

(21) Utilizing the National Corn-to-Ethanol Pilot Plant to Develop a Predictive Model for Distillers Dried Grain for the Fuel Ethanol and Animal Feed Industries

The objective of this two-year effort is to develop and validate a neural network predictive plant model for the composition of Distillers Dried Grain with Solubles (DDGS), a coproduct resulting from the dry grind fuel ethanol process.

Total project cost: \$807,221

Funding request: \$633,149

Project Lead: Southern Illinois University Edwardsville: National Corn-to Ethanol Research Center

Project Participants: Washington University, St. Louis, Missouri-Department of Chemical Engineering; Emerson Process Management; Illinois Department of Commerce and Economic Opportunity.

Start Date: May 23, 2005

End Date: May 23, 2007

Presentations/Publications

A publication based on Phase I trial data is in preparation.

A seminar based on the results of this project will be given on October 3, 2006 at the University of Missouri, Rolla Department of Chemical Engineering. A short description of the seminar will be presented in the next grant report.

Patents

None.

Progress in Past Quarter and Current Status

5.1 Amino Acid Compositions from the Phase I Trial

In creating an optimal animal feed, animal nutritionists need to know more than just the amount of protein in each feed component; nutritionist's need the composition of that protein. Proteins are polymers made up of 20 different amino acids bound together using peptide bonds. Ten of these amino acids cannot be synthesized by animals and are called *essential amino acids*. It is these essential amino acids that animal nutritionists use to formulate balanced diets.

In May, a plant trial was conducted at the NCERC that looked at post-distillation processes on the quality of animal feed. We determined that color, fatty acid content, and some physical properties of DDGS were affected by drying the animal feed in the presence of condensed thin stillage, called syrup. However, we did not find a strong correlation to the overall protein, fat, or fiber content. Despite this, there was still the possibility of the protein composition changing with processing conditions.

Three animal feed laboratories were contracted to analyze the samples from the May trial for amino acid composition. The results from all three labs were averaged and then analyzed statistically to determine if any correlations existed to account for differences in the composition between samples. Table 1 lists the values for the essential amino acids for each of these samples. The conditions at which these samples were generated can be found in the June 2006 STAC Grant Update.

Table 1: Essential amino acid compositions of DDGS samples from the May NCERC trial. Amino acids are expressed as a percent of the sample on a dry basis.

<i>Sample</i>	<i>Arg</i>	<i>His</i>	<i>Ile</i>	<i>Leu</i>	<i>Lys</i>	<i>Met</i>	<i>Phe</i>	<i>Thr</i>	<i>Trp</i>	<i>Val</i>
1	1.195	0.773	1.171	3.699	0.828	0.612	1.457	1.088	0.182	1.383
2	1.311	0.845	1.314	4.241	0.878	0.745	1.653	1.217	0.206	1.547
3	1.085	0.721	1.110	3.550	0.799	0.613	1.396	1.031	0.186	1.310
4	1.203	0.785	1.228	3.974	0.854	0.675	1.537	1.139	0.193	1.439
5	1.213	0.798	1.242	4.005	0.858	0.718	1.551	1.139	0.195	1.461
6	1.113	0.742	1.143	3.666	0.839	0.660	1.430	1.059	0.187	1.343
7	1.270	0.829	1.285	4.221	0.937	0.763	1.641	1.192	0.189	1.507
8	1.277	0.846	1.317	4.382	0.928	0.746	1.705	1.226	0.214	1.525
9	1.267	0.787	1.220	4.183	0.931	0.674	1.633	1.187	0.218	1.426

Statistical models for each of these amino acid compositions were constructed using the JMP software package from SAS, Inc. All of the amino acid compositions showed significant correlation to the variables in the trial. Using all seven independent and dependent variables, regression models for all of the essential amino acids had R^2 values greater than 0.90. Table 2 lists the R^2 values for each of these regression models in descending order of the strength of the correlation.

Table 2: R^2 values for regression models of each essential amino acid with respect to drying conditions.

<i>Amino Acid</i>	<i>Correlation using full set of variables</i>
Lys	1.00
Trp	0.99
Phe	0.98
Leu	0.97
Thr	0.97
Arg	0.95
His	0.95
Met	0.94
Val	0.94
Ile	0.92

Although these correlations are significant, the contributions of the individual factors are not significant. With only nine samples, seven variables in a regression model is too many to determine an individual factor's significance. Stepwise regression was used to reduce the number of variables, and to create individualized regression models for each amino acid. The results showed reasonable correlation for some of the essential acids using only 2-4 variables. The individual model correlations and the significance of each factor is shown in Table 3. As you can see, the three most important parameters in most models were the time the wet cake sat before drying, the amount of syrup applied, and the outlet moisture.

Table 3: P-Values for significant factors in regression models for the essential amino acids. The overall model R^2 is shown in the last column.

Amino Acid	Blower	Inlet Temp.	Outlet Temp.	Outlet Moisture	Percent Oxygen in Drum	Syrup Addition Rate	Time	Intercept	Model Correlation
Arg		0.0067		0.0001		0.0002	0.0002	0.0001	1
Phe		0.0538		0.0072		0.0086	0.0025	0.0005	0.97
His				0.008		0.0026	0.0284	0.0001	0.94
Thr				0.0048		0.0217	0.0072	0.0001	0.93
Leu				0.0116		0.0223	0.005	0.0001	0.92
Lys				0.0229		0.0886	0.0016	0.0001	0.92
Trp		0.0101		0.0427			0.0052	0.0128	0.90
Val	0.0302					0.0025		0.0001	0.84
Met						0.0055	0.0485	0.0001	0.80
Ile	0.0629					0.0061		0.0001	0.79
Green = 5% Confidence Interval									
Yellow = 10% Confidence Interval									

A brief discussion of why these variables are significant is warranted. While wet cake was stored until dried, the material is exposed to mold and other microorganisms. It is possible during this lag time, microbes change the amino acid composition of the material. Contrary to what one would expect, this lag time usually resulted in an increase in the essential amino acid composition, not a loss. Syrup has an essential amino acid composition less than that of wet cake. It is reasonable to expect a decrease in the essential amino acid composition with full application of syrup to the feed. Outlet moisture content is strongly correlated to the outlet temperature. At higher temperatures, amino acids are degraded and more moisture is released from the DDGS particles. Thus low moisture content would be indicative of increased protein degradation and a lower amino acid composition.

Although it was expected that outlet temperature would have a significant effect, the outlet moisture may actually be more indicative of the temperature experienced in the DDGS particle. Oxygen content also did not seem to have a significant effect in any of the essential amino acid compositions, implying small protein oxidation rates.

The overall conclusion of these results is that amino acid composition of the animal feed can be modeled and possibly predicted based on the process parameters used in de-watering and drying the animal feed. The fuel ethanol industry could apply the method used in this trial to create designer animal feeds that target a particular amino acid composition, while optimizing the overall cost of processing DDGS. In addition using neural nets, which will occur in the Phase III trial, would increase the predictive power of these methods further.

The final test needed to fully analyze the samples is a protein digestibility assay donated by Novus International. The samples obtained in May will be analyzed this quarter using the Novus kit.

5.2 Phase II NCERC trial to determine slurry DE based on process conditions

In August, the NCERC performed a plant experiment to create a neural net model of liquefaction and saccharification parameters that affect fermentation. The purpose of this experiment was to determine how changes in processes before fermentation affect the starting conditions of fermentation.

In order for corn to be fermented to alcohol, it first has to be ground, mixed with water and enzymes, and pH adjusted. This process, known as liquefaction, is responsible for the initial conditions of the fermentor and is likely responsible for the majority of variation in fermentation. Ultimately, the quality of the fermentation determines the resulting nutritional composition of the stillage that becomes DDGS.

The most important initial condition of fermentation is the concentration and form of the starch molecules. Initially, starch exists in long chains that must be broken up during liquefaction into smaller polymers. Once a fermentor has started filling with mash, gluco-amylase is added to convert these starch oligomers to glucose. Yeast is added shortly after to consume the glucose under anaerobic conditions to create alcohol.

There are a myriad of variables in liquefaction that can be adjusted to create a particular starting material for fermentation. Eleven were chosen and plant trials were designed to vary these parameters in a controlled manner. Unfortunately, during the trial pH control on the slurry was lost and had to be abandoned. The slurry pH continued to be measured but could no longer be considered a controlled variable. As these parameters were varied, samples were taken to measure the starch and sugar concentration.

The most common test for this initial starch concentration is called the dextrose equivalent (DE) test (details are given in the appendix). Using this test, we tested 100 samples of mash to determine its starch concentration while varying process conditions. The DE number, which corresponds to this starch concentration varied from a 6 to as high as 18, showing tremendous variation in this starch concentration with processing. In addition, to a large variation due to processing conditions, a stepwise regression model of liquefaction had an overall R^2 of 0.92, indicating strong correlation of plant factors to the DE number. Both of these results indicate a significant impact of plant processes on the initial starch composition critical for complete fermentation.

Table 4 shows significant control variables that impacted DE, their estimates, and their significance. In addition, two un-controlled but measured variables (ph, solids fraction) were included in the regression. The results indicate that the most important variable in the process is the temperature of secondary liquefaction. Secondary liquefaction occurs when enzyme is added a second time and allowed to sit for longer times at high temperatures. At higher temperatures the DE number decreased, a negative correlation. Possibly at higher temperatures, enzyme degradation is increased and ultimately depresses the conversion of the starch polymers to oligomers.

Table 4: Stepwise regression model for DE number with plant processing factors.

<i>Term</i>	<i>Estimate</i>	<i>Std Error</i>	<i>t Ratio</i>	<i>Prob> t </i>
950 Level	-0.03074	0.016112	-1.91	0.069
990 Enzyme Flow	0.033125	0.016672	1.99	0.059
990 Level	0.024566	0.010729	2.29	0.0315
Solids Loading	-0.50492	0.129306	-3.9	0.0007
Mash pH	6.13489	1.322264	4.64	0.0001
Intercept	54.78893	11.01006	4.98	<.0001
950 Temperature	-0.34252	0.028359	-12.08	<.0001

Other variables that are measured but not controlled could also be important to providing an estimate for the DE number. Examples of these are the densities of the slurry and wort as well as the pressures in the flash vessels and tanks. A neural network analysis is being done to incorporate both the controlled and measured variables and to create a predictive model for DE using time series data. The results of that analysis will be shown in the next grant report, but preliminary data suggests R^2 values greater than 0.95 can be obtained using neural nets and prediction of DE number based on these factors can be obtained.

5.3 Current Status

Samples taken from the Phase II trial are being fermented to determine alcohol yields and residual starch values. The fermented samples will be analyzed using an HPLC to obtain the starch oligomer, glucose, and ethanol concentrations. Fermented samples will also be frozen so that other tests can be performed later if necessary.

Plans for the next quarter

Preparations are being made for the final plant experiment sponsored by this grant. This experiment will look at significant factors identified in the previous plant trials and observe their impact on the DDGS nutritional values directly. This trial is scheduled to start on October 16, 2006 and will continue for 26 days.

Upon the completion of the final trial, the data from all three trials will be compiled and the focus will be to spend the remaining time in the grant disseminating the results from these trials to the academic and industrial communities.

References

8. Appendix

What does Dextrose Equivalent Mean?

The dextrose equivalent or DE test is widely used as a measure of the extent of conversion of starch to glucose. Chemically, the DE test determines the number of glycosidic bonds that have been broken relative to an initial number of intact glycosidic bonds.

$$DE = 100 \times \left(\frac{\text{Number of reducing ends}}{\text{Number of } \alpha-1,4 \text{ bonds}} \right)$$

To give an example, let's take a single 1000 glucose long polymer of starch. To simplify matters, we will assume it is amylose, which contains no branches. If we put that starch molecule in solution there are two ends to the chain, but only one of those can react chemically and is called the **reducing end**. Now let's add some α -amylase for a few moments and break that 1000 unit starch into 100 ten unit pieces. Now there are 100 exposed reducing ends of the polymer out of an initial number of 999 unexposed ends. This would correspond to a DE number of $100 \times (100/999) \approx 10$.

Since the DE test determines the fraction of available ends, it is closely related to the degree of polymerization (DP) of starch. To a good approximation,

$$DP = \frac{100}{DE}$$

Thus, in our previous example, the average size of the starch polymer chains is $100/10 = 10$ units of glucose. Now, in the real world not all starch chains are the same length. A distribution of chain lengths exists. However, DP is still the number average length of the chains as defined by a number weighted average.

$$DP = \frac{\sum_i n_i X_i}{\sum_i n_i}$$

The chain length of each type of starch chain, i , is X_i and the number of those chains have that molecular weight is n_i .

Thus, ultimately the DE test gives us the average length of the starch polymers in solution, but it does not give us an idea of the distribution of these molecules. For example, take a solution containing a 1000 unit starch molecule and break it into 25 chains of 20 glucose units each and 100 chains of 5 glucose units each. The DE test would find 125 reducing ends out of 999 for a value of 12.5. The predicted chain length would be $100/12.5 = 8$ units, but there are no eight unit starches in solution, only a mixture of 5 and 20 units.

To get an idea of the dispersion of the starch chains around the mean, one needs the polydispersity, P . Polydispersity is the ratio of a weighted average of molecular weight to a number average of molecular weight.

$$\bar{M}_n = \frac{\sum_i n_i M_i}{\sum_i n_i}$$

$$\bar{M}_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i}$$

$$P = \frac{\bar{M}_w}{\bar{M}_n}$$

Polydispersity is measure of the distribution of molecular weights of the polymer, similar to standard deviation in principle. Polydispersity can be determined using an HPLC column that can measure the distribution of sugar molecules sizes with a size exclusion column. With both the average chain length or average chain molecular weight and the polydispersity a suspension of small-chained starch molecules can be characterized using normal statistics.

DE is perhaps the most important initial parameter in fermentation and determines the extent of both sugar and alcohol production in fermentation. In addition, it can determine the extent to which the yeast metabolism occurs and changes the chemical composition of fermentation.