the effectiveness of various approaches.

A high-speed image taken of biomass flow through a working combine is shown in Figure 3. Overlaid on top of the image are the vector velocities of the different components. The vector velocities are used to provide the quantitative data necessary to visualize this flow field in a three-dimensional virtual reality environment. This visualization proves very useful in determining how the various biomass components separate in a working combine and serves as a physical benchmark for refinement and validation of the CFD results.

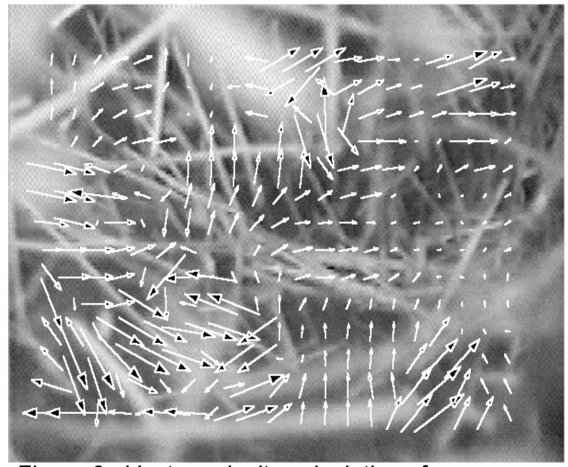


Figure 3. Vector velocity calculations from a series of high-speed images, represented by the arrows, on wheat straw moving through a combine.

We used a wind tunnel available at INL and the Case New Holland (CNH) prototype combine operating in a stationary mode (i.e., feed straw in by hand) to develop this imaging system within a controlled environment. Once this system was developed and validated for the controlled environment, it was used on a working combine (harvesting grain) to obtain quantitative data on biomass movement and separation. This numeric characterization data (vector velocities of biomass particles) was required to develop the subsequent visualization and computation simulations and models, which were expected to provide the fundamental experimental data benchmark for the development of a virtual simulation/engineering tool set. Additionally, this new understanding of the complex multi-component separation process was the foundation for an autonomous intelligent control system for the operation of the grain combine.

As part of other related research, a test was conducted at INL on the feasibility of using mechanical threshing methods to break up the anatomical structure of straw into its components (stems, nodes, and chaff). Some of the results are shown in Figure 4, illustrating the separation of nodes from the stem. Most of the nodes were separated with only a short piece of the stem attached on each side of the node. This breaking point in the vicinity of the node could potentially be exploited to disrupt the nodes and mechanically thresh the nodes from the stems. More detailed analyses were conducted in the laboratory on the structural properties of the straw. A model was built using the experimental data to computationally assess the effectiveness of various mechanical straw threshing methods. The model results can then be displayed in the three-dimensional virtual reality environment.

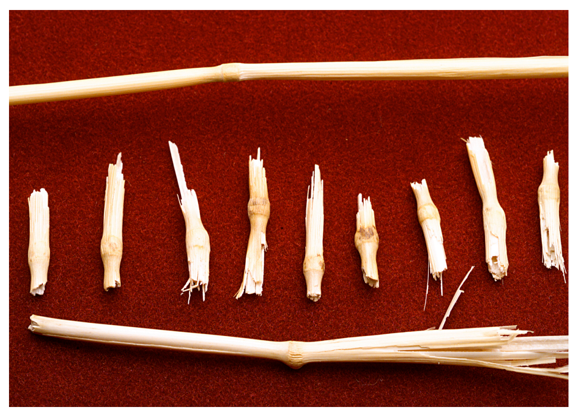


Figure 4. Nodes separated from the stem.

Experimental and CFD analysis were performed to understand and simulate the biomass fractionation in the turbulent air stream. Multiphase flows of this type are characterized by flows with a mixture of air and solids that have unique velocities. Interpretation of the different phases poses significant problems with traditional methods for handling flows with single phases or multiple phases with well-behaved interfaces. When this research was proposed, these challenging interpretation problems had just started being simulated and understood by specifically developed codes for multiphase flows. Controlled experiments were conducted at INL's wind tunnel to obtain the necessary data for development and first-stage validation of the multiphase models (Figure 5). After the CFD model was developed and validated for the controlled wind tunnel, a complete multiphase CFD model of the combine was developed. This CFD model output was then displayed in the three-dimensional virtual reality environment. Side-by-side comparison of the CFD results and the flow imaging results for both the wind tunnel and the working combine provided an excellent mechanism for enhancement and validation of the CFD results. MFIX (Multiphase Flow with Interphase eXchanges) was used to examine small regions of the flow and to develop the key relationships for the multiphase phase flow. These relationships were then implemented in large-scale codes to examine the interaction between the complex internal geometries of the combine and the multiphase particle flow.

Figure 5. The INL wind tunnel can accommodate air columns and full-size combine separation units (e.g., sieves) and provide analytical quality airflow for studies.

The two models, coupled with the experimental visualization data (derived from high-speed imaging system) in a virtual reality environment, provided an interactive engineering design environment that was used to identify and develop various integrated mechanical threshing/air fractionation system concepts. We used this virtual engineering environment to assess the benefits and limitations of various alternatives and identified the most promising candidates for further evaluation.

Virtual Engineering

Virtual reality, as understood in this project, includes all of the information technology and model development necessary to generate physically accurate simulations (Figure 6). The goal is to allow the user to experience the reality of the physical system by examining the computer-generated system (scene) in the same way as the physical system, with the additional capability to make changes and rapidly see the impact of the changes ("what if" scenarios).

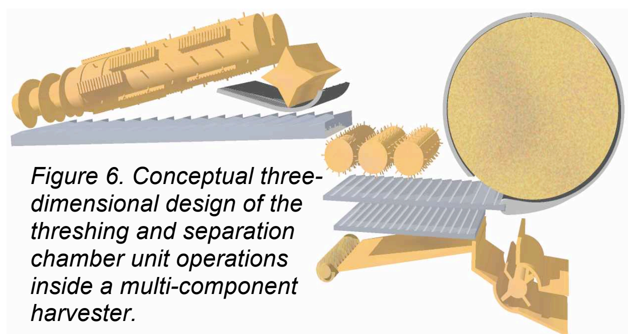


Figure 6. Conceptual three-dimensional design of the threshing and separation chamber unit operations inside a multi-component harvester.

Incorporating high-fidelity computational models in a real-time physics-driven virtual environment is a significant challenge. It requires the incorporation of high-performance visualization and high-performance computing and the development of new computational algorithms that can dramatically reduce the compute time for new solutions. Iowa State University's (ISU) Virtual Reality Applications Center (VRAC) is at the forefront of developing these integrated design and analysis tools.

This use of interactive immersive design tools, combined with computational and visualization modeling, represents a significant advancement in how investigation and design is performed. In a virtual model of the system, engineers are able to use their innate pattern recognition and intuitive skills to bring to light key relationships that may have previously been unrecognized. With these tools, significant breakthroughs can be made in our understanding of the complex three-dimensional relationships within the fluidized bed of a grain harvester.

Autonomous Intelligent Control of a Combine

Today's combine harvesters are already complex machines; operating and maintaining them at optimum performance under a wide variety of conditions is a challenge. Adding yet another process to the threshing and separating of crop residue components increases the complexity of the machine operation to the point where it can no longer be run under the operator's total control. To keep multiple synchronized systems running optimally, autonomous intelligent control of those systems is essential.

We built on previous INL research, expanding the development of an autonomous intelligent control system to address multi-component harvesting. This required both additional intelligent control system sensors to discretely identify the different biomass components and an understanding of the different effects of changing operating conditions on the different biomass components. Any change in operating conditions made by the intelligent system must take into consideration the change's effects on the different biomass components.

Currently manufactured combines have no sensors to identify the biomass components to be harvested. INL previously participated in research to develop an autonomous intelligent combine control system to minimize grain loss. From that research, fluorescence spectroscopy sensor technology appeared to be practical not only for recognizing the grain, but likely also for distinguishing various biomass components of the crop (Figure 7).

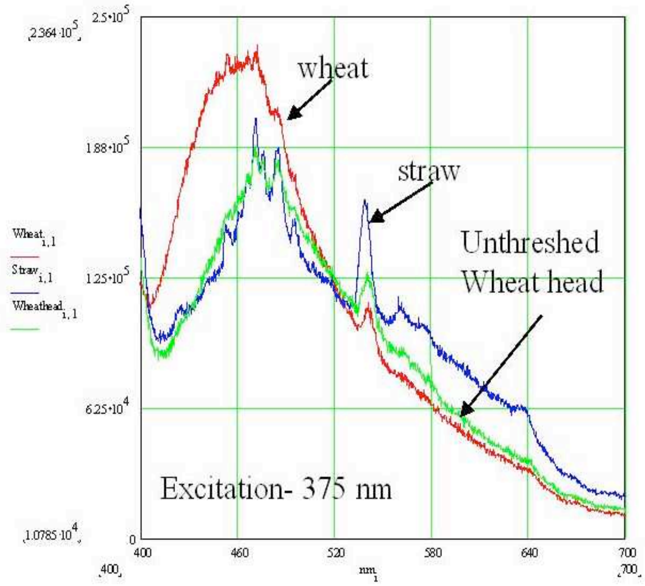


Figure 7. Differences in the fluorescent spectra of wheat and straw.

This project expanded that research to include development of a better intelligent control system sensor that can identify and measure the biomass components of interest in the multi-component harvest. We also integrated that sensor information into the control system, so that it can make intelligent control decisions (ground speed, fan speed, cylinder speed, concave/cylinder gap size, sieve adjustment, etc.) while simultaneously considering all the biomass components being harvested.

The intent of this research was to create a computer-based, software-driven intelligent control system that could make operational changes to the grain combine based on information from intelligent control system sensors, such that the combine minimizes grain loss and at the same time maximizes harvest of multiple desirable biomass components. And it needed to do it autonomously, without operator intervention.

Results

Experimental Data and Visualization of Biomass Flow through a Combine

Early in the research, INL engineers designed and installed sensors in a combine to measure airflow parameters through the machine. During the fall 2001 harvest season, INL tested a video imaging system using synchronized strobes in the CNH prototype combine. The camera and lighting system used in these initial tests demonstrated that particles could be tracked in the fluidized bed of the combine. However, specific problems associated with camera resolution, lighting, and accurate X-Y-Z positioning were identified.

To overcome the video imaging system's problems, the positioning system was redesigned in the first quarter of 2002 to allow accurate and repeatable placement of the camera and lighting systems within the combine separation chamber. The positioning system was planned for general-purpose installation in several makes and models of combine harvesters. The X-Y-Z positioning system was designed so that the camera could be positioned and repositioned accurately to within one centimeter. In addition, automation was incorporated into the positioning system to reduce harvesting downtime between imaging tests. The design of the system allowed for complete automation of the system across the entire space of the separation chamber, but to conserve costs, only the Z-axis was automated at that time.

The redesigned positioning system was capable of positioning the imaging system at any point across the cleaning shoe and was computer controlled, such that the imaging system could be positioned and repositioned remotely. The data collecting for the positioning, imaging, and combine operation system were all connected and cross-referenced such that for any given image the position and operating conditions could be determined.

INL also made airflow measurements (velocity and turbulence) on the prototype combine cleaning shoe. This involved collecting data at sensors throughout the combine separation chamber while the machine was operated over the range of adjustable airflows. The three-dimensional positions of the airflow sensors were recorded with respect to the geometric structure of the cleaning shoe. The experimental airflow measurement data were used to construct a two-dimensional model of the airflow through the cleaning shoe. The results of the model were used to direct more intensive imaging data collection and characterization of the cleaning shoe.

The robotic positioning system was set up prior to the fall 2002 harvest season to move the imaging system from side to side in the combine separation chamber via remote control from the combine's cab. The installed positioning and imaging system, which constituted the Particle Image Velocimetry (PIV) system, was operated in a combine throughout the harvest season. The lighting, camera, and positioning system proved sufficiently durable to withstand the harsh environment of the combine, and could capture images that could be analyzed to determine the vector velocities of material moving through the combine separation chamber.

The flow visualization images were analyzed with PIV software, and the results were sent to ISU for incorporation into the virtual models. At lower biomass flow rates, the images clearly captured unique flow phenomena that positively or negatively affected biomass component separation. While the new lighting and camera system dramatically improved imaging results in the combine under actual harvest conditions, image quality at full biomass loads continued to be a challenge. To overcome these challenges, CNH engineers provided a number of design concepts to modify biomass flow paths in such a way as to represent actual flows while not interfering with the camera's field of view. New hardware was purchased that allowed these new designs to be developed.

To allow for the comparison of experimental data to computational modeling results, the two sets of data must be relative to the same coordinate system. Therefore, the PIV data was transformed to the combine domain by changing the reference frame of the position vectors from the relative camera acquisition frame to the absolute combine geometry frame. This effort resulted in spatially accurate vector velocity data that could be compared to the output of numerical models of the combine concurrently under development at INL.

The PIV imaging techniques were later greatly improved due to hardware and software modifications and as a result of professional training. Improvements were made to the light sheet optics and camera, and methods were employed to control dust accumulation on the camera lens. The revamped system produced sharper and clearer images in the desired data plane. In addition, more advanced statistical analysis features were enabled in our software and our understanding of image timing techniques was improved due to on-site training from the equipment manufacturer. The effects of these improvements were evaluated during a series of stationary tests where baled wheat and barley were fed by conveyer belt into a parked combine and threshed and separated under static conditions. For purposes of evaluating the results from the stationary tests, comparative data were collected during the wheat harvest in the field. The images collected for both the static and dynamic test conditions after these improvements were of significantly higher quality.

A simple flow visualization system consisting of an artificial seed generator, an argon ion laser, and a high-speed camera was designed for the combine separation chamber to help in describing the difficult flow patterns that exist in the highly rotational vortex flows produced in a combine. The random nature, fine details, and time-dependent behavior of the fluid flow in the combine necessitated the use of qualitative flow visualization techniques in conjunction with common quantitative techniques such as PIV to provide a complete analysis of the flow field.

Virtual Engineering & Computational Modeling of Biomass Flow through a Combine

Virtual engineering systems enable all stakeholders in the multi-component harvester to understand and utilize the results of a design simulation. There are three simultaneous steps in developing such a system:

1. The development of CFD models of the grain harvester
2. The development of submodels for two-phase flow of grain
3. The development of the virtual engineering platform

This section will first discuss the progress toward the development of the virtual engineering platform.

In the first stages of developing an initial virtual combine model, CNH provided CAD files to ISU and INL for the original, non-modified combine. From these files, critical combine dimensions were determined for modeling the flow and for display purposes. The geometry of the cleaning shoe and crop flow area were converted to a format for display in the virtual reality software package then being used at ISU.

ISU soon expanded its virtual reality software package to read output files for CFX, MFIX, and Fluent 6. Two-dimensional MFIX output and two-dimensional and three-dimensional Fluent output were visualized in virtual reality. Work was initiated to read and display multiple vector and scalar data.

As ISU and INL continued to work jointly on developing a virtual model of the separation process within a grain harvester, a reader for three-dimensional MFIX data was completed. ISU's virtual reality software package was again expanded to handle multiple vector and scalar data. The package was used to visualize two-dimensional transient MFIX data on meshes containing 200,000 nodes for 200 timesteps. ISU began planning to extend the virtual engineering software to optimize and design components utilizing high-fidelity models.

In 2002, ISU began developing VE-Suite, an open source virtual engineering software toolkit that simplifies information management so users can simultaneously interact with engineering analyses and graphical models to create a virtual decision-making environment. It was developed to provide a mechanism for providing research results that industry can use directly to develop bio-based processes.

Version 1.0 was released on June 30, 2006, the first publicly available general purpose virtual engineering software package. Public release of the open source code was a critical step in creating and maintaining a software package and framework that can be sustained over a longer period of time. This will provide a needed ongoing bridge that links research results to applied projects. Version 1.0.2 was released on September 31, 2006 with corrections to several small bugs. A portion of the VE-Suite work won an R&D 100 award in October 2006.

VE-Suite can read, display, and couple together multiple data sets including experimental data, CAD data, and CFD/finite element analysis (FEA) results in both two and three dimensions. In its current form, VE-Suite consists of four main software engines:

- VE-CE is the software engine responsible for the synchronization of the data between the various analysis and process models and the engineer.
- VE-Xplorer is the decision-making environment that allows the engineer to visually interact with the equipment models.
- VE-Conductor, the graphical user interface, is the engineer's mechanism to control models and other information.
- VE-Open connects the core engines of VE-Suite and transfers data from user-defined information sources to VE-Suite software engines.

In addition, VE-Builder was developed as a set of tools for modifying three-dimensional data files for VE-Suite. It encompasses all the functionality needed to build the virtual engineering environment. VE-Suite can be used on Windows and Linux platforms from desktop computers to computer clusters to multi-wall virtual reality environments. ISU established an externally accessible CVS repository of the VE-Suite code, enabling INL and others to participate in the development of VE-Suite. The software is available, along with its documentation, at www.vesuite.org. The VE-Suite work completed for this project was presented at the VE2007 workshop held at ISU in May 2007. This was the first virtual engineering workshop/conference to be held.

Tools were also developed to be included in the VE-Suite package that enable visualizing and interacting with the engineering optimization problem as the design develops. These tools enable human-in-the-loop equipment design. From the interface of VE-Conductor, design parameters can be changed and returned to a computing resource for incorporation into the next analysis iteration. Communication between VE-Conductor and the other VE-Suite components was also improved so that multiple datasets can be loaded and quantitatively compared within the virtual environment.

The combine geometry was visualized in the virtual environment as well as the two-dimensional computational and experimental results. An interface was built to visualize the MFIX results (transient three-dimensional two-phase flow) expected from the computational modeling. Additionally, ISU developed a new technique to develop approximate analytical solutions of linear and nonlinear differential equations, and then extended this technique to two- and three-dimensional coupled nonlinear equation sets. This technique enables rapid analysis for engineering design and optimization.

Utilizing the PIV patches provided by INL, ISU developed an inverse engineering tool to enable complete flow fields to be developed from the PIV information. This enabled rapid review of the computational and experimental results as well as extending the information that could be obtained from the PIV patches. Efforts focused on developing a methodology on a simpler geometry that could be extended to the combine. We examined the use of quantitative trait loci (QTLs) with evolutionary algorithms as a means to speed up the inverse solution process. This is a concept borrowed from bioinformatics that has not previously been applied to engineering optimization. A QTL is a position on the genetic map of a chromosome that is associated with a quantifiable trait like weight gain, height, oil content, etc. This concept has been applied and demonstrated by ISU for an inverse radiation heat transfer problem. ISU performed a sensitivity analysis for a one-dimensional conduction problem to better understand how sensitivity analysis can be applied to inverse convection and extend the method to non-linear problems. The preliminary results, when compared to a standard evolutionary algorithm, have shown a significant speed up in time to solution.

INL sent a transient two-dimensional Fluent simulation of airflow through a combine separation chamber with oscillating sieve trays to ISU. The ISU team extracted data of interest and displayed the combine geometry and the animated cyclic data sequence in virtual reality.

A virtual environment was installed at INL, enabling its staff to run ISU's VE-Suite software to visualize CFD simulations and overlay collected PIV data in a three-dimensional interactive environment. This environment allowed ISU researchers to collaborate with INL researchers and CNH on the design and analysis of combine air flow. The model displayed a grain combine in virtual space along with computational and analytical air stream flow data. This virtual engineering tool provided the coupling of computational and analytical data, and with the interactive graphics, this data was viewable in the virtual combine environment, enabling it to be interpreted in the context of the physical bounds of the separations system.

CFD and experimental PIV data sets were inserted into VE-Suite. The integration of the CFD data set into VE-Suite was easily accomplished, but integration of the PIV data required considerably more work because the overall data set consisted of 39 discrete subsets of data. In the original implementation, each PIV data set had to be independently defined in a parameter file. This was cumbersome and functionally inadequate as it created regions of overlap between adjacent data sets. To partially solve this problem, a merge utility was developed to merge multiple data sets into a single data set. By averaging the data in the overlap regions with commercial software, the redundant data in the overlap regions were eliminated.

The development of computational models describing biomass flow in a harvester will now be discussed. Again, these models help determine what design requirements are needed to efficiently separate the biomass material during the harvesting process. Currently available separation options with combine harvesters are not able to achieve sufficient separation of the straw/stover and chaff streams to realize the full potential of selective harvest. Studies conducted at the National Renewable Energy Laboratory (NREL) (corn stover survey studies) and INL (cereal straw fractionation and storage studies) demonstrated that glucan and xylan sugar content variability can be greater than 10% for stover and cereal straw anatomical fractions. Lignin content variability between anatomical fractions can approach 6%. The NREL studies showed that total structural sugar content can vary between 45-88% as a function of variety and environment. Of further interest, the glucan and lignin content was least sensitive to these genetic and environmental factors; as such, the compositional variability of these constituents between anatomical fractions appears to be significant and have some degree of stable predictability that could be exploited to improve feedstock structural carbohydrate content. Since ethanol yield is a function of feedstock structural carbohydrate content, biomass anatomical fractions of higher product yield can have a significant beneficial impact on minimum ethanol selling price. However, tests conducted by INL showed that by using baffles and other machine adjustment measures, the best achievable anatomical fractionation of cereal straw stems was between 70-80% straw stem purity. This separation process also resulted in significant losses of the desirable stem material, and represented only a 2.2% increase in glucan content. However, based on analytical analysis of the discreet cereal straw anatomical fractions, a 6% glucan content increase is potentially achievable if a high-fidelity separation in excess of 80% stem purity can be achieved. Additionally, improvements in separations are needed to prevent yield losses of desirable biomass components resulting from simply losing the material during the harvest/collection. The purpose of these advanced biomass separation computation engineering models is to more effectively and efficiently engineer high-fidelity and high-throughput separation systems for biomass components.

Early in the project, INL and ISU developed a computational modeling strategy for simulating multi-phase flow with an integrated solver using various CFD codes. INL and ISU then began simulating the multi-phase flow with an integrated solver using various CFD codes: MFIX, CFX, and Fluent 6. ISU set up a classical multi-phase test problem to be solved by the various CFD codes. The benchmark case was based on experimental data for bubble gas holdup and bed expansion for a gas/solid fluidized bed. Preliminary fluidization experiments identified some unexpected fluidization behavior, where rather than the bed uniformly fluidizing, a “blow out” would occur where a hole would open up in the bed through which the air would preferentially flow, resulting in erratic fluidization.

ISU and INL also worked in parallel to develop detailed computational models of the grain separation process. These models were ultimately placed in a virtual engineering system that enables all stakeholders in the multi-component harvester to understand and utilize the results. INL developed a two-dimensional model of single-phase airflow through the harvester using Fluent 6. The analysis of simple single-phase models provided insight and a foundation for more complex multi-phase modeling studies. Meanwhile, ISU developed a CFD submodule to compute grain separation and used MFIX to solve two- and three-dimensional bench test cases.

INL constructed a multi-phase two-dimensional model of biomass and air flow through the cleaning shoe utilizing combine geometric structure data, airflow measurement data (velocity and turbulence), and biomass flow information (grain and MOG [material other than grain] density data). The model was enhanced to include the grain and MOG partitioning and their respective flow paths through the combine, and to include advanced numerical multi-component and moving mesh techniques. This modeling data was very useful in identifying positions and phenomena within the combine separation chamber to photograph with the imaging system. ISU determined physical parameters and interaction force models to allow the CFD submodule to describe solid flows in grain separation. This effort was also useful in identifying dynamic fluid flow characteristics and optimizing the computational resources needed to support the modeling effort.

INL also expanded their two-dimensional model of the combine to include advanced numerical multi-component and moving mesh techniques. The model predicted the flow of biomass and air through the cleaning shoe and was validated against INL’s flow visualization data. This effort was useful in identifying dynamic fluid flow characteristics and optimizing the computational resources needed to support the modeling effort.

ISU used MFIX to develop a simplified two-dimensional fluidized bed model for grain separation. As anticipated, because current multiphase models are not directly applicable to grain separation, this initial model was not successful in modeling dense phase grain separation. Rather, this model provided a starting point for developing a successful computational model that could be imported into a commercial software package. Issues identified in this model include grid resolution due to particle size and determination of an equivalent average grain diameter. Numerical simulations for gas-grain two-phase flow in a simplified two-dimensional fluidized bed configuration were performed to determine the fluidization state of the grain phase. A two-dimensional computational model of gas-grain-chaff flow using the multiparticle phases model was set up. This model successfully simulated the separation of grain from chaff in a fluidized bed configuration. This was a significant step forward in developing a three-dimensional model of grain and chaff separation.

Numerical simulations were completed for the fluidization of grain particles. The results indicate the appearance of large bubbles in the centerline of the fluidized bed (known as spouting), which is the typical characteristic of Geldart Group D particles, e.g., grain particles. In addition, the Massori radial distribution function for the particle-particle interaction model was examined; however, the model was incompatible with MFIX. Figure 8 shows the instantaneous velocity fields for (a) gas, (b) grain, and (c) chaff. It is evident that the grain tends to accumulate toward the bottom of the bed whereas the chaff moves upward toward the domain exit.

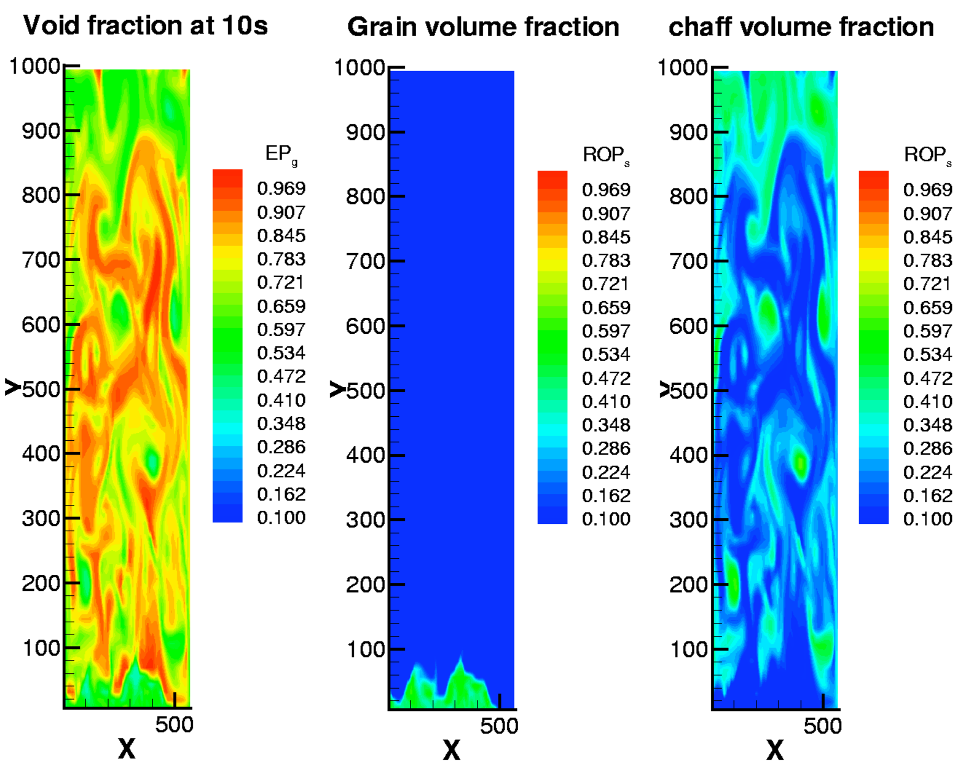


Figure 8. Instantaneous v at 10 s for (a) gas, (b) grain, and (c) chaff.

More numerical simulations of the fluidization process in a grain cleaning shoe were then conducted using a modified configuration. The new geometry more accurately modeled the cleaning shoe above the upper sieve. The effects of inlet air velocity and solid feeding velocity on the separation process were explored. Preliminary results suggested a correlation between the velocities and the separation extent. The correlation was explored to determine if it could be used to predict appropriate fan speeds to achieve the best separation. Periodic boundary conditions for the new fluidized bed configuration were studied and compared with wall (no-slip) boundary conditions. Both boundary conditions produced similar overall flow behaviors. The MFIX code was modified to accommodate different coefficients of restitution for different solid phases to account for the different collision properties of grain and chaff.

ISU also developed a two-dimensional model for predicting dilute multi-component flow phenomena and incorporated a moving mesh to conform to the physical separation process. A simplified fluidized bed model for gas-grain two-phase flow was set up and computations for gas-grain-chaff flow were extended, which led us to determine that standard multiphase flow models for fluidized beds are insufficient for this research.

The effects of restitution coefficient on the hydrodynamics of fluidized beds were investigated. The results demonstrated that bed hydrodynamics strongly depend on the amount of energy dissipated in non-ideal particle-particle collisions. Particle rotation effects on bed hydrodynamics were also investigated. The multi-fluid Eulerian model in MFIX was improved by incorporating particle rotation using a simple kinetic theory for rapid granular flow of slightly frictional spheres. Preliminary results indicated that particle rotation improved the fundamental hydrodynamics, thus providing more realistic fluidized bed dynamics and predicting the correct segregation trends. A new model for the momentum transfer between solid phases was derived based on a three-parameter inelastic, frictional collision model using kinetic theory. Parametric studies of the new momentum transfer model showed that the overall trends were dependent on the particle collision properties. However, the predictions based on the new momentum transfer model and the original MFIX model were within 1%, indicating that the new model was not advantageous. Additional theory for the radial distribution function was reviewed for predicting momentum transfer.

Results of the test of the discrete element method (DEM) model capability in MFIX showed that DEM simulations can provide valuable detailed information of individual particle motion and particle-particle collisions. However, the test for computational expense estimated that a DEM simulation for an industrial scale problem would be prohibitively expensive due to serial processing. Therefore, the use of DEM was constrained to performing small scale simulations and providing data to validate continuum models. A systematic scheme of DEM experiments was also designed to validate fundamental multiphase flow continuum models in MFIX and their capability to predict segregation.

Segregation of a single large particle from smaller particles (Brazil nut effect) was successfully simulated using DEM techniques. The segregation dynamics from the experiment were captured qualitatively with proper physical parameters corresponding to those used in the experiment published by Nahmad-Molinari et al. (2003). Quantitative agreement was achieved under certain simulation conditions. The segregation mechanism can be further enlightened by the simulation results, which revealed both microscopic and macroscopic information that were not available from experiments (e.g., force network and stresses among granules). Exploring DEM also provided useful guidelines for simulation techniques of segregation for future work.

Detailed analyses of the microstructures in granular media that characterize the force network were analyzed based on the Brazil nut effect. The data was used to characterize the spatial structure of the granular media and to quantify the force chain network using probability density functions. This was pioneering work toward a quantitative understanding of the function of force chains in dense granular media; most research has qualitatively shown force chains, also referred to as clusters. In addition, the microstructure analysis was extended to DEM simulations of gas fluidized beds using MFIX. Two-dimensional MFIX-DEM (discrete element model) cases were successfully run and the results from those simulations were analyzed and compared to the results from the continuum two-fluid model of MFIX without DEM. The comparison showed that MFIX-DEM simulations and two-fluid (MFIX only) simulations predict similar general fluidization behavior. However, MFIX-DEM simulations showed that the particle distributions in the bed are more heterogeneous and suggested that the models may require improvements.

MFIX-DEM functionality was later improved in terms of computational efficiency and numerical accuracy. Specifically, the following tasks were completed:

- A pressure correction numerical method for the Semi-Implicit Method for Pressure Linked Equations (SIMPLE) algorithm was developed and implemented in MFIX. This numerical method improved the coupling between the particle phase and the gas phase decreased the computational time by about four folds.
- A method of calculating drag force based on individual particle velocity was implemented into MFIX. This method differentiated the drag force between particles in the same computational cell while the original method assigned the same drag force. This method gave a more physically realistic description of the drag forces on particles.
- A method of estimating particle mean field using Monaghan's kernel function was implemented into MFIX. A fixed bandwidth, which is wider than the Eulerian grid spacing for the gas phase, was used for the kernel function. Therefore, the method eliminated the increase of statistical error with refining the Eulerian grid. However, the method predicted lower than expected particle mean values at wall boundaries due to the symmetry of the kernel function and the wide bandwidth.

Autonomous Intelligent Control of a Combine

During the fall 2001 harvest, initial data acquisition was completed for the first generation intelligent control decision system for the autonomous intelligent control system. After being verified and validated, these data were converted to engineering units. The data mining using artificial intelligence was completed on the whole dataset, which included all parameters, so as not to introduce any biases into the parameter subset that was used for intelligent control. In other words, we let the intelligent system tell us what parameters to use rather than using those we thought were important. The subset identified by the mining technique substantiated our inherent knowledge that ground speed, rotor speed, fan speed, etc. were the most important. We then re-mined the dataset of just those parameters and generated the initial decision-making criteria.

The initial facts (specifically for soft white wheat) for the autonomous intelligent control system were generated in early 2002. A flat-panel touchscreen computer was ordered for installation on the CNH prototype combine to be the autonomous intelligent control system. The initial control system screens were designed, and the controller-area network (CAN) setpoint system command protocol was received from CNH. The setpoint system allowed the intelligent control to command changes to a prescribed value instead of just being able to command a change as an increase or decrease. Farmer agreements were established with two farms in south-central Idaho to permit testing of the autonomous intelligent control system during the 2002 harvest.

The control system was then integrated onto a flat-panel display computer, which was installed into the cab of the prototype combine and integrated into the combine CAN bus. The previously developed control simulation was converted to provide direct control of the combine, and the system was bench tested in preparation for the harvest season. Design changes implemented later in the project included software revision and improved hardware. The systems were installed on two prototype combines and tested on both small grains and corn crops.

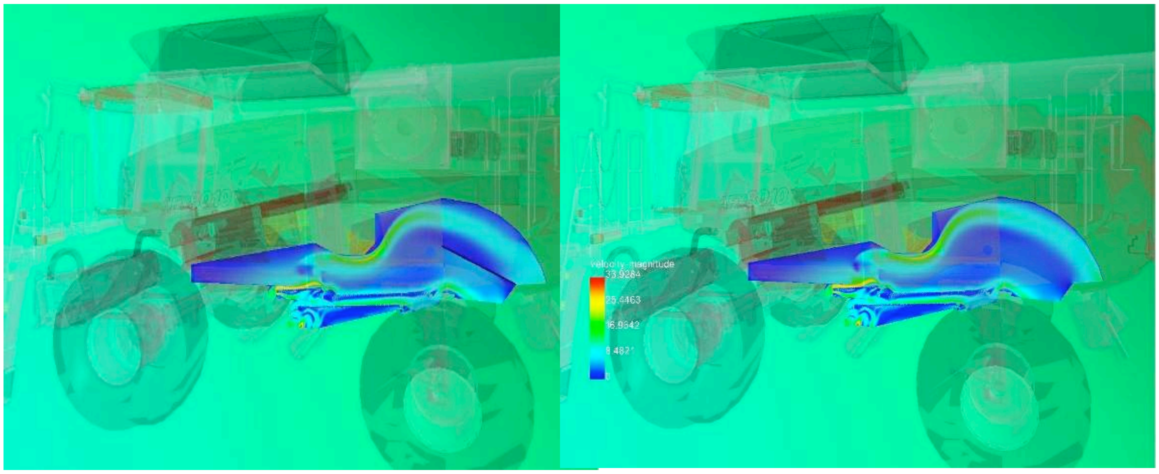
Field research was conducted in southern Idaho in August 2002 for three weeks at Grand 4-D Farms, one week on Martin Farms west of Idaho Falls, and one week on Hess Farms in Ashton, Idaho. The intelligent control system was able to make operating condition decisions and autonomously change the operation of the combine. Several different crop datasets were collected for later analysis.

The field data collected during the harvest season was then analyzed. Data was logged using both the INL data acquisition system and the CNH onboard data logging unit (DLU). The INL data was analyzed using both statistical procedures and the Arc/Info geographic information system (GIS). The DLU data was mapped using the CNH New Holland Application and the Management System Proprietary software. Problems were encountered during the harvest with the DLU; it was replaced with a new unit, but some problems continued through the harvest. As a result, the DLU data had only minimal value.

The Autonomous Control System (ACS) methodology flowcharts developed by INL and CNH were implemented into code, followed by extensive field testing and further development of the ACS. Testing occurred during the small grain harvest season in southern and eastern Idaho and revealed additional improvements in certain functions along with additional functionality to be added to the system. ACS prototypes were installed on two CNH field-test combines (one New Holland CR and one Case-IH AFX machine). The ACS user interface was completely redesigned to allow a combine operator to set up, monitor, and diagnose the ACS system. In addition, data-logging functionality was build into the ACS to allow data to be downloaded from the field and sent to INL for advanced analysis and troubleshooting. Operators successfully utilized the redesigned ACS user interface for combine setup, monitoring, and diagnoses of the ACS system. In addition, expanded data-logging functionality was successfully tested.

Virtual Engineering of Feedstock Assembly System Concepts

The virtual engineering techniques developed in this project and described above were tested and utilized to design a separation baffle in a CNH combine. Selective harvest performance targets were established, based on compositional analysis of anatomical fractions, that represented the best residue fractionation current combines are capable of achieving. A computational engineering approach involving modeling, analysis, and simulation was used in the form of virtual engineering to design a baffle separator capable of accomplishing the high-fidelity residue separation established by the performance targets. Through the use of the virtual engineering model, baffle designs were simulated to (1) determine the effect of the baffle on the airflow of the combine cleaning system, and (2) predict the effectiveness of the baffle in separating the residue streams. The virtual engineering simulation of the baffle design that was selected for testing is shown in Figure 9. A side-by-side comparison with the simulation that represents normal combine operation shows the baffle to have little effect on the flow field behavior. Although a partial baffle still allows some open space between the two



(a) (b)

Figure 9. Virtual engineering model simulation of the final baffle design (a) compared to the flow filed for normal combine operation (b).

residue streams in which mixing could occur, it satisfies the two main design requirements in that it eliminates the potential for mixing at the discharge and it does not impede the predominant air streams that are significant to combine performance.

Accordingly, this baffle design was selected, built into the INL selective harvest test combine, and tests performed to evaluate the separation fidelity and the resulting effect on composition and theoretical ethanol yield.

Field tests were run both with and without the baffle separator installed in the INL research combine, and the chaff and straw streams were collected in the wagons during harvest of Challis soft white spring wheat. The separation fidelity accomplished both with and without the baffle separator was quantified by laboratory screening analysis. The objective of screening was not to determine the particle size distribution but to determine the anatomical composition of the collected residue streams. Therefore, although the

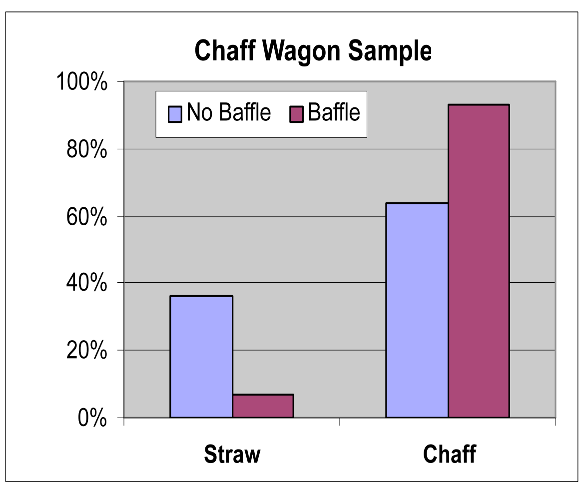


Figure 10. A before and after comparison of the separation fidelity of normal combine fractionation (no baffle) and the engineered selective harvest fractionation (baffle).

screen shaker separates the samples into six different fractions based on particle size, the fractions were consolidated into two fractions based on anatomical composition. The screening results showed that the engineered baffle separator did a remarkable job of effecting high-fidelity separation of the straw and chaff residue streams, improving the chaff stream purity from 64% to 93% (Figure 10) and increasing the straw stream yield by the corresponding amount.

The straw wagon and chaff wagon samples were also analyzed by wet chemistry to determine composition effects. The compositional analysis showed that as a result of the high-fidelity residue separations accomplished with the baffle separator, the corresponding compositional splits among the straw and chaff fractions were improved, significantly affecting the quality of the residue streams as quantified by the theoretical ethanol yield (see Figure 11). For the case of normal combine fractionation (i.e., no baffle separator), the straw fraction had the highest theoretical yield at 112.4 gallons of ethanol per dry ton of feedstock, with the chaff yielding only one gallon per ton less.

Because both residue streams are nearly identical with respect to theoretical ethanol yield, this case suggests that there is no value in selective harvest at this level of separation fidelity. In contrast, for the case using the baffle separator, the theoretical ethanol yield of the straw fraction was 115.6 gallons per dry ton, but the difference between the straw and chaff streams was considerable at 11 gallons per dry ton. This substantial difference in ethanol yield shows that high-fidelity selective harvest can have a significant effect on the quality of the two residue streams. Although the ethanol yield of whole wheat stover was not available for comparison because the composition of whole stover was not measured, ethanol yield of the whole stover must lie between the two fractions. Because the non-baffled straw and chaff yields were so close to each other, we assumed that the whole stover ethanol yield was halfway between at 112 gallons of ethanol per dry ton. This is illustrated by the dashed line in the chart. Based on this assumption, selective harvest increases the ethanol yield by 0.5% without the baffle separator and 3.3% with the baffle separator. The whole stover analysis was

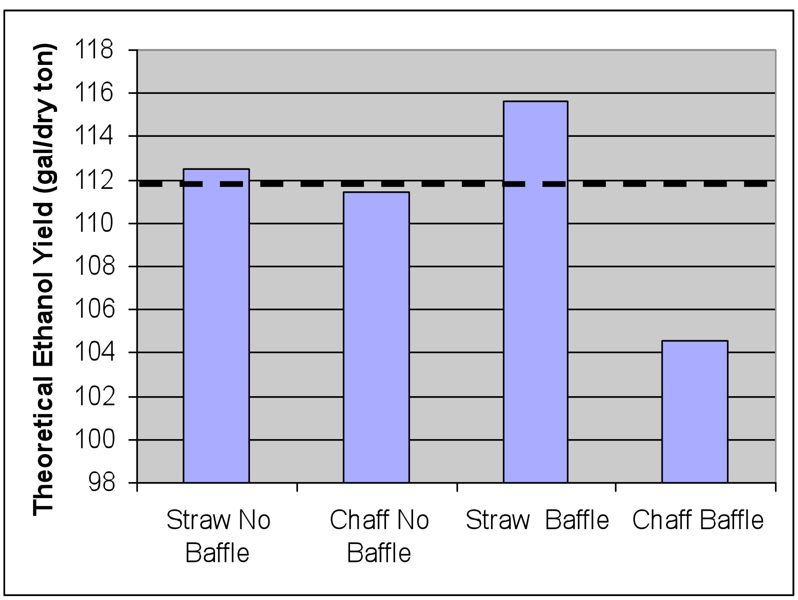


Figure 11. Comparison of the theoretical ethanol yield for each of the residue fractions. The dashed line represents an estimate of the ethanol yield of the whole wheat stover.

conducted to establish a firm comparison. Composition data obtained from this study was also submitted to NREL for input to the ASPEN model to determine minimum ethanol selling price. The results of this model are not yet available, but this will be a definitive metric for quantifying the value of selective harvest.

The other significant aspect of the high-fidelity separation obtained through this study is the resulting effect on pretreatment. Because different fractions respond differently to pretreatment, high-fidelity residue fractionation could lead to significant cost savings. While the composition and theoretical ethanol yield data suggest that the straw fraction is more valuable than chaff for ethanol production because of its higher sugar content, recent pretreatment severity studies have shown that chaff responds much better to pretreatment than straw. Consequently, even though the chaff fraction contains less digestible sugars, the cost of converting the available sugars may be so much less that the chaff stream may actually be the more valuable of the two residue streams. While this is only speculation at this point, there definitely appear to be competing factors that will ultimately define feedstock quality. This project provided strong evidence for the value of selective harvest, but whether this technology will be of commercial significance requires additional techno-economic analysis of the downstream effects on collection, transportation, preprocessing, storage, pretreatment and conversion to determine. Additional analysis should also include evaluating the value for selective harvest of other feedstocks (e.g., corn stover). Furthermore, although this study successfully demonstrated high-fidelity selective harvest, the harvester efficiency was dramatically reduced by the limitations of the residue handling systems. Therefore, selective harvest machinery efficiencies must also be improved before this technology can become commercially viable (we have already identified potential improvements in residue that may significantly improve harvester efficiency). Finally, this study demonstrated selective harvest using a rotary combine (the other main type of combine is a conventional combine), and because the residue fractionation may be significantly different for a conventional machine, some level of analysis to evaluate machine configuration effects should be performed before the value selective harvest can be broadly categorized.

Carbon Flow in Live Wheat Stock

In contemporary food, feed, and fiber systems, the processing infrastructure is mature; efforts to develop new crops or varieties of crops are constrained. For example, straw can be used quite successfully for the production of certain grades of paper, but the current paper-milling infrastructure cannot accommodate straw as a pulping feedstock because the infrastructure was developed around wood. However, the growing bioenergy industry provides a unique opportunity for advances in plant science and production to impact and even alter processing systems because this infrastructure is just being built. Improvement of crop residue biomass and development of machines expressly designed to harvest the new

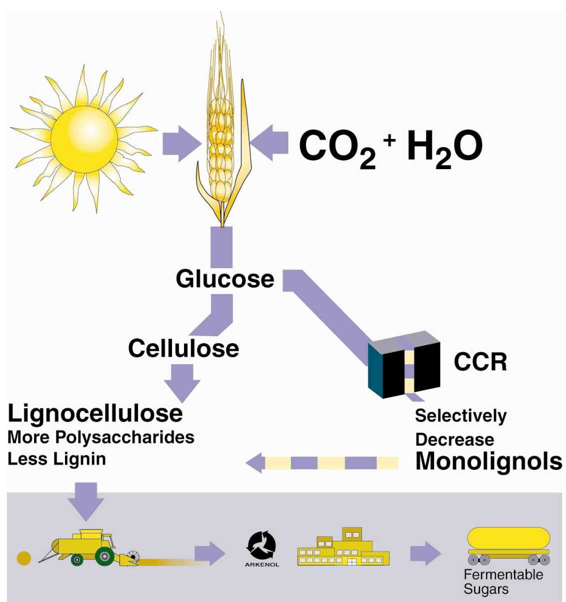


Figure 12. With control of lignin biosynthesis, more carbon can be diverted to higher-value polysaccharides.

multi-component crops may lead to more efficient and less costly processing system designs.

As machines are developed to harvest and separate specific biomass components, it becomes essential to develop a detailed understanding of the molecular and biochemical interactions and processes involved in the synthesis of biomass components in the live wheat plant. The structural/vascular tissues of crop residue stems are rich in cellulosic polysaccharides that can be converted by the Arkenol acid hydrolysis and other processes into five- and six-carbon fermentable sugars. However, these tissues are also heavily impregnated (22% to 28%) with lignin, an important strengthening component of cell walls. Unfortunately, lignin severely limits exploitation of agricultural residues for bioenergy and bioproducts. In the production of commodity sugars for fuels and chemicals, lignin presents serious obstacles:

- It limits accessibility to the cellulosic polysaccharides during processing, thereby increasing the cost of processing.
- It creates a large co-product/waste stream in fermentable sugar production.
- In the live plant, it competes with carbon flow to the more useful cellulosic polysaccharides.

The objective of this part of the project was to prove that wheat can be genetically engineered to modify and reduce lignin content in the wheat straw (Figure 12). The knowledge base of the regulation of lignin synthesis in grasses including cereals and our understanding of it are limited. Therefore, to better utilize cereal crop residues as renewable resources, it is important to establish the necessary background knowledge. The first major task—exploiting lignin regulation—involves better understanding of tissue-specific and developmental regulation of lignin biosynthesis genes by genetically engineering wheat plants to express reporter genes in the same plant tissues and during the same growth stages as lignin biosynthesis genes. Our understanding and ability to manipulate the expression of reporter genes will be exploited to down-regulate a gene for a key lignin biosynthesis enzyme.

Even though grasses constitute a large fraction of biomass in terms of carbon fixed annually, most of what we know about the regulation of lignin synthesis comes from research on trees and on herbaceous plants such as tobacco and alfalfa (Tyagi et al. 1999, Baucher et al. 1998). The limited scientific knowledge of lignin synthesis and regulation in grasses represents a large gap that needs to be filled. A better understanding of lignin biosynthesis in cereals will allow us better manipulate and exploit them as a renewable energy source.

Many genes of the generalized lignin synthetic pathway have been isolated in a number of plants species (Baucher et al. 1998, Boudet and Grima-Pettenati 1996, Campbell and Sederoff 1996). Even though a complete suite of genes has not been isolated from any single plant species, the regulation of lignin biosynthesis is understood well enough to provide a good starting point for wheat. For example, in trees, down-regulation of the O-methyltransferase (OMT) gene induced a dramatic reduction in monolignol, syringyl units (Dwivedi et al. 1994). Cinnamoyl alcohol dehydrogenase (CAD) down-regulated trees showed an odd phenotype in which the color of the plant tissues changed to red and the lignin composition was altered, without changes in either cell wall structure or morphology (Baucher et al. 1999). Lignocellulose from these trees was more easily pulped. This phenotypic change was also seen in alfalfa, in which the lignin quantity was not reduced, but the digestibility with alkali was substantially increased.

Altering lignin biosynthetic carbon flow was the second major task of this part of the project. Gene regulation elements were used to decrease the activity of genes for key lignin biosynthesis enzymes. Our hypothesis was that without deleterious structural and functional side affects, lignin content can be reduced and modified (not eliminated) in a way that results in an altered flow of carbon that favors an increase in production of wheat cellulosic polysaccharides that are also more readily extractable.

Hu et al. (1999) demonstrated that it is possible to down-regulate lignin synthesis in aspen through genetic manipulation, with startling results. In this study, lignin synthesis was reduced in modified trees. Unexpectedly, while the modified trees synthesized less lignin, the overall mass of the tree increased. In the trees induced to synthesize more cellulose, the overall structural characteristics of the cell walls did not change substantially. In addition, the transformed trees grew faster than the non-transformed control seedlings, although it was not known whether the trees were maturing faster due to the long growth cycle of aspen. Because decreased lignin content, increased cellulose content, and faster growth would all be significant improvements to production and exploitation of renewable biomass, determining whether the same phenomenon can be genetically engineered or bred (via molecular plant breeding) into wheat is an important research question.

To accomplish this task, we investigated suppression of the activity of cinnamoyl CoA reductase (CCR). The lignin biosynthesis is interconnected to many complex biosynthetic pathways for important secondary metabolites. We targeted CCR because it catalyzes the first committed step in lignin synthesis and because reduction in its activity by genetic engineering has been demonstrated (Baucher et al. 1998). This enzyme represents an important control point in lignin carbon flow, and its inhibition can be manipulated with no detrimental effects on synthesis of the other important plant phenolic compounds. Significant nucleic acid homology has been found between CCR genes from various plant species (Boudet and Grima-Pettenati 1996) and this homology was used to clone and characterize wheat CCR. The strategy described here has been successfully applied in tobacco, a herbaceous dicot (Sewalt et al. 1997), and we expect equal success in its application to wheat. These investigations will target a wheat variety that has a harvest index (ratio of grain to total crop biomass) best suited for our “multi-component harvester” regarding straw characteristics and properties.

In order to exploit lignin gene regulation, we identified, cloned, and characterized a wheat CCR gene. To clone the desired gene sequence for this wheat enzyme, INL/U of I first searched the Institute for Genomic Research TIGR) *Triticum aestivum* Gene Index (the wheat genome database) to locate tentative consensus sequences for CCR and identify homologous Expression Sequence Tags (ESTs). The INL used these sequences to probe wheat Bacterial Artificial Chromosome (BAC) genomic libraries. The ESTs and BAC libraries are available from UC Davis. The BAC clones showing homology were subcloned using standard recombinant methods, and subclones showing homology to the CCR gene were sequenced. Promoter regions and open reading frame sequences were determined by computer analysis of CCR gene sequencing data for future replacement of the constitutive promoters used in initial CCR1 anti-sense constructs for genetically modifying wheat. Replacement of these constitutive promoters with the CCR1 promoter and fusion with the green fluorescent protein gene could promote expression in different plant tissue types and at different stages of development. In this way, an epi-fluorescence microscopy assay could identify and define the CCR gene regulatory elemental needed to refine the level of tissue-specific site modification future lignin biosynthesis.

Gene constructs with constitutive peanut chlorotic streak caulimovirus (PClSV) and cauliflower mosaic virus (CaMV) promoters were used to express the CCR1 anti-sense sequence along with the kanamycin selectable marker gene to select genetically transformed embryogenic wheat tissue and regenerated transformed plants. Wheat tissue cultures were transformed by the microprojectile method, and co-transformants selected using kanamycin for further analysis. Stable kanamycin resistant transformants were regenerated into plants and analyzed for lignin and structural sugars modification, as well as for changes in the efficiency of conversion to ethanol.

Constitutive promoters were fused to antisense sequences of CCR to investigate and influence the suppression of lignin biosynthesis. The standard antisense method for reducing gene activity is well established in plant systems and has been shown to specifically alter lignin content and quality in other plant species (Baucher et al. 1998). Using antisense gene sequences from various plant species, lignin synthesis was altered in non-wheat species by taking advantage of significant DNA homology found among CCR genes (Boudet and Grima-Pettenati 1996). This strategy was successful in tobacco (Sewalt et al. 1997). Using a homologous wheat-specific CCR antisense sequence should work efficiently for this purpose.

Using wheat tissue cultures initiated from immature embryos, the U of I transformed these calli cultures using antisense CCR vector constructs. Cultures co-transformed with a selectable marker gene for phosphinothricine resistance were regenerated into plantlets, rooted, and grown out so that their lignin content could be assayed and compared to non-transformed controls. The chemical composition of straw from genetically modified plants was screened by the U of I and INL using the Quan Sac method. Seeds from transformed plants selected for their improved straw composition were bulked up (propagated) by U of I to produce wheat straw to test lignocellulose extractability and processing improvements that could correlate with less lignin and/or more easily and less expensively processed lignocellulose. The technical hurdle that had to be overcome in the lignin biosynthesis was to successfully genetically alter the plant to reduce lignin content while at the same time maintaining the plant's viability, production, and harvestability.

Simultaneous saccharification fermentation (SSF) analysis at the INL was done to approximate Arkenol's complete feedstock assessment (CFA) analysis and to assess the characteristics of the modified feedstock using a process similar to the Arkenol Concentrated Acid Hydrolysis process. The SSF analysis was done to determine the enhanced processability/convertibility of lignin-modified wheat straw stems.

The test of suitability for fermentation or biological utilization was to actually run this fermentation test at the INL. The SSF tests identified the percentage of theoretical ethanol conversion produced by the biomass structural sugars extracted from the modified wheat plants. More definitive pilot-plant testing by Arkenol, using larger biomass samples, is required to set future equipment variables and determine larger scale conversion processing optimization opportunities.

The benefits of down-regulating this key enzyme activity were twofold. First, a portion of the carbon and metabolic energy used to produce lignin can be diverted to increase the yield of sugar-producing cellulosic polysaccharides. Second, even though the down-regulation of the CCR1 gene by the gene constructs used appeared to only slightly decrease in lignin content in T2 transgenic plants, these plants have shown important variation in the ease and efficiency with which their fermentable structural sugars can be converted to ethanol.

Results

INL and the U of I identified, characterized, and modified a key plant biosynthetic lignin gene, CCR, to assess its influence on the efficiency with which the resulting biomaterials interact with and are processed by engineering systems. The characterization of CCR genes and resulting antisense gene constructs allowed us to modify and assess the impact of this cell wall biosynthetic gene in wheat. This allows the model systems needed to study the structural and functional post-harvest properties of straw to be created and assessed. Post-harvest properties and qualities associated with key genes involved in cell wall biosynthesis like CCR are important in developing engineered systems that can most effectively harvest and extract value from crop residues.

Bioinformatics analysis led us to important CCR genes. A gene expression sequence tag from a corn gene database was used to find analogous genes for CCR in wheat. Gene sequence information allowed us to identify, acquire, and characterize CCR as a key gene for attempting to alter lignin content in plant residues. A wheat gene library was successfully screened and related CCR genes were cloned and acquired. Genes from various sources were characterized and used to modify and influence harvestability, storage, and processing characteristics of wheat.

Progress was made in modifying CCR gene expression. Key anti-sense CCR gene constructs were generated and tested. Resulting transgenic materials were examined for changes in their structural and processing characteristics of interest. The degree to which anti-sense modification of this gene alters biomass characteristics, including sugar accessibility were correlated and explored. Gene constructs were refined and improved as we assessed the action of CCR regulatory sequences. Constructs were transformed into receptive wheat tissues and plants were selected for further analysis. Transgenic biomaterials were grown into plants to assess their molecular biology characteristics as they related to gene expression and harvestability.

This anti-sense approach to lowering lignin content allowed us to study and understand the contribution and impact that key genes make to harvestability, storage, and bioprocessing of crop residues. We anticipated that lowering lignin content in plant residues would result in a reduced lignin/cellulose ratio within straw stem and influence its harvestability, storage, and ease of post-harvest processing. The genetic, chemical and physical properties data derived from these altered biomaterials will be important to improving future crop residue harvesting, storage, and processing engineered systems.

The desired gene sequence for a wheat lignin gene were successfully identified by searching the TIGR *Triticum aestivum* Gene Index (the wheat genome database) and an appropriate tentative consensus sequence for the CCR gene was identified. The corresponding cDNA was obtained and used successfully to probe and identify CCR clones within a wheat BAC genomic library. The U of I has also cloned and identified CCR genes from wheat. These clones contain the gene sequences for open reading frame sequences and regulatory regions for optimizing constructs for transform of embryogenic wheat tissues in order to alter CCR gene expression with antisense constructs.

Embryogenic wheat tissue was transformed with CCR antisense constructs driven by a constitutive promoter and plants regenerated. When these plants reached maturity, their half seeds were analyzed for the presence of integrated CCR antisense sequences in their genomic DNA. Transformed plants expressing antisense constructs were further characterized for changes in their lignin content and the impact of these changes on harvestability and processability. Lignin levels in initial transgenics appeared to have been reduced by up to 25% of levels observed in the non-transformed control plants.

The U of I identified and characterized four clones of putative CCR genes from wheat and the INL identified and characterized two BAC genomic clones of CCR1 from a *Triticum monococcum* library. The U of I completed sequencing four CCR1 gene clones from the cultivar Hubbard. Two of the four clones appeared identical after sequencing, so only three of the original clones were sequenced and error-checked, and then submitted to GenBank.

INL overcame problems associated with subcloning DNA fragments containing the CCR1 gene of *Triticum monococcum* from large bacterial artificial chromosome genomic clones by taking a shotgun cloning approach. A 7.9 KB CCR1 subclone was successfully identified using polymerase chain reaction (PCR) screening. Using a transposon insertion approach, a major portion of the CCR1 gene within the subclone was sequenced, analyzed, and compared with CCR genes from other plants. The identity of the CCR1 gene has been confirmed. In addition, this analysis provided CCR1 gene components for testing the modification of lignin biosynthesis within wheat stems.

With the objective of suppressing expression of CCR1 in wheat, the U of I continued production of a construct involving a carrier sequence and a 150 bp sequence from the CCR1 gene. Orientation on the 150 bp sequence was switched to “sense” to match the direction of the carrier sequence. The U of I was successful in creating two orientations of the 150 bp CCR1 gene sequence for construct 2 and made two versions of the construct that will have the 150 bp sequence in either sense or anti-sense orientation to the carrier sequence. These constructs were successfully transformed into wheat callus and the regenerated plants are under seed increase for further evaluation.

The sequences from four gene clones were used by the U of I to identify an intron from the CCR1 gene for use in the development of a “stem-loop” construct (construct 3) for inducing siRNA gene silencing of the CCR1 gene. Using the gene sequence identified in this project, the U of I has identified four candidate sequences for use in the “stem loop” construct. These sequences are currently being evaluated using virus induced gene silencing (VIGS) to identify the sequence that provides the optimum level of reduction of lignin in the straw.

We also developed primers to confirm the orientation of the carrier sequence and CCR1 gene sequence. To complement this expression work, the CCR1 promoter region was PCR amplified by the INL with the specific cloning restriction sites on either end. This custom-designed promoter fragment was inserted into the U of I CCR1 plasmid in an attempt to replace the 35s promoter in the U of I construct 1. However, the promoter did not insert as expected and, because of limited funds allocated for this specific INL effort, this promoter replacement effort will not be continued at INL.

The INL completed the sequencing of a major 7.9 Hind III fragment containing most of the CCR1 gene of *T. monococcum*. This Hind III fragment, derived from a CCR1 containing a BAC library clone, contains about 250 basis pairs of the promoter region with important gene regulatory components and runs three feet into the intron that separates Exon IV and V coding regions. The sequenced promoter was synthesized with specific restriction enzyme sites for ease of replacement of the constitutive 35S promoter in the U of I construct 1, which drove the expression of the antisense CCR1 gene in putatively transformed wheat plants. Replacement with this CCR1 promoter region allowed the assessment of antisense gene regulation under controlled developmental and tissue specific expression. This allowed us to look for reduction in CCR1 gene expression, and potential reduction in lignin, when and where it is typically expressed in wheat plants.

Using primers homology to a barley CCR1 Exon V sequence, the INL identified fifteen clones containing putative copies of the CCR1 Exon V from the BAC plasmid containing *T. monococcum* CCR1. Of these fifteen putative Exon V clones, two contained an appropriate single Hind III fragment. The remaining thirteen clones appeared to contain multiple copies of BAC Hind III CCR1 fragments. INL sequenced the two putative CCR1 Exon V clones of *T. monococcum* of CCR1 and confirmed the sequence of the entire gene as required for submission to GenBank. The complete sequence of this CCR1 included its promoter region and about 400 base pairs past its stop codon. This genomic sequence consisted of 5 exons and 4 introns with a total length of 3983 bp. Comparing the coding region of the genomic sequence to the mRNA sequences of other monocot CCR1 genes allowed the assembly of a putative amino acid sequence. The *T. moncoccum* CCR1 gene has strong homology to similar CCR1 mRNA and amino acid sequences in *Triticum aestivum, Hordeum vulgare, Lolium perenne*, and many other monocots. This confirmation of the entire gene sequence allowed for a detail comparison and contrast of it with the three CCR genes from the cultivar Hubbard that were cloned and sequenced by the U of I.

Progress was also made in the transformation of wheat to produce transgenic plants with reduced lignin production and increased sugar production. T_1 seed was harvested off of spring wheat that was transformed with construct 1, planted, and grown in a greenhouse. Winter wheat plants transformed with construct 1 were transferred to soil and vernalized. Additional transformations were done introducing construct 1 into callus derived from winter wheat. To assist the U of I in identifying transgenic T_1 winter wheat plants, the INL sent primers based on the CCR1 clone WHE2338 and used successfully to identify CCR1 BAC clones. The spring material was screened using PCR to identify individuals carrying the CCR1 construct. The T_1 generation of transgenic spring wheat was harvested for both seed and straw. The straw was sent to the U of I analytical lab for analysis of total lignin content using the Klason analysis for acid insoluble and acid soluble lignins.

The U of I submitted node and internode tissue from the T_1 generation of transgenic spring and winter wheat to the U of I Analytical Lab for analysis of lignin content. Initial analysis of the internode tissue of the T_1 generation of transgenic spring wheat carrying construct 1 was done on five of eight plants for each T_1 population. Several individuals were identified with a lower level of acid insoluble lignin and total lignin than the non-transformed check cultivar. Greatest reduction was shown in the progeny of transformant 307E with one line showing a 40% reduction in percent acid insoluble lignin and in total lignin. Constructs 2 in the sense and anti-sense orientation were completed and introduced into wheat through particle bombardment and pollen-mediated transformation. Expression of the green fluorescent reporter protein was observed in the callus, indicating that expression of the constructs was occurring in the wheat callus. Transgenic winter wheat lines were harvested and the straw partitioned into nodes and internodes for analysis. The sequences of the three CCR1 gene clones from the cultivar Hubbard underwent additional confirmation in preparation to be submitted to GenBank. In addition, an intron sequence was identified for use in construct 3.

T_2 generation plants of T_1 spring wheat plants that showed the greatest reduction in acid insoluble lignin content were then planted in the greenhouse. DNA was collected from these plants for PCR analysis for construct 1. As the plants matured, stem internode tissue was collected for analysis of insoluble and total lignin. T_1 winter wheat lines were submitted to the U of I for lignin analysis. The first three populations examined did not show a significant reduction in lignin content. Putative transgenic wheat plants that potentially carried construct 2 in both the sense and anti-sense orientations were recovered. Seed produced by pollen-mediated transformation was harvested. Upon reaching maturity, the T_2 generation plants were harvested and processed for lignin analysis. Variations were observed among the populations for soluble and insoluble lignin content. The decreases observed were greater for the soluble lignin content than insoluble lignin content. Progress was made toward identifying and using key CCR lignin biosynthesis gene sequences for controlling lignin biosynthesis with the goal of altering lignin content and composition in wheat. This was important because of the potential impact that reduced lignin could have on feedstock harvesting, transportation, storage, pretreatment, and processing.

Modified Feedstock

The third important objective of this project was to assess the benefit of modified feedstock and to assess the resulting conversion processing enhancements that could be effected using a specifically developed feedstock prepared for conversion into low-cost fermentable sugars. The genetics of the plants producing straw feedstock was modified with the goal of reducing the cost of producing fermentable sugars and making the use of improved straw more economically and environmentally attractive. Straw from these plants was modified at the U of I and assessed for changes in lignin and fermentable sugar content at the INL. Changes in these components may favorably impact straw feedstock processability and convertibility to ethanol, the ultimate test of feedstock quality.

The assessment focused on analysis of directed changes in structural composition of stems and the production of fermentable sugars from the altered straw product. Near infrared (NIR) analysis of all samples was used to rapidly assess variation. This promising assay is still in development. Even so, it allowed for the down-selection of samples for further analysis using more difficult and costly analysis methods. Methods for lignin and sugar composition used the standard quantitative saccharification (Quan Sac) assay and a more recently developed pretreatment/fermentation assay called simultaneous saccharification/fermentation (SSF). SSF measures the relative theoretical percentage of ethanol produced from relatively small samples (0.5 to 1.0 gram per sample). Genetically modified feedstock samples were assessed for possible compositional changes in structural glucan, xylan, galactan, arabianan, mannan, acid-soluble lignin, and inorganic fractions. To assess the potential impact on the operation of a biorefinery, straws with modified lignin composition were further down-selected into a subset of samples for SSF analysis to assess their relative processability and conversion to ethanol.

The goal of this work was to assess the potential of modified wheat biomass feedstock in regards to its ultimate conversion to ethanol. Feedstock with improved quality and conversion characteristics has the potential to impact the design of harvesting and processing equipment as well as future biorefineries and their supporting infrastructure.

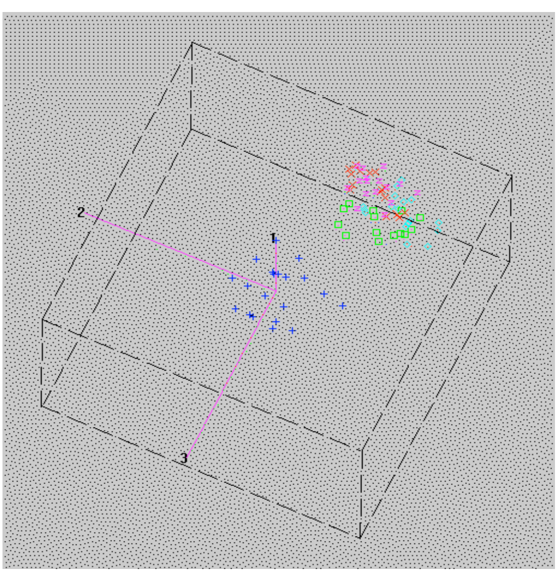
Centennial and Brundage wheat cultivars were used for this study. Centennial is spring wheat that is a high tillering cultivar with moderate straw strength. Brundage is a soft white winter wheat that has a much stiffer straw than Centennial. Brundage is currently one of the major soft white winter wheat cultivars grown in southern Idaho and a reselection of Brundage, Brundage 96, has gained acreage in northern Idaho and eastern Washington. Embryogenic wheat callus cultured from these two different varieties were each transformed with an anti-sense gene construct of cinnamoyl- CoA reductase (CCR1) at the U of I. As a key gene that regulates the flow of carbon through the lignin biosynthesis pathway, CCR1 was positioned in an anti-sense direction within a gene construct that was designed to lower the lignin content in transformed and selected wheat lines. Seeds from selected regenerated plants were grown out into putative transgenic T_2 plants. Straw from these T_2 plants was assayed by NIR spectral, wet chemistry, and SSF analyses.

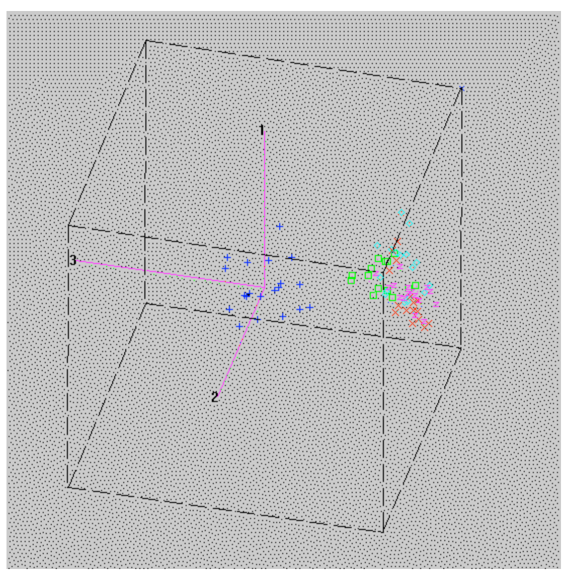
Results

NIR Analysis: Near infrared spectroscopy (NIRS) can provide a rapid indirect measurement of plant composition once calibrated with suitable representatives of a specific feedstock type like corn stover or wheat straw. Over 150 NIR spectra were collected in duplicate from mature transgenic T_2 and control parental wheat plants that were greenhouse grown, dried, and ground to pass through a two-millimeter screen. These ground biomass materials were analyzed using a FOSS 6500 NIR spectrophotometer. Collected spectra were compared by principal component analysis (PCA).

Mature wheat tillers (approximately five grams per plant) were harvested and ground in a Wiley mill for analysis. NIR-assessed Centennial lines included transgenic lines 3077A-1-b-1 through 3077A-1-b-100 as well as ten Centennial parental controls. Transgenic Brundage lines assessed by NIR analysis included 3163-G-2 A-E & G, H; 3163-G-5 A-H; 3163-G-6 A-H; 3163-G-7 A-H; plus ten Brundage 96 controls. All assessed plants were grown at the same time in the same greenhouse under the same growth conditions.

Figures 13 and 14 show the Global-H (Malhalanobis Distance) in three-dimensional space based on Principle Component Analysis of the NIR spectral data obtained for Brundage 96 control plants and Brundage transgenic plants that were modified to alter CCR1 gene expression. Dark blue/plus sign symbols represent Brundage 96 control plants; green symbols represent transgenic 3163-G-2 Brundage plants A through H; light blue symbols, Transgenics 3163-G-5 A-H; red symbols, Transgenics 3163-G-6 A-H; and purple symbols, Transgenics 3163-G-7 A-H. Transgenics 3163; G-2, G-5, G-6, and G-7 represent plants derived from individual transformation events. Note that these different sets of transgenic plants group more tightly among themselves. All the transgenic plants group separately away from the blue control Brundage 96 plants in terms of relative nearest neighbor positions.

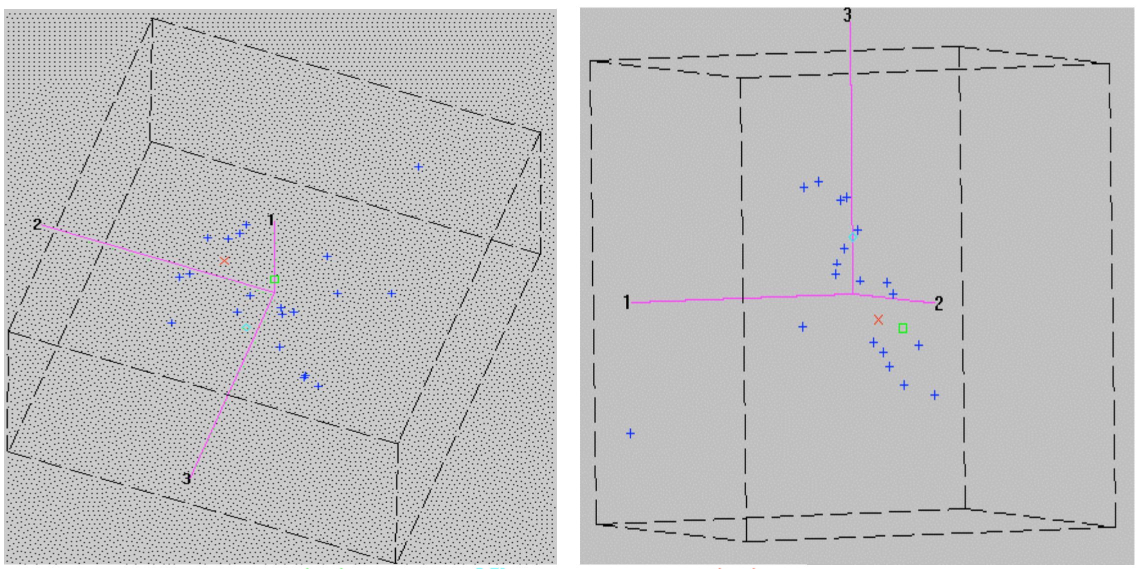




+ Brundage 96 □ G2 ◇ G5 × G6 z G7

Figures 13 and 14. Global-H data distribution in three-dimensional space based on Principle Component Analysis of the NIR spectral data obtained for Brundage 95 control plants and transgenic plants modified to alter CCR1 gene expression.

Figures 15 and 16 below show the Global-H relationship in three-dimensional space based on PCA of the NIR spectral data obtained for Centennial control plants and Centennial transgenic plants modified with the CCR1 anti-sense gene. In these figures, displaying the same data points in two orientations shows the relative positions of the Global-H ordered data obtained for transgenic Centennial plants in dark blue symbols. These same SSF-analyzed transgenic parental control Centennial 02b.nir, 07b.nir, and 09b.nir plant NIR data are shown in green, light blue, and red.



Figures 15 and 16. Global-H relationship in three-dimensional space based on Principle Component Analysis (PCA) of the NIR spectral data obtained for Centennial control plants and Centennial transgenic plants.

These specific control parental plants were further analyzed by standard quantitative saccharification (Quan Sac) assay and a pretreatment/fermentation assay simultaneous saccharification/fermentation (SSF) that measures relative ethanol percentage theoretical ethanol production. A smaller set of transgenic and parental control samples representing the wide breadth of distribution in Global-H values as sorted by the PCA were down-selected for these chemical and SSF analyses.

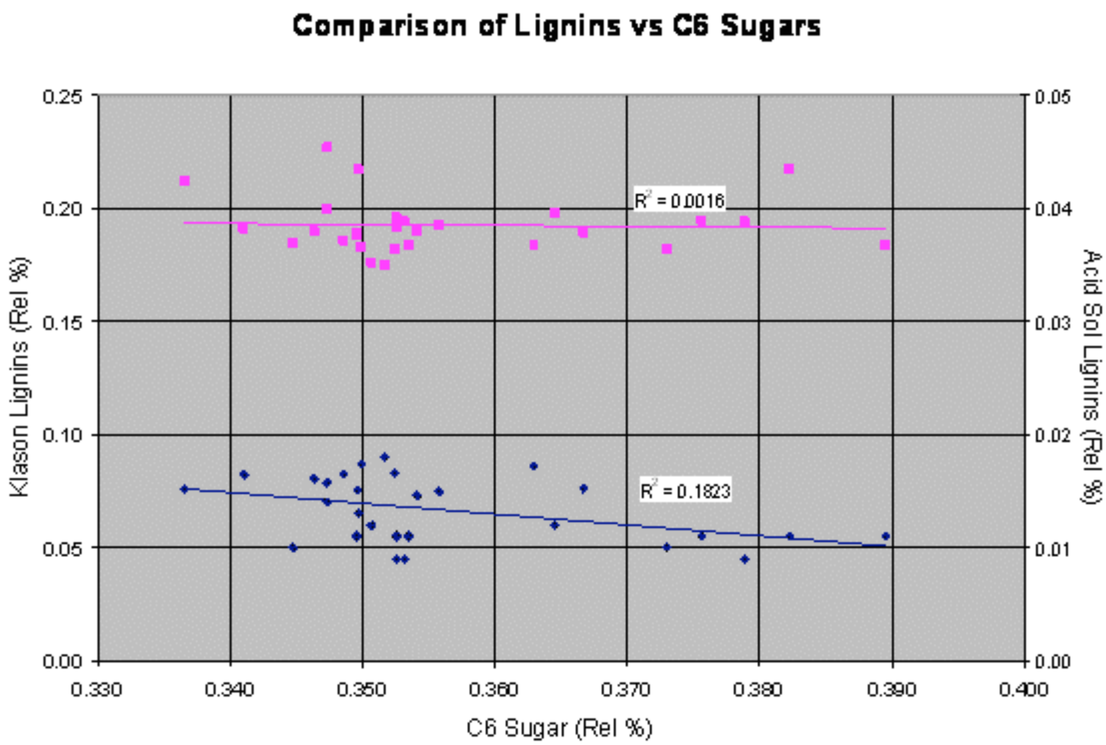


Figure 17. Comparison of Lignin verse C6 sugar content for Centennial plants

Chemical Composition Analysis: Results from the Quan Sac analysis are shown in Figure 17 for Centennial and Figure 18 for Brundage plants. The correlation of the relative percentage of lignins with the relative percentage of six carbon (C6) sugars in transgenic Centennial plants is very weak (Figure 17). More data is needed to more clearly interpret the trends in the relationship of lignin and C6 sugars for Centennial samples. Observable C6 structural sugars ranged from 33.6% to 38.2%, or about 4.5% even though a trend up or down in the relative percentage of associated Klason lignins was not clear.

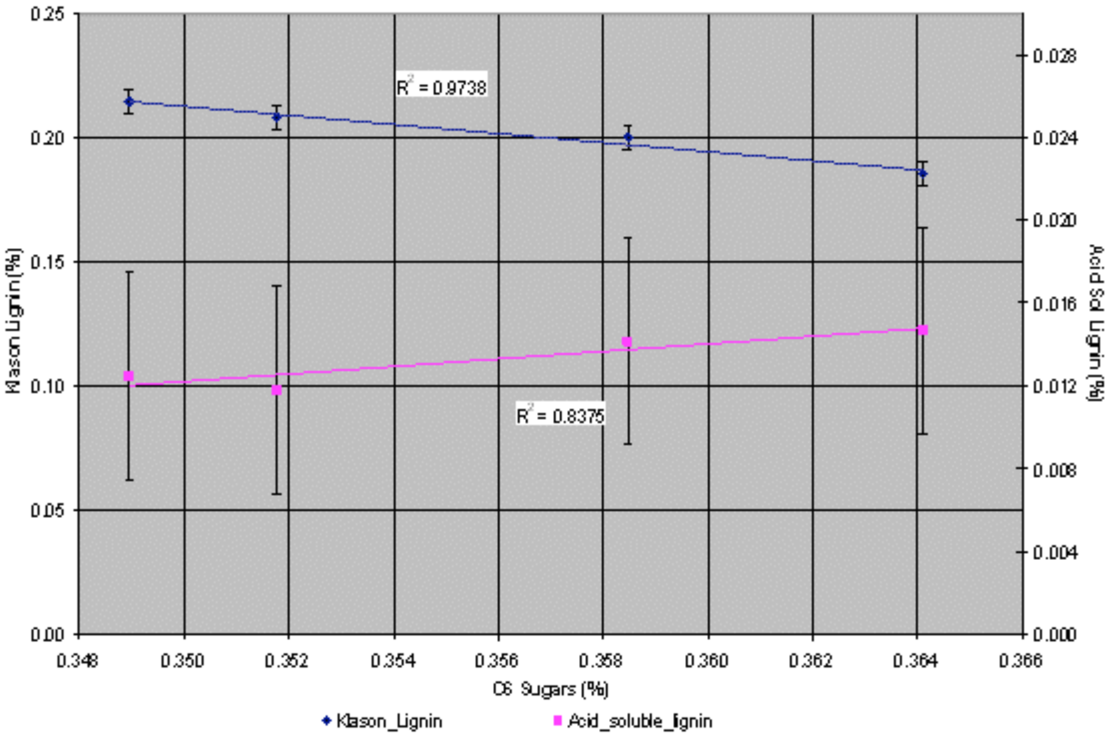


Figure 18. Comparison of Lignin verse C6 sugar content for Brundage transgenic lines (TG) and Brundage 96 plants.

The relationship between sugar composition and lignin content for Brundage transgenic lines (TG) and Brundage 96 (GGB-96) is shown in Figure 18. In addition, Table 1 lists the specific relative fraction values for C6 sugar, Klason lignin, and acid soluble lignin. The percentage of structural C6 sugars ranged from 34.9% to 35.2%, Klason lignin from 20.0% to 21.4%, and acid soluble lignin from 1.18% to 1.41% among the Brundage transgenic plants. The observed values for the Brundage 96 control were 36.4%, 18.5%, and 1.47%, respectively (C6 sugars, Klason lignin, and acid soluble lignin). This indicated that the lignin content was modified in the transgenic straw but the Brundage 96 plants used as the controls have a relatively lower Klason lignin value at 18.5%. However, Brundage 96 may differ from its Brundage parent. Even though it is a closely related isogenic line of Brundage, 96 was selected as a distinct cultivar because of its resistance Stripe Rust. Brundage 96 was used in this study as the greenhouse plant control because it is an isogenic line of Brundage and because Brundage was not available at the time for comparative analysis. Interestingly, Brundage 96 rust resistance may possibly be associated with its observed lignin composition. Because the lignin content of Brundage 96 appears to be lower than that of the related Brundage CCR1 transgenic plants (as shown in Figure 18 and Table 1), the lignin content of the actual Brundage parental line—the source of material for the transgenic plants—will need to be assessed to make a definite comparison between Brundage parental and the derived transgenic lines.

Brundage Plants	C6 Sugars	Klason Lignin	Acid soluble lignin
Mean TG-2 N=4	0.349	0.215	0.0125
Mean TG-5 N=4	0.352	0.208	0.0118
Mean TG 6 N=4	0.359	0.200	0.0141
Mean GGB-96 Parents N=5	0.364	0.185	0.0147

Table 1. Comparison of structural C6 sugars, Klason lignin and acid soluble lignin composition among a subset of Brundage plants, a smaller subset of which, were later assessed by SSF.

However, even though the relative amount of Klason lignin appears lower in the Brundage 96 "controls," two of the transgenic plants TG-2-1 and TG-05-1 have a higher observable ethanol yield (Figure 19).

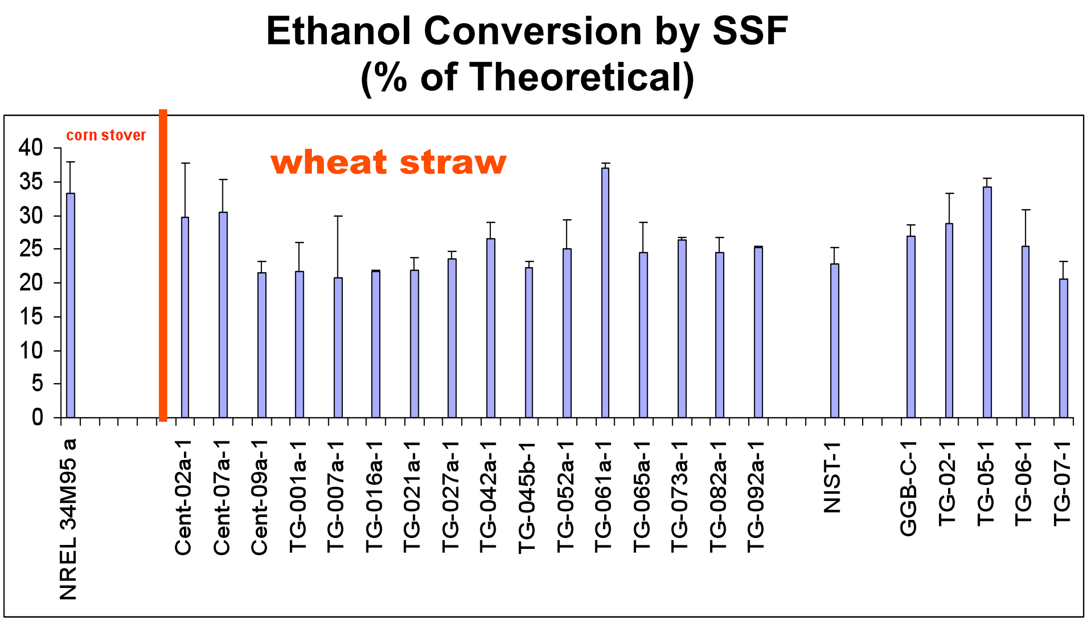


Figure 19. Potential Ethanol Yield as determined by SSF assay.

SSF Analysis: For the SSF results shown in Figure 19, all samples were assayed in duplicate. The percentage of theoretical ethanol conversion obtained with the NREL 34M95a control indicates that the SSF assay performed as expected. NIST-1 wheat straw (RM 8494) was also included as an internal experimental standard. RM 8494 was included because it has been rigorously characterized by NREL in terms of its chemical composition and its analysis using their ASPEN model. It represents a relative minimum ethanol selling price (MESP) of $1.146 per gallon of ethanol.

Figure 19 shows the relative ethanol conversion efficiency among a subset of the original 151 samples assessed by NIR. The most interesting result of this SSF analysis is the wide range of promising variation in the relative percentage of theoretical ethanol among the 13 Centennial transgenic lines tested, TG -001a-1 through TG -092a. A similar range of variation in feedstock conversion to ethanol was observed among the four assessed transgenic Brundage lines TG-02-1, TG-05-1, TG-06-1, and TG-07-1 and the Brundage 96 control (GGB-C-1). Ethanol conversion efficiency differed by over 16% (between TG-061a-1 and TG-016a-1) among the Centennial plants and by over 13% among the Brundage plants (Table 2).

SSF Assessed Sample	% Theoretical Ethanol
TG-02-1	28.7
TG-05-1	34.1
TG-06-1	25.4
TG-07-1	20.6
GGB-C-1	26.8

Table 2. SSF results for specific plants within the sets representing transgenic Brundage plants (3163-G-2, 3163-G-5, and 3163-G-6) and Brundage 96 (GGB-C-1).

The variability among the transgenic plants indicates that differences in expression due to the insertion site of the CCR1 anti-sense sequences may influence suppression of the CCR1 gene in wheat and that segregation is still occurring among the transgenic plants for number of copies of the inserted sequence. This observed variation in ethanol conversion among the altered transgenic plants once stabilized highlights the potential for genetically modifying straw composition to enhance ethanol conversion efficiency.

Conclusions

The significance of the high-fidelity separation obtained through this study is the resulting effect on pretreatment. Because different fractions respond differently to pretreatment, high-fidelity residue fractionation could lead to significant cost savings. While the composition and theoretical ethanol yield data suggest that the straw fraction is more valuable than chaff for ethanol production because of its higher sugar content, recent pretreatment severity studies have shown that chaff responds much better to pretreatment than straw. Consequently, even though the chaff fraction contains less digestible sugars, the cost of converting the available sugars may be so much less that the chaff stream may actually be the more valuable of the two residue streams. While this is only speculation at this point, there appear to be competing factors that will ultimately define feedstock quality. This project provided strong evidence for the value of selective harvest, but whether this technology will be of commercial significance requires additional techno-economic analysis of the downstream effects on collection, transportation, preprocessing, storage, pretreatment and conversion to determine. Additional analysis should also include evaluating the value for selective harvest of other feedstocks (e.g., corn stover). Furthermore, although this study successfully demonstrated high-fidelity selective harvest, the harvester efficiency was dramatically reduced by the limitations of the residue handling systems. Therefore, selective harvest machinery efficiencies must also be improved before this technology can become commercially viable (we have already identified potential improvements in residue that may significantly improve harvester efficiency). Finally, this study demonstrated selective harvest using a rotary combine, and because the residue fractionation may be significantly different for a conventional machine, some level of analysis to evaluate machine configuration effects should be performed before the value selective harvest can be broadly categorized.

The virtual engineering capability within the INL was also enhanced as a result of this project. Since the completion of this project, the INL has successfully utilized virtual engineering technology and capabilities on other bio-energy research projects. In addition, the INL is actively involved in the development and support of VE-Suite. The VE-Suite toolkit continues to be an effective tool for enabling the investigation of complex problems at the INL.

Recommendations

Our proposed straw separation system removes the large stems (which tend to clog modern tillage equipment) from the field, but leaves the chaff and nodes in the field (environmental benefit). This strategy ensures sustainable agriculture by preventing the depletion of soil minerals, and it restores organic matter to the soil (environmental benefit) in amounts and particle sizes that accommodate farmers' needs to keep tillage and fertilizer costs low (economic and energy benefits). A ton of these nutrient-rich plant tissues contains as much as $10.55 worth of fertilizer (economic and energy benefits), in terms of nitrogen, phosphorus, potassium, and other nutrients provided to the soil when incorporated by tillage instead of being burned (environmental benefits).

Economic Benefits

In 1999, American farmers harvested 53,909,000 acres of wheat. The straw from this acreage of wheat represents over 100 million tons annually. Currently, some of the straw is harvested (baled) for use as livestock bedding or low-grade animal feed. However, these low-grade uses provide only a minimal return. Nationally only about 3.2% of the economic return on wheat is from straw. Wheat is an expensive crop to grow. National average costs to produce the national average yield of 38 bu/acre are: $16.95 for fertilizer, $7.22 for chemicals, and $6.59 for fuels, lube, and electricity, for a total of $30.76 per acre for these out-of-pocket production costs. In a more comprehensive look at production costs, a U of I study examined expenses for a typical sprinkler-irrigated southcentral Idaho wheat farm with a target yield of 110 bu/acre for hard red spring wheat; that study specified a preharvest cash operating cost (i.e., excluding harvest, trucking, interest, land rent, overhead, depreciation, etc) of $201.46 per acre. Considering these high production costs, selective harvesting of the wheat stems as a feedstock for low-cost fermentable sugars represents a higher-value use for wheat straw stems and an attractive secondary revenue stream for the grower.

Low-cost fermentable sugars are a valuable feedstock for a wide range of chemicals, such as polylactic acid and fuels such as ethanol. Use of wheat straw and other cellulosic plant biomass, derived from the significant volumes of crop waste produced annually, is an attractive alternative for increasing production of low-cost fermentable sugars. These crop wastes are available throughout the agricultural areas of the U.S. For example, if corn stover were exploited to produce ethanol, an additional 7 to 12 billion gallons of ethanol could be produced per year. An equivalent amount of ethanol could be produced from wheat and other straw residue. Use of cellulosic plant biomass in the western U.S. would provide a ready feedstock to increase local production of fermentable sugars for fuels and chemicals.

Use of lignocellulosic plant biomass as a feedstock for the low-cost fermentable sugar market has been limited by barriers relating to collection, transportation, and processing. Production of fuels and chemicals from starch feedstocks such as corn or direct sugar feedstocks such as sugarbeets is much simpler than production from lignocellulosic feedstocks. However, sugars and starch have significantly higher economic value as food compared to plant biomass that has a low, sometimes negative value. In contrast, lignocellulosic plant biomass has significant environmental benefits. For example, ethanol produced from lignocellulosic biomass has a better net energy balance than ethanol produced from starch. The net energy balance is calculated by subtracting energy necessary to produce a gallon of ethanol from the energy contained in a gallon of ethanol (~76,000 Btu). Starch-based ethanol has a net energy balance of 20 to 25,000 Btu/gallon, whereas ethanol produced from lignocellulosic biomass has a net energy balance of more than 60,000 Btu/gallon. The main advantage of lignocellulosic plant biomass is lower cost. More expensive feedstock (starch, etc.) is replaced with less expensive lignocellulosic plant biomass; the result is an improvement in the economics of ethanol compared to gasoline.

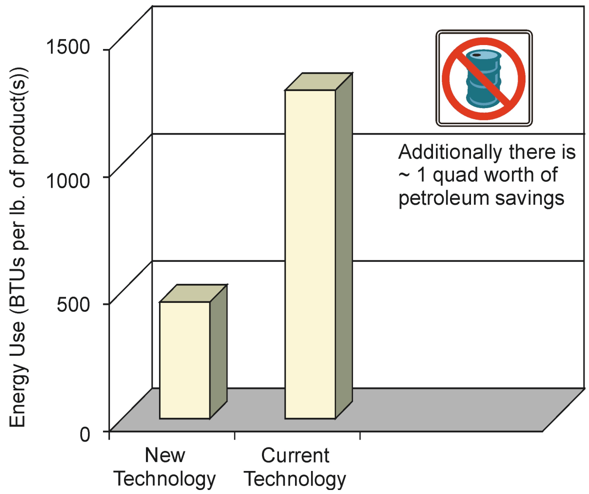


Figure 20. Our proposed technologies will save an estimated 830 BTU per pound of product produced, and create a commercially viable crop residue feedstock that could displace an estimated 1 QBTU of petroleum per year (Appendix A).

Energy Benefits

Large-scale displacement of petroleum-based products will come primarily from low-cost lignocellulosic feedstocks like wheat straw. Producers, including the National Association of Wheat Growers, have long recognized the potential economic and environmental benefits in producing bioenergy and bioproducts from excess wheat straw. The harvesting and fractionation improvements that will be possible as a result of our research will reduce the energy necessary to produce, harvest, and process lignocellulosic crop residues and contribute to the creation of a viable petroleum feedstock (Figure 20).

Energy savings will be realized in several areas:

1. Selecting and harvesting the energy-valuable stems will result in much more efficient use of the energy expended to produce the wheat crop.
2. Eliminating the second pass through the field with the baler (by deploying a single machine that harvests both wheat and stems) will result in a significant reduction in fuel consumption.
3. Returning the soil-replenishing nodes and chaff to the field will reduce the total amount of fertilizer used in the production of the wheat crop and thus reduce the energy consumed in fertilizer production.
4. Removing the stems from the field will eliminate the need for separate application of nitrogen fertilizer in the fall (to promote decay of the straw), thus reducing fuel consumption for fertilizer application.
5. Reducing the tillage necessary to incorporate the straw into the soil, thus reducing the amount of fuel needed to produce the crop. Tillage that is capable of incorporating large amounts of straw into the soil is time, energy, machinery, and

labor intensive. Removal of the stems will eliminate energy intensive and/or double tillage operations, thereby reducing tillage costs and energy consumption by as much as 50%.

The production cost for fertilizers, chemicals, fuels, lube, and electricity represents not only an economic cost, but also a significant energy cost, both for the fuel used to produce the crop and for production of the fertilizers and chemicals. It is estimated that it takes 9326 Btu to produce one pound of nitrogenous fertilizer and 6712 Btu to produce one pound of phosphatic fertilizer using the wet process.

Additional energy is consumed in the production of the fertilizer applied to offset the immobilization of N by carbon in the biomass, and in the extra trip across the field in the fall to apply that fertilizer. The tillage to incorporate the straw into the soil also uses a significant amount of energy. Removal and harvesting of the stems, which reduces tillage by 50%, represents energy savings of approximately 160,000 Btu/acre, considering diesel fuel consumption alone.

3.3 Environmental Benefits

With the creation of a new market for crop residues, farmers will voluntarily harvest and sell the separated straw stem product rather than burn it in the field or use energy intensive tillage/chopping operations to remove it from the field surface. Considering all crop residue sources, 150 million metric tons per year of fossil fuel carbon could be displaced, which is greater than 6% of the total 1997 U.S. carbon emissions. Eliminating the need for intensive tillage operations will promote environmentally friendly conservation tillage practices.

Open field burning of agricultural residue injects pollutants into the atmosphere. Gaseous pollutants include CO, unburned hydrocarbons (HC), NOx, and SOx. Estimates of pollutant emissions from open burning of field crops in California are 63.8 kg CO per ton of crop residue, 4.85 kg of HC per ton, 2.9 kg of NOx per ton, and 1.65 kg of SOx per ton. Open field burning also injects polycyclic aromatic hydrocarbons (PAHs) into the atmosphere. Researchers in California found that wheat and barley straw produced over 2,000 mg of PAHs per kg of smoke particulates – significantly higher than other cereal and wood crops. Based on an estimate of 3.7 kg of particulate matter per ton of field crop residue, an estimated 7,400 mg of PAHs are released per ton of straw residue. Harvesting wheat straw stems and processing them into chemicals and fuels will eliminate release of harmful NOx, SOx, and PAHs from open field burning of herbaceous residue.

In short, the following considerable environmental benefits will be realized from the proposed program: (a) the impact of open field burning is removed; (b) carbon dioxide, a greenhouse gas, is retained and recycled or fixed into durable products; (c) conservation tillage practices are encouraged; and (d) nutrients associated with the nodes and chaff are left in the field.

Impact Table

	New Technology	Current Technology	Difference	Comment
Energy Use (BTUs per lb. of product(s)) (Assume Ethanol is product)				
Electricity	190	474	284	Reduced Fertilizer Energy Savings
Natural Gas	190	474	284	Reduced Fertilizer Energy Savings
Petroleum	78.1	341.26	263.2	Reduced Diesel Fuel Usage for Baler and Tillage
Coal	0	0	0	
Biomass	0	0	0	
Other	0	0	0	
Total	458.1	1290	831.9	
Feedstock Use (per lb. of product(s)) (Assume Ethanol is product)				
Biomass (lbs)	2.78 lbs Wheat Straw/ lb Ethanol	Not Commercially Viable		1 lb ethanol replaces 0.8 lbs of gasoline
Petroleum (lbs)	0.0	0.8 lbs gasoline		
	7.54×10^{14} BTUs Ethanol /year			Total potential if all 100 million tons of wheat straw is converted to ethanol
Environmental Impact : Non-Combustion Related Emissions (per lb. of product(s))				
CO_2 (lbs)	0	53.3		Assume wheat straw to ethanol is carbon neutral
SOx (lbs)	0	5×10^{-3}		New technology eliminates open field burning
NOx (lbs)	0	8.8×10^{-3}		New technology eliminates open field burning
Particulates (lbs)	0	1.1×10^{-2}		New technology eliminates open field burning
VOCs (lbs)	0	1.1×10^{-2}		New technology eliminates open field burning
Other (lbs) (PAHs), CO	0	10.2 mg PAH, 1.8×10^{-1} CO		New technology eliminates open field burning

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Appendix A – Project Publications

Publications/presentations

Ashlock, D. A., K. M. Bryden. (2003). “Thermal Agents: An Application of Genetic Programming to Virtual Engineering.” Proceedings of the 2003 Congress on Evolutionary Computation, Canberra, Australia, December 8-12, pp. 1340-1347.

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