Biorefinery concept development based on wheat flour milling

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Abstract -- We are developing an innovative process for the recovery of a starch-rich product from millfeed, the low-value byproduct of wheat flour milling; enzymatic processing of the starch to glucose; and the subsequent processes for conversion of that glucose into a value-added product by either a catalytic or a fermentation process. We have completed the development of the starch recovery step with enzymatic processing and the assessment of its economic viability. The processes to use the glucose product as feedstock for catalytic processing and fermentation processing have been tested in the laboratory. Catalytic processing of the glucose from the extracted starch for polyol production is based on catalytic hydrogenation to sorbitol. Alternatively, fermentation of the extracted starch-derived glucose also provides a pathway to value-added chemical products via a platform chemical, lactic acid.

The paper includes results from all the processing areas addressed. Starch extraction and glucose generation from wheat milling byproducts are presented with laboratory and scaled-up processing results. Results of fermentation of the glucose product to lactic acid in shaker flask tests are presented, documenting the minimal requirements for nutrient addition. Stirred batch reactor tests of catalytic hydrogenation of the glucose product to sorbitol are presented with a discussion of contaminant effects on the catalyst.

Key Words: wheat, millfeed, starch, glucose, extraction, hydrolysis, fermentation, hydrogenation

Background

Wheat is the second largest agricultural commodity in the United States with a net production of 56.1 million tonnes in 1995 (2.1 billion bushels). Of that production, about half is exported; and of the balance, 85% is dry-milled within the U.S. to produce 17.8 million tonnes of flour for human food consumption. The remaining 5.9 million tonnes are low-value byproducts, which

are disposed as animal feed, for the most part. These byproducts, collectively referred to as millfeed, contain the bran, germ, and a portion of the endosperm, which contains a significant amount of starch not recovered in the milling process.

Millfeed is a combination of cellular structures and components left from the wheat kernel after the flour removal; it contains about 75% carbohydrate, 17% protein, 3% fat, and 5% mineral matter. Millfeed is not a single homogeneous stream but a collection of several streams from the process of making flour. In addition, millfeed composition varies depending on the variety of wheat being processed into flour. Millfeed production varies slightly depending on mill operation and variety of wheat processed. Flour extraction ranges from 73 to 77% resulting in an average millfeed production of about 25% by weight of the wheat introduced to the mill. Considering that the wheat kernel contains 83% endosperm (the starchy source of white flour), it is apparent that the millfeed contains, in addition to the bran, a significant portion of the starchy endosperm. A rough calculation suggests that millfeed is composed of up to 35% starch that originated from the endosperm.

Wheat Milling Byproducts Used for Chemical Production

We are developing an innovative process for the recovery of a starch-rich product from millfeed, the low-value byproduct of wheat flour milling; enzymatic processing of the starch to glucose; and the subsequent processes for conversion of that glucose into a value-added product by either a catalytic or a fermentation process. We have completed the development of the starch recovery step with enzymatic processing and the assessment of its economic viability. The processes to use the glucose product as feedstock for catalytic processing and fermentation processing have been tested in the laboratory. Catalytic processing of the glucose from the extracted starch for polyol production is based on catalytic hydrogenation to sorbitol. Alternatively, fermentation of the extracted starch-derived glucose also provides a pathway to value-added chemical products via a platform chemical, lactic acid.

In either pathway to value-added products (catalytic or fermentation), use of the residual millfeed will be an important consideration. If the residual material has value as a premium animal feed, it will reduce the actual cost of the extracted starch. By extracting the starch from the millfeed, the food value of the remaining material may actually be increased by concentrating the protein content. The improved value of this starch-extracted millfeed for animal feeding has undergone preliminary evaluation but needs to be validated in animal feeding tests.

Technical Results

The paper includes results from all the processing areas addressed. Starch extraction and glucose generation from wheat milling byproducts are presented with laboratory and scaled-up processing results. Results of fermentation of the glucose product to lactic acid in shaker flask tests are presented, documenting the minimal requirements for nutrient addition. Stirred batch reactor tests of catalytic hydrogenation of the glucose product to sorbitol are presented with a discussion of contaminant effects on the catalyst.

Starch Extraction and Glucose Production

This section describes the results of the starch extraction and glucose production process development phase. The primary tasks under this phase were 1) millfeed analyses, 2) bench-scale testing, 3) pilot-scale testing.

Wheat Millfeed Analyses: Several different wheat millfeed types and fractions were received for testing purposes. Five fractions were derived from soft red winter wheat including "midds, bran and screenings", "midds and bran", "red dog" (similar to midds), "bran", and "screenings". From four other wheat types: soft white winter, hard red winter, hard amber durum, and hard red spring, the fractions included millfeed stream with or without the screenings, "shorts," (similar to midds) and "bran" from each wheat type and a combined screenings sample.

These byproduct millfeed samples were analyzed and the data are presented in Table 1.

Bench-scale Testing for Process Optimization: The process optimization testing was conducted on the bench-scale. Process optimization testing was conducted, using a systematic approach to evaluate each key-processing step in the order that it appears in the process (see Figure 1). After testing each of the individual unit operations, the optimized conditions were then tested together to arrive at an optimized starch extraction process.

A specified amount of water was added to a 250-mL flask, and then placed in an incubator (and constantly shaken at 300 rpm) for heat-up to a predetermined temperature. Millfeed was then added to the 250-mL flask, and it was placed back into the incubator for a predetermined time ("initial water wash"). The millfeed slurry was then removed from the incubator and "coursed filtered" via vacuum filtration for 10-minutes through a 4-inch Buchner filter (no filter paper used). The filtercake from the course filtration was typically dried and retained for analyses. The filtrate was collected, pH-adjusted if necessary, and placed back into the incubator for heat-up to a predetermined "liquefaction" temperature. After reaching, the specified liquefaction temperature, α-amylase was added, and "liquefaction" was initiated.

Following the liquefaction step, the incubator temperature was adjusted to a predetermined "saccharification" temperature and pH adjustments were made if necessary. After reaching the desired "saccharification" temperature, glucoamylase was added to the solution, and it was placed in the incubator for a specified "saccharification" time.

Following a specified time, the product from "saccharification" was removed, and again filtered. This second filtering operation, termed "fine filtration" was conducted via vacuum filtration for 10-minutes, using a 4-inch Buchner funnel with Whatman #4 filter paper. Following "fine filtration", the filtercake was typically dried and retained for analyses. The filtrate from the "fine filtration" step was also collected for analytical purposes. All of the optimization testing was conducted using "soft white winter all inclusive without screenings" millfeed".

Table 1. Analytical Results of Wheat Millfeeds.

Millfeed Type	Component Concentration (wt%)								
	Moisture	Ash	Carbon	Hydrogen	Nitrogen	Protein[a]	Fat	NDF[b]	Starch[c]
Soft Red Winter									
Midds, Bran & Screenings	11.2, 11.6	5.23, 5.08	45.7	6.12	2.71, 2.82	16.9, 17.6	3.64	39.2	33.5, 34.6
Midds & Bran	11.0, 10.9	5.36, 5.49	45.3	6.36	2.88, 2.86	18.0, 17.9	3.95	41.7	29.8, 31.1
Red Dog	9.77, 9.72	3.70, 3.73	46.2	6.51	2.89, 2.85	18.1, 17.8	4.98	27.1	34.6, 46.4
Bran	11.0, 10.2	6.61, 6.83	45.3	6.15	2.46, 2.71	15.4, 16.9	2.33	48.4	22.4, 25.6
Screenings	12.4	2.40	45.4	6.20	2.45	15.3	NA	NA	NA
Soft White Winter									
All Inclusive	9.32, 9.85	4.71, 4.79	45.5	6.15	2.77, 3.07	17.3, 19.2	2.82	34.4	31.7, 38.9
All Inclusive without Screenings	9.05	5.27	45.5	6.39	2.65	16.6	3.05	41.5	22.0, 31.3
Shorts	8.78, 9.46	4.44, 4.67	46.1	6.05	2.73, 2.91	17.1, 18.2	4.19	33.1	30.5, 39.8
Bran	9.25, 10.2	6.12, 6.11	45.3	6.07	2.89, 2.84	18.1, 17.7	2.59	44.7	18.8, 28.9
Hard Red Winter									
All Inclusive	8.75, 9.57	4.45, 4.77	45.8	6.06	2.74, 3.04	17.1, 19.0	3.07	37.8	31.3, 35.3
All Inclusive without Screenings	8.38	5.23	45.8	5.98	2.76	17.3	4.55	43.9	16.1, 27.3
Shorts	8.94, 9.24	4.75, 4.82	46.2	6.12	3.05, 3.05	19.1, 19.1	4.41	39.1	25.6, 32.7
Bran	9.10, 9.67	6.28, 6.58	45.8	6.21	2.70, 2.91	16.9, 18.2	3.68	53.1	11.8, 18.4
Hard Amber Durum									
All Inclusive	9.63, 9.84	4.16, 4.58	46.4	6.28	2.94, 3.05	18.4, 19.1	4.77	33.0	37.0, 38.5
All Inclusive without Screenings	8.77, 9.67	4.87, 4.71	46.0	6.00	3.18, 3.31	19.9, 20.7	5.03	31.1	31.3, 38.4
Shorts	8.93, 9.54	3.91, 3.75	NA	NA	3.34	20.9	4.73	22.3	43.7, 48.4
Bran	9.09, 9.50	5.77, 5.85	46.4	6.09	2.79, 3.02	17.4, 18.9	5.82	43.6	18.0, 25.8
Hard Red Spring									
All Inclusive	9.25, 9.62	4.91	NA	NA	2.98	18.6	3.02	39.4	30.4, 34.0
All Inclusive without Screenings	8.80, 10.3	6.17	NA	NA	3.30	20.6	3.83	48.6	23.0, 20.8
Shorts	9.00, 9.49	3.94	NA	NA	3.20	20.0	4.56	28.1	35.6, 43.5
Bran	8.95, 8.10	6.45, 7.02	NA	NA	3.04	19.0	3.41	54.9	13.7, 15.7
Combined Screenings									
	9.16, 9.05	2.68, 2.77	45.9	6.31	2.71, 2.91	16.9, 18.2	2.27	23.7	53.7, 53.1

(a): Protein obtained by multiplying nitrogen value by 6.25.
(b): NDF = neutral detergent fiber (~ cellulose + hemicellulose + lignin).
(c): First listed starch value obtained via direct analyses, second listed starch value obtained indirectly by taking 100-NDF-Fat-Protein-Ash.

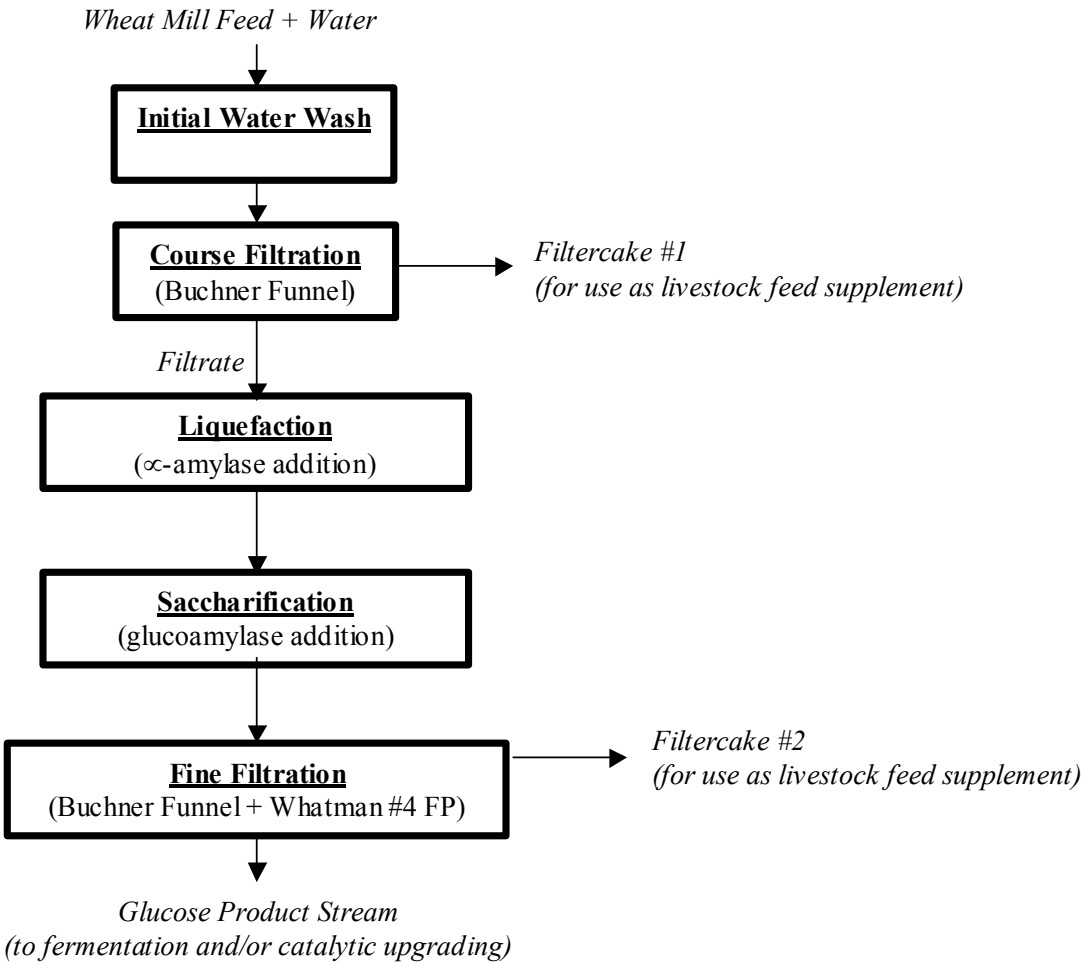

Figure 1. Starch Extraction Key Processing Steps.

Initial Water Wash: The key initial water wash parameters investigated were time (0.25-24 hrs), and temperature (25-80°C). During the testing 30 g wet basis (~27 g dry basis) of "soft white winter all inclusive without screenings" millfeed was added to 150 g deionized water for the specified water wash temperature and time. The subsequent course filtration, liquefaction, saccharification, and fine filtration steps were then carried out (see Figure 1). The final filtrate was then analyzed for glucose concentration. In addition, the filtercakes from the course and fine filtration were dried to determine the solids concentration. During this testing, the liquefaction and saccharification parameters were held constant.

The primary purpose of the initial water wash is to enhance the separation of the starch-containing solids away from the rest of the material. This starch-containing material is typically smaller than the other "branny" material and is very rich in starch, but also contains some protein. The water wash acts to liberate the starch-containing material by breaking weak hydrogen bonds that hold some of the starch-containing material to the other solids. Thus, a

fairly good measure of the effectiveness of the initial water wash is the amount of starch-containing material recovered after the water wash/course filtration step. Another good measure is the total glucose yield (assuming all steps downstream of the water wash and course filtration steps are conducted at the same conditions).

It was observed that operation at solids (millfeed) loadings greater than approximately 15% was difficult. For example, at a 25% solids loading, there was very little free water available, which caused problems with mixing and filtering the slurry solution during the course filtration step.

Liquefaction: The key liquefaction parameters investigated were time (1-4 hrs), temperature (65-85°C), and enzyme type and concentration (0 to 0.05 wt%, dry millfeed basis). Two different α-amylase enzymes were evaluated; a low-temperature and a high-temperature version. No pH adjustment was made to the feed solution to liquefaction; the pH was approximately 7 in all cases.

During the testing 30 g wet basis (~27 g dry basis) of "soft white winter all inclusive without screenings" millfeed was added to 150 g deionized water for the specified water wash temperature and time. The subsequent course filtration, liquefaction, saccharification, and fine filtration steps were then carried out (see Figure 1). The final filtrate was then analyzed for glucose concentration. In addition, the filtercakes from the course and fine filtration were dried to determine the solids concentration. During this testing, the water wash and saccharification parameters were held constant.

The primary purpose of the liquefaction step is to partially hydrolyze the starch, so that it is for the most part solubilized. This allows the glucoamylase, used in the saccharification step downstream, to more efficiently convert the starch to glucose.

From our test results we concluded that conducting the liquefaction step, using the high temperature ∝-amylase results in both the highest glucose conversion and recovery in the product stream (i.e., efficient dewatering in the fine filtration step).

Saccharification: The key saccharification parameters investigated were time (1-4 hrs), temperature (55-65°C), and enzyme concentration (0.04 to 0.22 wt%, dry millfeed basis). The feed solution to saccharification was pH adjusted to approximately 5, using a buffer solution. The primary purpose of the saccharification step is to complete the hydrolysis of starch to glucose.

During the testing 30 g wet basis (~27 g dry basis) of "soft white winter all inclusive without screenings" millfeed was added to 150 g deionized water for the specified water wash temperature and time. The subsequent course filtration, liquefaction, saccharification, and fine filtration steps were then carried out (see Figure 1). The final filtrate was then analyzed for glucose concentration. In addition, the filtercakes from the course and fine filtration were dried to determine the solids concentration. During this testing, the water wash and liquefaction parameters were held constant

Baseline Process Definition: The baseline millfeed starch extraction process, shown in Figure 2, was developed using the bench-scale and feed analyses results discussed above. The millfeed to the starch extraction process is assumed to be "*soft white winter without screenings*".

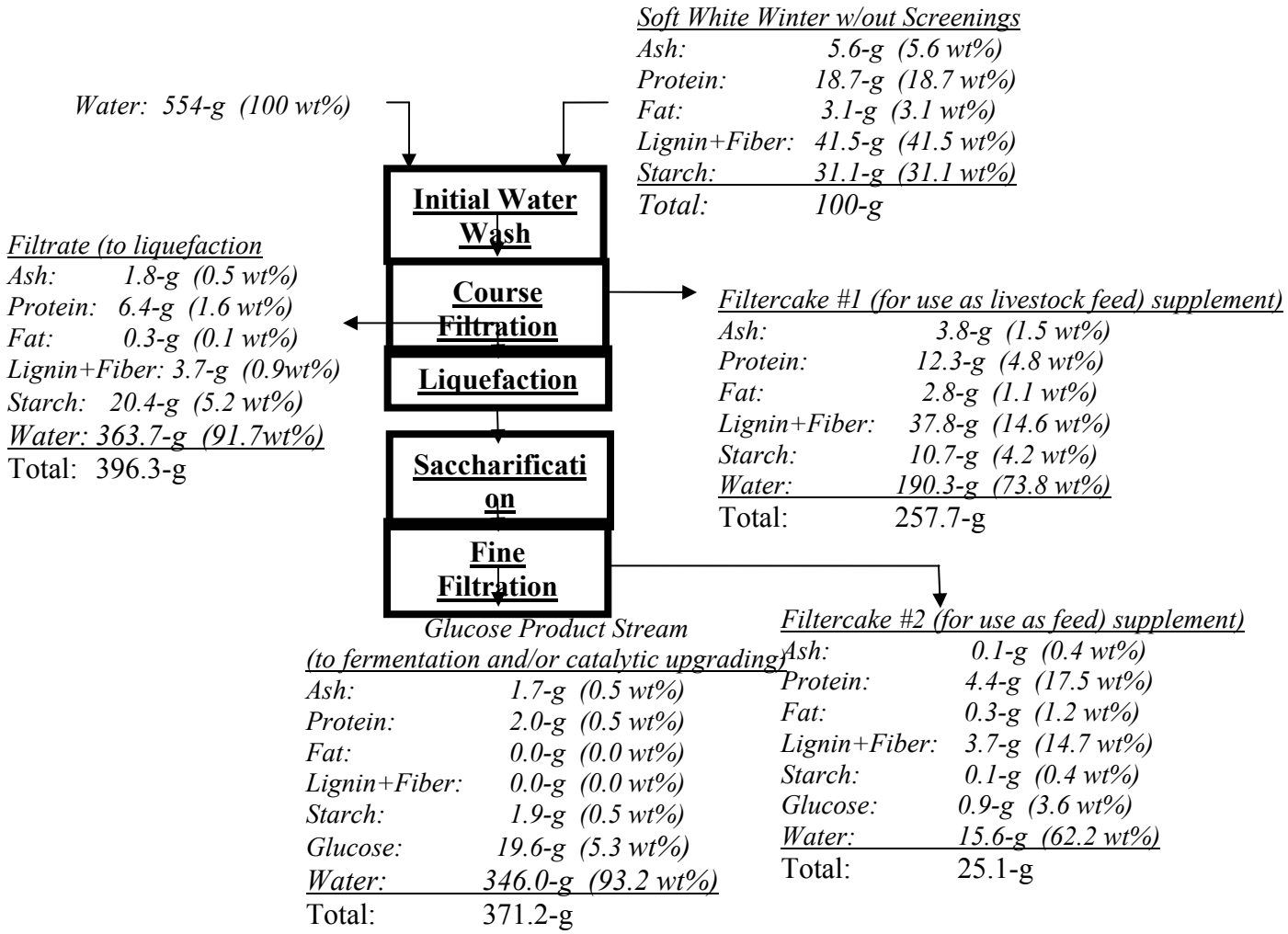

Figure 2. Baseline Process

Approximately 33% of the original millfeed is recovered in the filtrate following the water wash and course filtration steps. In addition, approximately 66% of the original starch is recovered for further processing. The remaining 34% leaves with filtercake #1, and is primarily associated with the water in the filtercake. Recovery of this starch is key to improving the baseline process. The majority of the lignin + fiber, fat, and most of the protein exit the process in filtercake #1. The protein content of filtercake #1 is approximately 18% (dry solids basis), which is comparable to the initial millfeed protein content. Thus, based on protein content, filtercake #1 should be comparable to the original millfeed for use as a livestock feed supplement.

The glucose product stream following the liquefaction, saccharification, and fine filtration steps contains approximately 5.3 wt% glucose (total stream basis). Increasing the concentration of glucose in this stream is another process improvement that is being developed.

Approximately 90% of the starch entering the liquefaction process is converted to glucose, and conversions of greater than 95% were achieved during some of the testing. In addition, greater than 95% of the glucose produced is recovered in the product stream, (primarily due to the excellent dewatering of filtercake #2). The baseline process shows that approximately 19.6% of the original dry millfeed is recovered as glucose. The test data also suggests that the higher starch content analysis (see Table 1) of the original "*soft white winter without screenings*" millfeed is the more accurate value.

Filtercake #2 has a protein content of approximately 46% (dry solids basis). Therefore, based on protein content this material should be superior to either the original millfeed or filtercake #1 as a livestock feed supplement. This is a relatively small stream however (i.e., <10% of the original millfeed stream, dry solids basis) as compared to filtercake #1 (i.e., >67% of the original millfeed stream, dry solids basis).

<u>Millfeed Effects:</u> All of the initial process optimization testing discussed above was conducted using *""soft white winter all inclusive without screenings"* millfeed. Testing was also conducted, using other millfeed varieties and fractions. The millfeed varieties included soft red winter, soft white winter, hard red winter, hard amber durum, and hard red spring. The soft red winter wheat included five fractions: "midds, bran and screenings", "midds and bran", "red dog" (similar to midds), "bran", and "screenings". The other millfeed variety fractions included "all inclusive", "all inclusive without screenings", "shorts," (similar to midds) and "bran". In addition, a combined screenings sample was evaluated.

The results are given in Table 2. The starch content of the millfeed is given in Table 2 (also see Table 1), along with the total glucose yield on a dry millfeed basis. The total glucose yield is the glucose in the product filtrate and wet filtercake streams from the fine filtration step (see Figure 2).

The results indicate that there is not a dramatic difference in processing different millfeed varieties, given that the fractions are the same (e.g., comparison of soft white winter bran and hard amber durum bran). When processing any of the "all inclusive" fractions of the millfeed varieties evaluated, via the baseline starch recovery process (see Figure 1), between 14% to 20% of the millfeed was recovered as glucose (i.e., glucose in the fine filtration filtrate and filtercake streams).

A more significant difference is observed when comparing the fractions within a certain variety of wheat. For example, from a filtering viewpoint, the bran and shorts are very different from one another. The glucose yields appear to be slightly different for these as well, with the glucose yield from bran being significantly lower than that of shorts in most cases. This difference may in part be due to the differences in filtering the two millfeed fractions. Two somewhat inconsistent results were obtained for "hard red spring, all inclusive without screenings" and "hard red winter shorts".

Table 2. Wheat Millfeed Effects on Glucose Recovery.

Millfeed Type	Starch (wt%, Dry Millfeed Basis)[a]	Total Glucose Yield (wt%, Dry Millfeed Basis)
Soft Red Winter		
Midds, Bran & Screenings	33.5, 34.6	19.5
Midds & Bran	29.8, 31.1	18.4
Red Dog	34.6, 46.4	7.4
Bran	22.4, 25.6	13.3
Screenings	NA	7.0
Soft White Winter		
All Inclusive	31.7, 38.9	17.8
All Inclusive without Screenings	22.0, 31.3	19.6
Shorts	30.5, 39.8	20.7
Bran	18.8, 28.9	11.2
Hard Red Winter		
All Inclusive	31.3, 35.3	15.3
All Inclusive without Screenings	16.1, 27.3	14.4
Shorts	25.6, 32.7	10.8
Bran	11.8, 18.4	6.2
Hard Amber Durum		
All Inclusive	37.0, 38.5	14.2
All Inclusive without Screenings	31.3, 38.4	18.0
Shorts	43.7, 48.4	27.5
Bran	18.0, 25.8	9.3
Hard Red Spring		
All Inclusive	30.4, 34.0	13.7
All Inclusive without Screenings	23.0, 20.8	7.9
Shorts	35.6, 43.5	18.6
Bran	13.7, 15.7	4.3
Combined Screenings		
	53.7, 53.1	16.3

Pilot-scale Testing: A pilot-scale test was conducted primarily to produce sufficient amounts of solid fiber material from the course and fine filtration steps to supply for analyses and animal feed studies. Secondary purposes for conducting the test were to compare glucose yields with the small-scale testing results, to evaluate some of the processing steps (i.e., pumping, mixing and filtration) at a larger scale, and to provide material for downstream catalytic processing.

An existing system at PNNL (after slight modifications were made) was used to conduct the pilot-scale testing. The system is shown in Figure 3. It contains two 200-gallon tanks with impellors, and an inline heater/circulation system for each tank. The system has the capability of processing material at temperatures up to approximately 90°C. The system also has a small acid feed tank. Temperature and pH controls exist in the system.

Figure 3. Pilot-scale Starch Recovery System.

At the beginning of the test, 113 gallons (~940 lbs) of tap water was fed to one of the 200-gallon tanks and heated. After reaching temperature, 131 lbs of a 50/50 (wt/wt, dry weight basis) blend of hard red winter without screenings + soft white winter without screenings millfeed was gradually added through a port-hole in the top of the tank. The slurry was held at temperature for the water wash step in the agitated tank. The solution was then course filtered by first passing it across a pilot-scale screen, and then further dewatered using 400 and 600 micron filter bags. A portion of the course filtercake was then rinsed with tap water and again filtered using 400 and 600 micron filter bags, and then dried at 70°C for 44 hrs. This dried filtercake was then sent for analyses and animal feed testing.

The filtrate (containing starch) from the course filtration step was returned to the processing tank, heated, and α-amylase was added to liquefy the starch.

The solution was then cooled, the solution pH adjusted by adding sulfuric acid, and glucoamylase added to convert the liquefied starch to glucose. A portion of the solution was then fine filtered by using 25 micron filter bags. This fine filtercake was then rinsed with tap water and again filtered using 25 micron filter bags, and then dried at 70°C (atmospheric pressure) for 34 hrs. This dried filtercake was then sent for analyses and animal feed testing.

The material balance around the entire pilot-scale test was >99% (not including the filtercake rinsing steps). The material balance around the course filtercake rinse step was 97.5% and around the fine filtercake rinse step was 98.1%.

Operationally, filtration was the most difficult to implement during the pilot-scale testing. The course filtration progressed fairly well, with the exception that more of the large mesh size fibrous material was passed through in the feed to liquefaction than was desired. This can be corrected by using smaller screen/mesh sizes. The fine filtration step was very difficult to implement. It was extremely difficult to dewater the fine filtercake during the pilot-scale testing.

Glucose concentrations and yields are given in Table 3. For comparison, glucose concentrations and yields are also given for small-scale testing using the 50/50 blend of hard red winter without screenings + soft white without screenings. Same solids loading and similar processing conditions were used for both tests. The only difference between the small-scale testing and the pilot-scale testing in this case is that the soft white winter without screenings and hard red winter without screenings were obtained from different mill runs (i.e., the two materials used for the pilot-scale testing was received as a separate aliquot at a later time).

Table 3. Glucose Concentration and Yield.

	Small-scale Testing	Pilot-scale Testing
Glucose Concentration[(a)]	3.5%	3.4%
Glucose Yield[(b)]	18.7%	20.3%

(a): Glucose concentration in stream from saccharification after filtration through a 0.45 micron filter.
(b): Glucose yield is on a dry millfeed basis, and are also based on the solution exiting saccharification (i.e., does not include glucose contained in the wet course filtercake, but does include glucose in the wet fine filtercake

The values given in Table 3 were obtained by taking a small sample of the solution after saccharification, filtering it through a 0.45 micron filter and then analyzing the solution for glucose. This was then combined with material balance information to obtain the glucose yields that are reported. The glucose yields are on a dry millfeed basis, and are also based on the solution exiting saccharification (i.e., does not include glucose contained in the wet course filtercake, but does include glucose in the wet fine filtercake). As can be seen, the small-scale and pilot-scale testing results are in agreement with one another.

Incorporation of the glucose estimated to be lost in the water associated with the wet course filtercake during the pilot-scale tests gives a total glucose yield of approximately 29.9 wt% (dry millfeed basis). This is the maximum glucose yield expected (i.e., using process improvements such as thorough rinsing/recovery of rinsate, and/or countercurrent washing).

<u>By-Product Stream Evaluation:</u> The recovered fine filtercake solids and course filtercake solids were analyzed and used in animal feed testing. The analytical results are shown in Table 4 along with reported values for other typical feedstock supplements (wheat midds, corn gluten feed, corn, and soybean meal) for comparison.

The analytical results indicate that the course filtercake contained approximately 2.4 wt% residual starch, and the fine filtercake material contained approximately 3 wt% residual starch.

The protein analyses show that the fine filtercake material had a protein content of 33.9% (dry solids basis) and the course filtercake material had a protein content of approximately 17.1% (dry solids basis). The protein content of the fine filtercake material is significantly lower than

that achieved in some of the small scale testing (i.e., 50% protein and higher have been attained). This is likely due to less than ideal course filtration during the pilot-scale test. It is anticipated that more of the large mesh size fibrous material (i.e., low protein content) passed through the course filtration than is desired.

Table 4. Chemical Compositions of Coarse and Fine Filtercake By-products in Comparison to Other Feed for Ruminant Animals.

Nutrients	Coarse Filtercake By-product	Fine Filtercake By-product	Wheat Midds[(a)]	Corn gluten feed[(a)]	Corn[(a)]	Soybean meal[(a)]
Dry Matter (DM), %	90.60	94.50	89.30	90.00	90.00	90.00
Crude Protein (CP), %DM	17.10	33.90	17.20	23.80	9.80	51.80
Available CP, %DM	15.90	32.50	---	---	---	---
Unavailable CP, %DM	1.20	1.50	---	---	---	---
Adjusted CP, %DM	17.10	33.50	---	---	---	---
Soluble CP, %CP	22.00	9.70	40.00	49.00	11.00	20.00
Acid Detergent Fiber (ADF), %DM	22.00	18.90	11.70	12.70	3.30	7.00
Neutral Detergent Fiber (NDF), %DM	75.30	53.90	35.90	36.20	10.80	10.30
Fat, %DM	4.00	8.50	4.70	3.91	4.06	1.67
Ash, %DM	4.34	1.58	5.00	6.90	1.46	6.9
Total Digestible Nutrients (TDN), % DM	60.00	79.00	69.00	83.00	85.00	87.00
NE_L, Mcal/kg[(b)]	1.39	1.89	1.57	1.91	1.96	2.01
NE_m, Mcal/kg[(b)]	1.19	1.91	1.60	2.03	2.10	2.16
NE_g, Mcal/kg[(b)]	0.62	1.28	1.00	1.37	1.43	1.48
Non-structural Carbohydrate (NSC), %DM	0.60	3.60	33.50	28.30	73.88	29.30
Starch, %DM	2.40	3.00	---	---	---	---
Ca, %DM	0.22	0.06	.17	0.07	0.03	0.46
P, %DM	1.12	0.38	1.01	0.95	0.32	0.73
Mg, %DM	0.61	0.08	0.40	0.40	0.12	0.32
K, %DM	0.36	0.19	1.81	1.40	0.44	2.42
Na, %DM	0.00	0.01	0.02	0.26	0.01	0.07
S, %DM	0.13	0.29	0.19	0.47	0.11	0.46
Fe, ppm	156.00	215	170	226	55	277
Zn, ppm	107.00	40	102	73	24	67
Cu, ppm	16.00	6	13	7	3	19
Mn, ppm	194.00	23	124	22	55	48
Mo, ppm	0.00	2	2	2	1	7

(a): Book values from Beef NRC (1996), Dairy NRC (1987) and DHI Lab.
(b): NE_L = net energy for lactation; NE_m = net energy for maintenance; NE_g = net energy for growth.

The feed analyses and feed testing results indicate the following:

- In ruminant diets: coarse byproduct (course filtercake) is estimated to be 65-96% of wheat midds value; fine byproduct (fine filtercake) to be 46-61% of soybean meal value.

- In poultry diets: fine byproduct (fine filtercake) is valued to be 167-204% of wheat midds. In swine diets: fine byproduct (fine filtercake) is estimated to be 171-232% of wheat midds value.

Fermentation Processing to Value-Added Products

The hydrolysate of wheat milling byproduct was used as the primary carbon source for the lactic acid fermentation process development. Batch cultures of lactic acid fermentation were primarily conducted to assess the feasibility of the fermentation process development.

Summary of Fermentation Results: Three lactic acid producing strains, *Lactobacillus rhamnosus* (ATCC 10863), *Lactobacillus delbrueckii subsp.lactis* (ATCC 12315), and *Lactobacillus rhamnosus* (ATCC 11443) were tested for their performance of lactic acid production. All strains showed favorable lactic acid production rates at the temperature of 43°C while there was some lactic acid fermentation inhibition at 45°C for strain *L.lactis*. At 43°C, most of the tests showed complete conversion of the glucose in the wheat millfeed hydrolysate into lactic acid. In general, a higher glucose concentration in the wheat byproduct hydrolysate required a longer fermentation time. In all the tested strains, *L rhamnosus* (ATCC 11443) had the best fermentation performance under the testing conditions in terms of shorter fermentation time and higher sugar tolerance. In the nutrient addition tests, results showed that the pure hydrolysate of wheat millfeed could entirely support the lactic acid fermentation process without lowering the conversion yield (The theoretical yield is 100% by weight). Based on the mineral analysis, manganese was the only critical element lacking in the hydrolysate medium. The fermentation batch culture without an addition of manganese could result in lower lactic acid yield than the one with the addition of manganese. Concentrated seed inoculation could speed up lactic acid fermentation and reduce lag time. Most encouraging of all, under the testing condition, the hydrolysate medium with a minimal manganese addition had better performance than the culture medium containing reagent grade glucose and other nutrients such as yeast extract and peptone. The superior performance of the hydrolysate medium is probably due to the presence of various amino acid and other nutrients in the wheat millfeed hydrolysate. Experimental results also indicated that a semi-batch or even a continuous culturing mode could provide higher fermentation performance than the batch culture due to the elimination of a culture lag phase and the maintenance of top fermentation activities. There were only low levels of fermentation byproducts, acetate and ethanol present in the culture broth. Crude wheat millfeed hydrolysate, pre-sterilized in the hydrolysate process step, could be directly used in the fermentation process, which could potentially lower the production cost.

Lactic Acid Fermentation Without the Addition of Growth Nutrients and Mineral Salts: The supplement of additional nutrients such as yeast extract and peptone and mineral salts is critical to both lactic acid production and its associated cost. The major objectives of this test were to

determine whether pure wheat byproduct hydrolysate could directly support lactic acid fermentation without the addition of yeast extract, peptone, and mineral salts. The elimination of nutrient addition to the fermentation medium can significantly reduce the cost of lactic acid production. Most of the minerals were available for lactic acid fermentation except manganese. Four different batches of wheat byproduct hydrolysate were used in the fermentation test; the glucose concentration in the feedstock ranged from 36 to 126 g/L prior to sterilization and inoculation. The bacterial strain ATCC 11443 was used for all the fermentation tests. Results show that pure wheat byproduct hydrolysate can support the entire lactic acid fermentation process, and the overall yield of glucose to lactate varied from 97 to 104% (± 3.0 – 7.5%) depending on the batch cultures. The slightly higher conversion yield was due to the utilization of other sugars such as fructose present in the hydrolysate. The highest lactate concentration reached 69.7 ± 2.4 g/L with an overall conversion yield of 102.3 ± 3.0%. In general, it took about 34 to 40 hours to complete the entire fermentation process when the glucose concentration in the hydrolysate ranged from 34 to 80 g/L. In the case that the culture had a glucose concentration of 98 g/L, the fermentation process lasted about 64 hours. However, the glucose in the tested cultures was all converted to lactic acid at levels ranging from 97-104 %.

To compare the productivity, Table 5 and Figure 4 summarize the batch fermentation results among the conditions with and without the addition of yeast extract, peptone, and mineral salts. The results had suggested that there were no effects of yeast extract and peptone on the lactic acid productivity, but the mineral salt addition might affect the productivity. The productivity plots in Figure 4 show that the most active productivity occurred during a 20 to 40-hour period for the cultures with initial glucose concentrations less than 80 g/L, indicating that the culturing age also affected the productivity. Based on the analysis of the concentrations of metals in the regular hydrolysate feed as shown in Table 6 there is apparently a lack of Mn and an insufficient amount of Fe in the pure hydrolysate as compared to the supplemented level of these minerals in the culture medium. In addition, there were threefold differences of the concentrations of K and P between the pure hydrolysate and the modified medium. In the next section, the fermentation tests were conducted to determine the effects of these metal concentrations.

Table 5. Lactate productivity comparison of batch cultures with and without additions of yeast extract, peptone, and mineral salts.

Hydrolysate Batch No.	Initial Glucose Concentration (g/L)	Yeast Extract and Peptone Addition	Addition of Mineral Salts	Lactic Acid Productivity* (g/L/hr)
WHC (concentrated)	70.2	Yes	Yes	3.15
WHC (concentrated)	72.4	No	Yes	3.13
WH (regular)	34.0	No	No	0.91
WHC1 (concentrated)	68.1	No	No	1.58
WHC2 (concentrated)	80.1	No	No	1.42
WHC3 (concentrated)	97.6	No	No	1.52

*Productivity was calculated when over 90% of the glucose was consumed in the culture.

Table 6. Mineral ion comparison in hydrolysate medium with and without mineral supplement.

Mineral Ion	Hydrolysate Filtrate (mg/L)	Hydrolysate Filtrate with Supplemented Mineral Ions (mg/L)
Mg	233	292
Mn	NS*	39
Fe	0.9	6.9
K	1,170	3378
P	561	1777
S	406	506

*NS: not significantly detected.

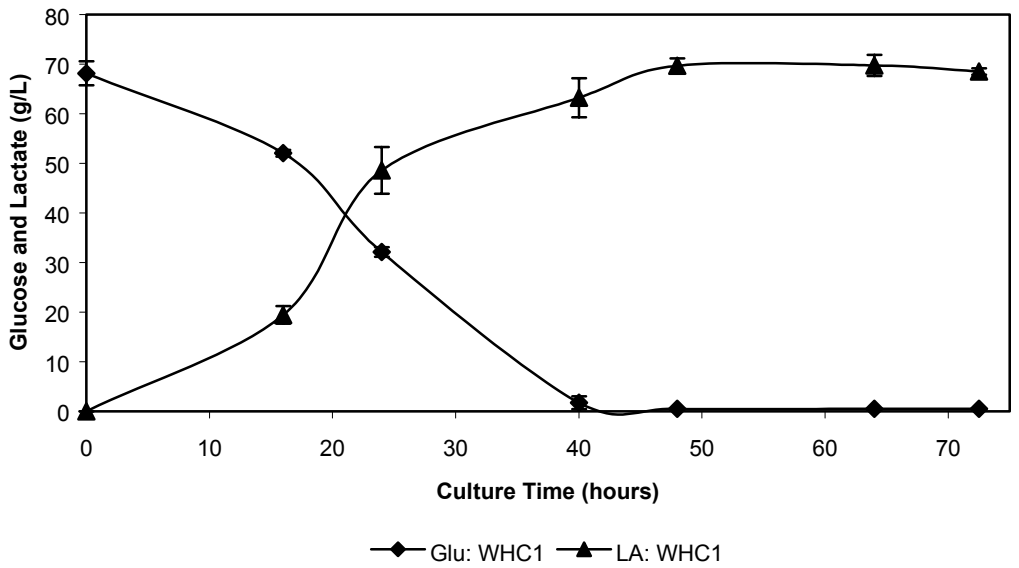

Figure 4. Lactic acid fermentation in batch cultures
Each data point represents the data averaged from triplicate cultures,
Error bars stand for standard deviation. Glu: glucose; LA: lactate.

Effects of metal ion additions on lactic acid fermentation: The hydrolysate medium has less iron and an insufficient amount of manganese, which might have significant effects on lactic acid fermentation and its productivity. The objective of this test was to determine the effects of Mn, Fe, and Mg levels on lactic acid fermentation. In addition, preliminary tests were also conducted to study the effects of seed inoculum concentration on lactic acid fermentation.

Table 7 summarizes the normalized overall lactate yield of glucose to lactate under different metal ion supplemental conditions. The normalized yields were calculated under the assumption that the yield in the batch culture supplemented with all mineral ions was 100%. As shown in Table 7, only the culture without the addition of Mn had a decrease of 6.8% in the normalized yield while others maintained the normalized lactate yield at 101.5 – 103.0 % (± 0.1 – 2.7%). The results imply that Mn is an important factor on lactic acid fermentation although the yield

difference is small under the current testing condition. It should be noticed that the seed inoculum, which comprises 10% of the fermentation volume, might bring in some levels of Mn, which needs to be determined.

Table 7. Normalized overall lactate yield from glucose without the addition of certain metal ions

Initial Metal Ion Concentration in Hydrolysate*			**Final Metal Ion Concentration (in Hydrolysate + Supplemented)**			**Normalized Overall Lactate Yield (%)**
Mn (mg/L)	**Fe (mg/L)**	**Mg (mg/L)**	**Mn (mg/L)**	**Fe (mg/L)**	**Mg (mg/L)**	
NS**	1.7	466.0	39.0	7.8	525.2	100.0
			NS	7.8	525.2	93.2
			39.0	1.7	525.2	101.5
			39.0	7.8	466	103.0

*The hydrolysate was concentrated by two-fold. Therefore, the initial metal ion concentrations were twice the ones before concentration.
**NS: not significantly detected.

The preliminary tests were made of seed inoculum concentration on lactic acid fermentation. After a two-day growth, seed culture was concentrated by two and fourfold through centrifugation. Consequently, the batch cultures were inoculated with 10% volume of concentrated seed culture (5 ml seed culture to 50 ml culture medium). Results show that during the initial 24 hours of fermentation, the batch cultures inoculated with 2x and 4x seed culture had higher fermentation productivity (2-6 % and 10-14% higher, respectively) than the cultures with only 1x seed inoculation. The preliminary results here imply that fed batch culture or even continuous culture could increase productivity since the culture can always be maintained at the top productive level due to elimination of the lag phase (for biomass accumulation) in the fermentation process.

Feedstock Comparison on Lactic Acid Fermentation: To compare the lactic acid fermentation performance between reagent grade glucose and wheat millfeed hydrolysate, batch fermentation cultures were set up using anhydrous D-(+)-glucose (99+%, ACROS Organics, NJ). The culture medium contains 7.2% glucose supplemented with yeast extract 0.5%, peptone 1.0%, and mineral salts. The comparison results are shown in Figure 5a and 5b for both types of batch cultures using either reagent glucose or wheat millfeed hydrolysate as primary substrate. The data indicates that the wheat millfeed hydrolysate has better performance than the pure glucose medium supplemented with growth nutrients and minerals. All the hydrolysate cultures completed lactic acid fermentation within 40 hours when the initial glucose concentration was equal or less than 7.2% while the culture with pure glucose took about 63 hours to complete the entire fermentation process, which had a similar fermentation ending time as the culture with 9.7% glucose in the hydrolysate medium. Therefore, the hydrolysate medium has equal or even better performance under the current testing conditions, probably due to a more complete set of growth nutrients contained in the wheat millfeed hydrolysate than that in the supplemented yeast extract and peptone.

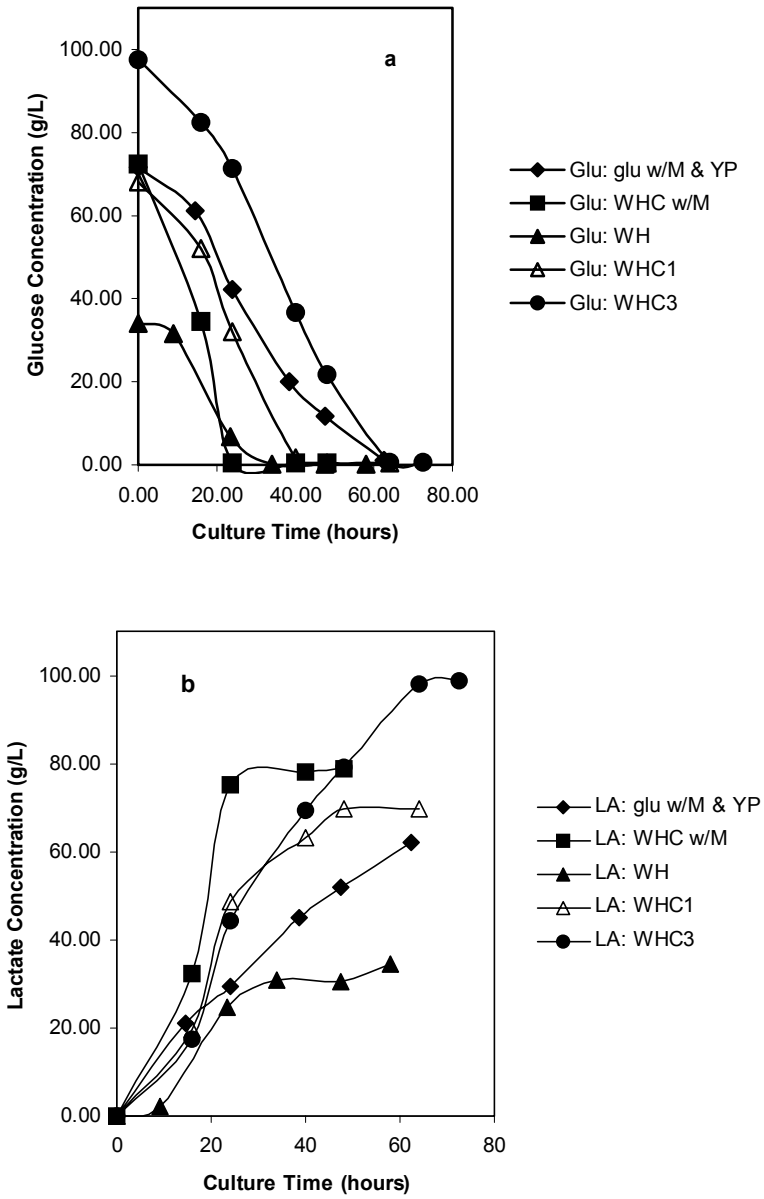

Figure 5. Lactic acid fermentation in batch cultures
a: glucose utilization in batch cultures;
b: lactate production in batch cultures.
Glu: glucose; LA: lactate; w/M: hydrolysate supplemented with all minerals; w/M & YP: hydrolysate supplemented with minerals, yeast extract, and peptone.

Catalytic Processing to Value-Added Products

The catalytic process evaluated using wheat millfeed-derived glucose solution was the hydrogenation of the glucose to sorbitol. The reaction is a straightforward reaction of elemental hydrogen gas with the carbonyl functional group on the glucose to form an alcohol. In the process, the sugar is converted to a polyol (C6-hexitol). The reaction occurs with a high

selectivity of 95% or greater to the preferred product. Numerous metal catalysts for the process have been reported in the literature. The process is commercially important as the sorbitol product is produced domestically at over 500 million pounds annually.

These tests were an extension of much earlier work with reagent grade glucose and corn-derived dextrose (d-glucose). The tests involved the ruthenium on rutile catalyst (U.S. patent #6,235,797), which was developed in our laboratory and has been used extensively in earlier tests. The main issue involved in the tests was the effect of the trace contaminants carried over from the wheat and the wheat processing. The wheat millfeed-derived glucose was tested directly and also following various cleanup steps. Numerous tests were made to evaluate the several contaminants identified in the wheat millfeed glucose by adding reagent chemical models to reagent glucose for catalytic testing.

Test Procedure: Glucose hydrogenation to sorbitol was chosen as the catalytic processing test case for the wheat-derived glucose. The reactor was a stirred batch reactor made of 316 stainless steel. The reactor volume was typically 300 mL with the vessel filled half full of aqueous sugar solution. The catalyst was a ruthenium on titania (rutile form) extrudate ground to powder (5 g per 150 g of feed solution). The processing conditions were 100°C and 1500 psig with hydrogen atmosphere. The slurry of feed solution and catalyst particles were stirred to maximize hydrogen contact. The reaction was allowed to proceed for 6 hours, typically, with multiple samples of product solution withdrawn throughout the reaction period. The product solutions were analyzed by liquid chromatography to determine primarily the glucose and sorbitol concentrations, but also to monitor for byproducts. The vented gas product at the end of the test was analyzed by gas chromatography to look for other byproducts (hydrocarbons or carbon oxides).

Baseline Test Results: Initial tests were performed to quantify reaction rate and reproducibility using reagent glucose (5 wt%) in deionized water as the feedstock. The process test was repeated in order to be able to evaluate the reproducibility of the reactor system. The used catalyst was reused in two subsequent tests in order to be able to evaluate the catalyst lifetime. The effect of stir rate was also assessed by testing at higher rpm. The glucose solution was also tested at two other concentrations, 2.6% and 7.4%, in order to be able to evaluate the effect of concentration. The results of these tests are graphically depicted in Figure 6.

The glucose conversion curves in Figure 6 show a common trend in all the tests. The tests that repeat the use of the catalyst (reagent 1A and 1B) show a slight inhibition in the early portion of the test that suggests that the catalyst must be reactivated (re-reduced). The test at higher stir rate shows little improvement. The tests of varying concentration of glucose (high = 7.4% and low = 2.6%) show a slight trend of increased rate of glucose conversion at lower concentration, but the middle concentration of 5% does not follow the trend. Overall, these results show the range of variability achieved with this test. In all cases, the glucose conversion has reached 95% within 2 hours at temperature.

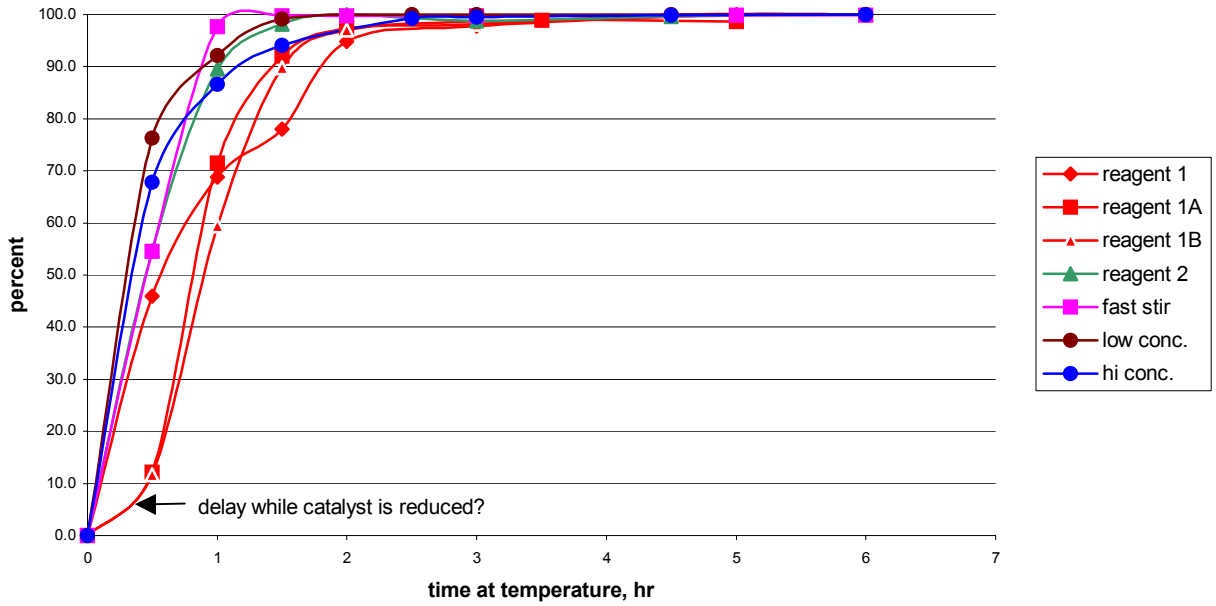

Figure 6. Glucose Conversion by Catalytic Hydrogenation

Wheat Millfeed-Derived Feedstock Tests: Tests were performed with the wheat millfeed-derived glucose solution to determine its reactivity relative to reagent glucose. Tests were made with three versions of the glucose solution: the hydrolysis filter press product, the hydrolysis ultrafiltration (UF) permeate, and the nanofiltration (NF) concentrate from the UF permeate. The results of the tests are graphically depicted in Figure 7. In each of these cases, the glucose was not reacted as readily as the reagent glucose. An overwhelming inhibition or catalyst poisoning occurred, apparently due to one or another of the trace components in the wheat-derived products. Recovery and reuse of the catalyst with a reagent glucose feedstock showed that the effect on the catalyst was not permanent as a high activity for hydrogenation to sorbitol was found upon reuse.

The wheat millfeed hydrolysis products (filter press product, UF permeate, and NF concentrate) were treated with a carbon adsorption bed to determine what components could be removed by such a process and what effect on the catalyst there might be by such a removal. The carbon treatment involved passing the hydrolysate solution through a column of carbon particles. The passage was by gravity flow downward and required a contact time of about 2 minutes. The carbon was Norit RO 0.8, ~80 mL, in the first case, and Norit ROX 0.8, ~100 mL, in the second case. They are similar steam-treated carbon extrudates with the ROX being acid-washed as well.

The first carbon was used to treat the UF permeate. The carbon bed was first wet with deionized water. The hydrolysate was added as a light yellow, slightly cloudy liquid; it was recovered with

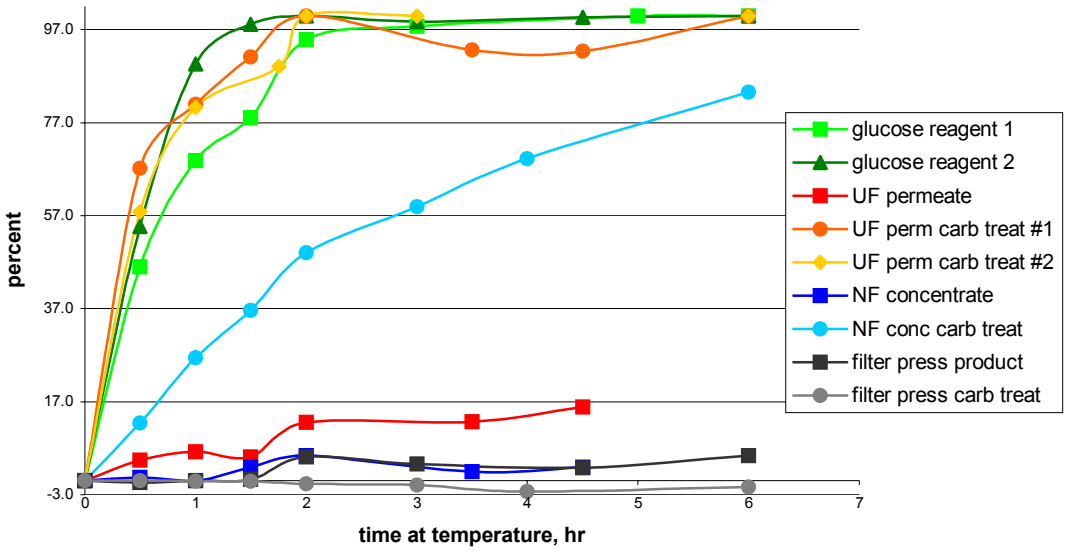

Figure 7. Millfeed Glucose Conversion by Catalytic Hydrogenation

carbon powder and required a filtration (0.45 micron nylon filter) to produce a clear, off-white liquid. With the second carbon, the UF permeate was recovered without carbon powder carryover as a slightly cloudy, off-white liquid. The carbon bed from the second case was subsequently used to treat both the NF concentrate and the filter press product. The NF concentrate was dark yellow and cloudy with a bit of light colored precipitate and was decolorized to a light yellow, slightly cloudy liquid. The filter press product was dark orange, cloudy with light colored precipitate and was just about the same (as dark colored, slightly less cloudy) after the carbon treatment. The results of the chemical analyses completed before and after the carbon treatments are given in Table 8. These results clearly show that the carbon bed did remove some of the components, most significantly the protein and sulfur. The sulfur effect was confirmed by the sulfate analyses. The metals were less affected. The phosphorus also appeared to be little affected based on both elemental phosphorus and phosphate analyses. Chloride (and possibly sodium) content was increased by the treatment with the carbon, most noticeably in the nanofiltration concentrate, which was essentially chloride-free following the membrane filtration.

These carbon treated products were used as feedstock for catalytic testing and compared to the untreated products and reagent glucose. The results (see Figure 7.) show greatly improved conversion with the carbon treatment of both the UF permeate and NF concentrate, while the filter press product was still unconvertible. The combination of ultrafiltration and carbon treatment appears to produce a glucose feedstock nearly equivalent to reagent glucose based on these short-term batch test results. The nanofiltration concentrate shows value in increasing the concentration of the glucose product stream. However, it is apparent in the more concentrated product that the residual materials from the ultrafiltration still inhibit catalyst activity.

Table 8. Analyses of wheat millfeed hydrolysates before and after carbon treatment

component	UF permeate	UF perm carb treat	NF concentrate	NF conc carb treat	Filter press product	Filter press carb treat
protein	0.3 %	0.12 %	0.62 %	0.08 %	0.75 %	0.35 %
nitrate	NA	0.6	NA	2.2	NA	1.5
calcium	39	33	165	117	44	36
magnesium	200	210	523	490	233	280
potassium	1050	1500	2370	2600	1170	1600
sodium	18	70	23	43	17	19
chloride	56	200	0	72	56	96
orthophosphate	NA	377	NA	991	NA	477
total phosphorus	510	536	1180	1201	561	587
sulfate	1080	164	2940	361	1100	160
total sulfur	305	74	819	117	406	65

Contaminant Model Chemical Tests: Based on the analyses done on the wheat millfeed-derived products, there were numerous potential problem components. These (shown in Table 8) included sulfate (potential for metal sulfide formation); calcium, magnesium and phosphate (potential for catalyst pore plugging by insoluble salt precipitation); sodium or potassium (alkali attack on the catalyst support); organic nitrogen components, such as amino acids (thiol source for metal sulfide formation), proteins (pore plugging by precipitation of denatured forms) or urea (metal complex formation); chloride (reaction with the metal); phytic acid or sodium phytate (decompose and precipitate as phosphate) or maltose and maltodextrins (pore plugging or catalytic site plugging).

We completed more definitive analyses of the hydrolysate UF permeate for nitrogenous compounds. Our attempts at protein analysis appeared to show little protein in the hydrolysate UF permeate (50 ppm by the Bradford dye-binding assay), contrary to the nitrogen analysis (0.27-0.30% by Kjeldahl nitrogen). The remaining nitrogen is apparently present as individual amino acids or as very low molecular weight proteins or protein hydrolysis fragments (e.g. urea). A measurement of the cysteine content showed it below the level of detection.

Tests were completed with chemical models of the various components to attempt to identify the cause of catalyst inactivity. The results of the tests with these components are summarized in the Table 9 below. No component has yet been identified as the offending material. The inorganic components were added as salts to the feedstock solution. The phytic is a phosphorylated cyclohexanehexitol, which is found in wheat. It had no effect in either the acid or sodium salt form. Only the cysteine shows a deactivation of the catalyst; however, its deactivation is not reversible as was found with the wheat-derived material. Although cysteine is identified as a component of wheat protein structures, it is not likely that it would be found as the cysteine in the filtration products. More likely, it would be present as the bridged disulfide cystine following the oxidative reactions in the processing. As cystine, there is no free thiol available to react with the catalyst metal and therefore, no mechanism for catalyst deactivation.

These tests and the companion chemical analyses suggest that the catalyst activity problem does not result from any of the inorganic components. The carbohydrate structures also appear to have little affect on activity. The nitrogenous materials are the one component significantly affected by the carbon treatment, which may not be adequately modeled in the tests thus far.

Table 9. Testing results with trace contaminants in glucose

contaminant	form	amount, ppm	amount in hydrolysate UF permeate	reduction in conversion
Sodium	sulfate	1000	18	None
Calcium	hydroxide	100	77	None
Magnesium	hydroxide	400	209	None
Potassium	sulfate	1050	1050	None
Phosphorus	phosphoric acid	950	510	None
Phosphorus	phytic acid	430	510	None
Phosphorus	dodeca-sodium phytate	550	510	None
Sulfate	sodium sulfate	1000	1050	None
Chloride	sodium chloride	49	56	None
Kjeldahl N	urea	470	425	None
Cysteine	l-cysteine	200	200, based on wheat analysis; none expected	95-99%
Cysteine	dl-cystine	200	200, based on wheat analysis	None
Maltose	crystalline	10,000	3000	None
Maltotriose	crystalline	10,000	2000	None
Glucose oligomer	maltodextrin 4-7	10,000	NA	None
Starch oligomer	maltodextrin 13-17	10,000	NA	None

Process and Product Assessment from CRADA Partners

Initial economic modeling of the starch extraction and glucose recovery process was done as part of this project. The model development included material and energy balances built into a virtual process simulator based on Excel™ and Visual Basic™. Equipment sizing and costing calculations were dynamic in nature and adjusted with changes in the mass balance. Capital investment costs and operating costs were also estimated. Using the model, sensitivity analyses could be undertaken as well as comparisons with other technology options.

The model was used to cost a midwest green field site processing a moderate 227,273 tonne per year of millfeed. The yield of glucose was 68,182 tonne per year with 149,091 tonne per year of animal feed and 17,727 tonne per year of high-protein feed supplement. The plant capital cost estimate is $34 million. Using a range of values for the byproducts, a range of costs and prices for the millfeed-derived glucose product were generated. As given in Table 10, these range around the current costs and prices for corn-derived glucose, which are given at the bottom of the table for comparison. The corn costs and prices are based on a much larger (typical) corn wet mill producing 1,081,818 tonne per year of glucose. Capital costs at the different scales of operation are the major differentiating factors between the two glucose sources.

Table 10 Millfeed-Derived Glucose Costs and Prices

By-Product Credits	Variable Cost	Production Cost	Selling Price
Low-end of estimate	7.5 cents/lb (16.5 cents/kg)	9.1 cents/lb (20.0 cents/kg)	12.3 cents/lb (27.1 cents/kg)
High-end of estimate	5.8 cents/lb (12.8 cents/kg)	7.3 cents/lb (16.1 cents/kg)	10.6 cents/lb (23.3 cents/kg)
Corn-derived glucose	6.4 cents/lb (14.1 cents/kg)	7.0 cents/lb (15.4 cents/kg)	7.6 cents/lb (16.7 cents/kg)

Acknowledgement

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[1] Pacific Northwest National Laboratory is operated by Battelle for the U.S. Department of Energy under Contract DE-AC06-76RL0 1830