FINAL REPORT
COMPREHENSIVE RESEARCH ON RICE
June 1, 2007 – June 1, 2008

PROJECT TITLE: Enzymatic hydrolysis and fermentation of broken rice kernels and rice straw

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OBJECTIVES:

Objective 1 – Enzymatic hydrolysis and fermentation of rice straw

A. Prove that MSW is viable as a feedstock for the production of ethanol.
B. Complete installation of autoclave pilot plant in Salinas, CA.
C. Prove that liquid hot water pretreatment can improve the hydrolyzability of rice straw.
D. Optimize conditions for liquid hot water pretreatment of rice straw.
E. Evaluate the pretreated rice straw for glucose content and inhibitors.
F. Demonstrate that liquid hot water pretreated rice straw can be easily fermented.

Objective 2 – Enzymatic hydrolysis and fermentation of broken rice kernels

A. Gelatinize broken rice kernels by traditional methods and by autoclaving.
B. Optimize enzymatic hydrolysis and fermentation conditions.
C. Commingle rice flour with MSW during hydrolysis and prove that co-hydrolysis is possible.

FOREWORD

At a presentation before the Rice Research Board in 2006, the USDA received interest from some of the board members pertaining to the feasibility of using broken rice kernels as substrate for ethanol production. Therefore the USDA presented a proposal to do a study on fermentation of broken rice kernels. This seems like a long time ago, and indeed this date precedes the fervor over production of liquid transportation fuels from food starch sources. The Rice Research Board made the request that in addition to broken rice kernel fermentation they would like to see some data presented on production of ethanol from rice straw. The data presented in this report will therefore address both research directions and also attempt to predict the feasibility of incorporating rice straw as an amendment to the feedstock for the MSW-based integrated biorefinery that has been proposed by the USDA.

INTRODUCTION

As the nation transitions from fossil fuels to renewable bio-based energy, it is unclear from where we will obtain our liquid transportation fuels. Up to this point, ethanol has served merely as an oxygenate and has been produced solely from food starch fermentation. With the current backlash against food for fuel, we as a nation must look in different directions for fuel alternatives. The Energy Independence and Security Act of 2007 renewable fuel standards call for the production of 36 billion gallons of advanced alternative biofuels by 2022 with 16 billion gallons deriving from cellulosic sources. [1] Even at optimal ethanol yields (~100 gal/mt biomass) this will require utilization of multiple different types of cellulosic materials.

Much work has been published on the utilization of agricultural wastes as standalone feedstocks for cellulosic biorefineries yet the first facility has not been built due to the tight economics associated with seasonal crops. Ironically, the economics will only get worse as the price of oil once again rises permanently to levels recently seen (~$150/barrel) and the cost of transportation of feedstock increases. As a result the USDA has been working on a new concept for an MSW-based integrated biorefinery capable of accepting multiple feedstocks as they are produced locally thereby reducing the cost of transportation.

The use of MSW as the base feedstock shifts the burden of justification for capital expenditure away from seasonal crops and to a reliable low cost substrate. The MSW-based biorefinery opens alternative disposal methods for agricultural by-products that are

grown much less densely than would be otherwise required for a biomass-based ethanol facility. This report will present some data on hydrolysis and fermentation of broken rice kernels as well as the effects of pretreatment that are compatible with a "green" biorefinery on rice straw. A brief economic analysis will also be included.

MATERIALS AND METHODS

Materials. Rice straw (M202) was collected and baled at the LeGrande farm in Williams, CA at the time of harvest in 2006. The straw was milled to pass a 20-mesh screen and stored in an air-dried condition prior to usage.

Broken rice kernels (M202) were supplied by Dr. Kent McKenzie, Plant Breeder Director at the California Rice Research Station (Biggs, California). Hydrolysis and fermentation of unmilled broken rice kernels was attempted and then abandoned as it was determined that the biochemicals were inefficient at such large particle sizes. The broken rice kernel data reported here as a result is based upon liquefaction and saccharifcation of broken rice kernels that were milled in a cyclone mill (UDY Corp.) to pass 20-mesh. Milled rice flour was stored in a dessicator over P_2O_5 prior to use.

All chemicals were purchased either from Fisher Scientific or Sigma Aldrich and were used as purchased.

Pretreatment of rice straw. Rice straw was pretreated mainly by autohydrolysis (liquid hot water) and selective CAFI pretreatments [2], including lime, ammonia, acid prehydrolysis, and organosolv.

Pretreatment experiments were performed on 25 oven-dried (OD) g batches of ground rice straw that were diluted to 5 % solids and added to a Parr 4521M pressure reactor controlled with a Parr 4843 temperature controller (Moline, IL) reactor equipped with an

overhead stirrer, pressure transducer, cooling coils, and a release valve at the bottom of the pressure vessel. For the chemical experiments, all reagents were dissolved prior to initiation of the reaction. For the hot water pretreatments, nothing additional was added. Mixing commenced and the reactor was allowed to ramp as fast as possible to the predetermined setpoint temperature. Temperatures used in this study were varied between 190 and 230 ^{o}C. Ramp time varied from 52 to 60 minutes increasing incrementally from lower to higher residence temperature. After the dwell time was completed, the cooking liquor was removed using the bottom relief valve and the reactor was flushed with cool H_2O to cool the reactor and materials, as well as rinse the fibers clean. The materials were then air dried prior to storage to avoid microbiological growth.

Lignin Content. Acid soluble and insoluble lignin analyses were performed by the standard techniques. [3]

Carbohydrate Analysis. Sugar monomers from above hydrolyzates were analyzed using an Agilent 1200 Series LC equipped with a Biorad Aminex HPX-87P column and an RI detector. The hydrolyzates were neutralized by $CaCO_3$ addition to a thymol blue endpoint, pH 2-3, centrifuged, and filtered through a 0.2 μm PVDF filter. Water was the mobile phase and the flow rate was 0.6 mL/min.

Ash content. Ash content was performed according to TAPPI test method T-211 om-93.

Enzymatic hydrolysis of rice straw. Hydrolysis was performed using Celluclast 1.5 L and Novo 188 enzyme solutions (Novozyme) in 500 mL shake flasks at 55 ^{0}C. The activities of the enzyme preparations were determined by standard assays [4, 5] to be 56 FPU/mL and 1440 CBU/mL, respectively. Each flask was charged with 5 g OD pulp fiber at 5 % solids in 50 mM citrate buffer (pH 4.5), and enzyme addition was varied according to the glucose content of the particular sample. Cellulase addition was fixed at 50 FPU/g cellulose and cellobiase was automatically added at a 1:4 cellulase/cellobiase ratio.

In order to monitor the hydrolysis, 1 mL samples were withdrawn at 3, 6, 24, 48, and 72 hours and frozen prior to analysis. The samples were then thawed and centrifuged to separate hydrolyzate from any suspended solids, the supernatant was filtered through a 0.2 μm PVDF filter, and the progress of the enzymatic hydrolysis was monitored by directly injecting 10 μL of the hydrolyzate into the HPLC described above.

Fermentation. Fermentation was done using an overnight seed culture of Ethanol Red yeast ™ (Fermentis, France); 0.0125g dry yeast was added to 50ml of seed media. The seed media consisted of glucose 150 g/L, yeast extract 7.5 g/L, ammonium sulfate 22.5 g/L, dipotassium phosphate 15 g/L, magnesium sulfate heptahydrate 3 g/L; pH was adjusted to 4.5. [6] Seed Media was sterilized for 20 min at 121˚C. Yeast cells were grown at 30˚C and at 200 rpm using a shaker incubator until glucose concentration of the seed culture was below 2 g/L. Glucose concentration was monitored by HPLC. Cell density was also monitored by spectrophotometry. Once the cells reached the desired glucose

concentration, the amount of culture to be transferred was determined using the formula presented in NREL technical report/TP-510-42630 [7]. The calculated initial optical density for the new fermentation flasks was 0.5. Inoculum from seed culture was centrifuged at 4,500 rpm for 10 minutes and the supernatant was decanted. Cells were resuspended in the same volume of distilled water, centrifuged again, and supernatant was discarded. The cells were then resuspended in $1/10^{th}$ of the volume of water and an aliquot of this cell suspension was used to inoculate the hydrolyzed biomass culture flasks. Before addition of the inoculum biomass feedstock shake flasks were supplemented with 1 % yeast extract and 2 % peptone and sterilized for 20 minutes at 121˚C. The shake flasks were incubated for 24 hours at 30˚C. Glucose and ethanol concentrations in the fermentation were monitored using HPLC.

Broken rice kernel liquefaction and saccharification. Contrary to the proposal objectives, broken rice kernels were ground to pass a 20-mesh screen prior to liquefaction. This step was performed because it was found that liquefaction proceeded at an inefficient rate from whole broken rice kernels.

Broken rice kernel hydrolysis was ultimately accomplished by mixing in a 1 L stainless steel cup with an overhead mixer mounted to scaffolding for support. The same volume (300 mL) was used for each experiment with the amounts of rice flour added varied depending upon target solids concentration. Deionized water was first added and heated to 90 ^{0}C and then 60 ppm $CaCl_2$ was added to enhance α-amylase activity. The rice flour was added to the desired concentration and thoroughly mixed until the temperature had

stabilized. α-amylase (from *Bacillus licheniformis*) was added at a level of 0.02 % (w/w) via a 1 mL addition from a 3 mg/mL stock solution and the contents were mixed at 90 ^{0}C for a period of one hour. The temperature was decreased to 85 ^{0}C with a subsequent second addition of α-amylase, 0.06 % (w/w) and incubation continued for one more hour to complete the liquefaction.

The temperature was lowered to 55 ^{0}C, the pH was adjusted to 4.5, and amyloglucosidase was added at a level of 0.08 % (w/w) from a 12 mg/mL stock solution. The saccharification process was allowed to proceed to completion (~17 hours) and was sampled at 0, 2, and 17 hours for LC analysis.

The saccharified starch was then divided into three fractions and transferred to shake flasks for fermentation in triplicate. The fermentation was performed identical to the above description for enzymatic hydrolysis.

RESULTS AND DISCUSSION

Pretreatment of rice straw. The USDA's efforts have focused on "green" techniques thus far for pretreating biomass, including rice straw. The integrated biorefinery is heavily dependent upon reuse of water and production of methane as a by-product of solubilizing food and other waste materials. As a result it is highly desirable to minimize the use of chemicals that may permeate the system and ultimately result in the destruction of the volatile solids in the anaerobic digester. The USDA has therefore worked primarily with hot water pretreatment (HWP) for enhancing the enzymatic hydrolysis of MSW and other biomass sources.

HWP is referred to by multiple names including autohydrolysis, which explains the fundamental reaction mechanisms that occur as cellulose and hemicellulose are broken down and made more accessible to enzymes. Autohydrolysis is essentially subjecting the given substrate to high temperature (~200 ^{0}C) and high pressure (~300 psig). Although the pH is neutral at STP (standard temperature and pressure) acetyl groups from the hemicellulose are cleaved at higher temperature to produce acetic acid groups, dropping the pH slightly to ~4.5 and make the solution slightly acidic. These conditions at high temperature prove favorable for two mechanisms of cellulose hydrolysis: one is a "peeling" reaction which removes the end groups thus solubilizing a glucose monomer. The other reaction involves random chain scission, breaking apart the crystallinity of the cellulose, lowering the overall molecular weight, and creating two new reducing end groups from which the enzyme can attack the cellulose. Finally, the hemicellulose portion of the rice straw has a low degree of polymerization and is easily solubilized at

these temperatures. Since hemicellulose is interspersed between the cellulose microfibrils in the cell wall, its hydrolysis creates pores between the cellulose chains. The result from these reactions is that there is more accessibility for the enzyme to hydrolyze the substrate.

Table 1. Pretreatment conditions, severity factor of the pretreatment, % yields, and glucose contents of resultant rice straw samples.

	Residence temp. (^{0}C)	Residence time (min)	Severity Factor (R_o)	% Yield	% Glucose	% Xylose
Untreated	--	--	--	--	39.8	18.3
RS-6	190	10	3.65	57.9	48.1	5.5
RS-1	195	30	4.27	52.7	58.0	0
RS-5	195	60	4.58	52.1	69.4	0
RS-2	205	30	4.57	54.7	75.8	0
RS-7	210	15	4.41	55.0	52.4	0
RS-8	210	20	4.54	55.4	51.2	1.5
RS-3	215	30	4.86	54.0	74.8	0
RS-4	225	30	5.16	62.0	67.6	0
RS-9	230	20	5.13	51.6	44.4	0

Residence temperatures were varied between 190 and 230 ^{0}C with residence times at temperature varying from 10-60 minutes. The severity factors range from 3.65-5.16 (Table 1) and provide a basis for comparison of each experiment. The severity factor takes the effects of residence temperature and residence time into consideration and is determined from the following equation [8]:

$$R_o = t * (\exp(T-100)/14.75)$$

where,

T = residence temperature
t = residence time

All total yields were in the range of 51.6 -62 % of the original rice straw and the glucose contents ranged from 44.4-75.8 %. In each case the glucose content was higher than the untreated rice straw glucose content of 39.8 %. Glucose contents tended to be lower at low residence temperatures and low residence times with the exception being the pretreatment at 230 ^{0}C which provided the lowest glucose content of all pretreated materials. Physical observation of the rice straw from this experiment along with the glucose content indicates that degradation of the cellulose is occurring and that either residence time should be minimized or lower residence temperatures should be used.

Table 2. Effects of pretreatment upon glucose recovery, and lignin and xylan removal.

	Residence temp. (^{0}C)	Residence time (min)	Severity Factor (R_o)	Glucose Recovery (%)	Lignin Removal (%)	Xylose Removal (%)
Untreated	--	--	--	100	0	0
RS-6	190	10	3.65	77.8	22.4	82.4
RS-1	195	30	4.27	76.8*	7.0	100
RS-5	195	60	4.58	78.7*	0	100
RS-2	205	30	4.57	79.7*	3.1	100
RS-7	210	15	4.41	80.5	8.2	100
RS-8	210	20	4.54	79.2	8.1	95.5
RS-3	215	30	4.86	90.3*	2.2	100
RS-4	225	30	5.16	75.9*	0	100
RS-9	230	20	5.13	64.0	1.3	100

* Values estimated, must be reconfirmed.

Table 2 compares the different pretreatment experiments as they pertain to the most important attributes: glucose recovery and lignin removal. HWP is slightly acidic and hence is not a lignin removing pretreatment. Lignin is typically removed by alkaline pretreatments since the phenolic hydroxyl groups tend to ionize at pH above 9.5. This is reflected in the very low efficiencies in lignin removal across all experiments (0-8 %). The single exception is the lowest temperature, short residence time pretreatment, which removed 22 % of the lignin. A small portion of the lignin polymer is of lower molecular weight and is acid soluble. It has been hypothesized that any lignin solubilized will redeposit on the cellulose during extended reaction under autohydrolytic conditions. [9]

Glucose recovery of course is of primary importance when considering biofuel applications and the recovery was quite good for all pretreatments (75-80 % recovery) with the exception of the 230 ^{0}C pretreatment (RS-9), which results in a recovery of only 64 %. This is likely due to degradation as mentioned previously. One other anomaly is the RS-3 pretreatment with a glucose recovery yield of 90.3 % which in comparison with the other treatments seems to be a bit high.

Xylose removal is nearly complete for all conditions investigated with the exception of the low temperature experiment in which xylose removal is ~82 %. One anomaly is the slight amount of xylose remaining in the RS-8. As mentioned previously, cellulose and hemicellulose are degraded by end group removal and random chain scission. In a typical ag-waste dependent biorefinery which requires 100 % recovery of all available sugars to achieve economic viability, the filtrate from these pretreatments would be

recycled to enzyme hydrolysis. The main disadvantage of doing so would be carryover of inhibitors. For the integrated biorefinery the filtrate would likely be recycled back to autoclaving and not enzyme hydrolysis to avoid inhibition. The sugars would still be converted to bioenergy but in this case it would be biogas instead of ethanol.

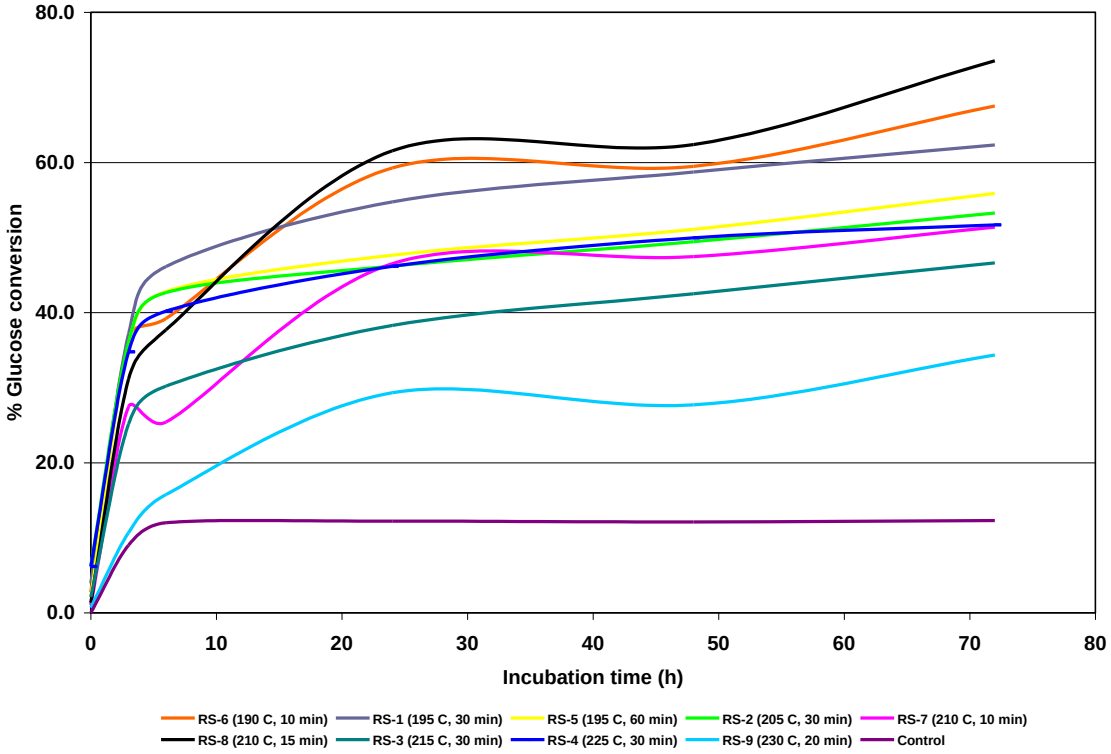

Figure 1. Conversion of glucose during enzymatic hydrolysis for each pretreatment.

Figure 1 shows the enzymatic hydrolysis curves and conversion of cellulose to theoretical glucose. The data is also tabulated in Table 3. The rice straw control experiment produced approximately 12 % of the theoretical glucose available in the rice straw. Theoretical glucose represents the glucose polymerized in the cellulose microfibrils and potentially small amounts of glucose in the glucomannans. For the pretreated straws, yields varied from 34-73 % of theoretical with the low point being the high temperature

(230 ^{0}C) pretreatment discussed previously and the high point being the experiment at 210 ^{0}C residence temperature and 20 minutes residence time.

Table 3. Yields from enzyme hydrolysis and fermentation and estimations of the yields of ethanol from rice straw on a 1000 tpd OD basis.

	Residence temp. (^{0}C)	Residence time (min)	Severity Factor (R_o)	Glucose Converted (%)	Glucose to fermentation (t/1000 tpd basis)	Ethanol yield (gal/d)
Untreated	--	--	--	**12**	**43**	**7,747**
RS-6	**190**	**10**	**3.65**	**67.5**	**188**	**33,868**
RS-1	**195**	**30**	**4.27**	**62.3**	**190**	**34,307**
RS-5	**195**	**60**	**4.58**	**55.9**	**169**	**30,432**
RS-2	**205**	**30**	**4.57**	**53.3**	**169**	**30,465**
RS-7	**210**	**15**	**4.41**	**51.4**	**145**	**26,202**
RS-8	**210**	**20**	**4.54**	**73.5**	**208**	**37,560**
RS-3	**215**	**30**	**4.86**	**46.6**	**146**	**26,263**
RS-4	**225**	**30**	**5.16**	**51.7**	**186**	**33,494**
RS-9	**230**	**20**	**5.13**	**34.4**	**79**	**14,199**

The three different pretreatments at 195 ^{0}C show an interesting trend in the effects of residence time on glucose conversion, which was varied from 15-60 min. Hydrolysis yield varied from 67.5 % at 15 min to 55.9 % at 60 min residence time. A decrease in both overall yield and glucose conversion over time indicate the dissolution of glucose over the reaction time period and likely oxidation of the polymer which would inhibit enzymatic activity on the substrate. In general glucose conversion also decreased with increase of temperature with the exception of RS-8 which had the highest conversion rate of 73.5 % of theoretical. This number may be an anomaly and needs to be repeated for verification. The example of the 195 ^{0}C experiments also points out the need to perform

experiments at higher temperature and short residence time to determine whether there is an improvement in yield from enzymatic hydrolysis.

Table 3 also lists the glucose available for fermentation and the expected production of daily ethanol, which are directly related, from the different pretreatments of rice straw. The theoretical maximum of glucose available from 1000 tpd of rice straw is 398 mt which could produce 71,000 gal/d. 43 mt/d of glucose are available from untreated rice straw on a 1000 tpd basis without any type of pretreatment which would result in <8,000 gal/d of ethanol. The yield of ethanol is dependent upon the total yield of pretreated biomass, the % glucose retained, and the yield of glucose from the pretreated biomass after enzymatic hydrolysis. A low yield of biomass from pretreatment will therefore necessarily produce a low amount of ethanol after fermentation even if the conversion of glucose is near the theoretical. On the other hand, an ineffective pretreatment will likely produce a high yield of pretreated biomass but a low yield of ethanol since the enzymatic conversion will likely be low.

The data provided in this report from the pretreatments explored indicates ethanol yields are roughly half that of theoretical. RS-8 is once again the high point for ethanol production at 37,000 gal/d and RS-9 (230 ^{0}C) is the low value at 14,000 gal/d. All other pretreatments vary between 26,000 and 34,000 gal/d. In general, ethanol yields decrease with pretreatment temperature and residence time. Two exceptions are RS-8 with the high enzymatic hydrolysis yield and RS-4 which has a high yield of pretreated biomass (62 %).

MSW-based biorefinery. The MSW-based biorefinery shifts the burden of capital justification from seasonal ag-wastes such as rice straw to MSW which is produced year-round near population centers, already collected and transported to transfer stations with the cost built-in to sewer service charges, and provides a diversion fee (tipping fee) for usage other than landfilling. Figure 2 shows a simplistic flow diagram for the MSW-based biorefinery, including inclusion of rice straw into the flow scheme. Rice straw does not require autoclaving and can be incorporated via aqueous pretreatment and commingled with MSW prior to saccharification (enzymatic hydrolysis) if necessary. Seasonal production of rice straw in the local area may necessitate running the monosubstrate during peak times of agricultural activity.

MSW is autoclaved, screened, and then diluted and cleaned prior to enzymatic hydrolysis. (Figure 2) The filtrate from the cleaning process is sent to a high-rate anaerobic digester to produce biogas which can be combusted to produce electrical energy and steam or further purified to sell as a replacement natural gas. The pulp can be hydrolyzed and fermented either as a commingled feed or a single substrate. The stillage can be separated from the ethanol either by distillation or membrane separation. All insoluble residue from hydrolysis and fermentation can be combined with the Trommel screen rejects and either gasified or combusted depending upon prevailing environmental regulations. Gasification is the likely option in CA since the thermal conversion process is cleaner and more complete and along with the gas cleanup the emissions are generally minimized.

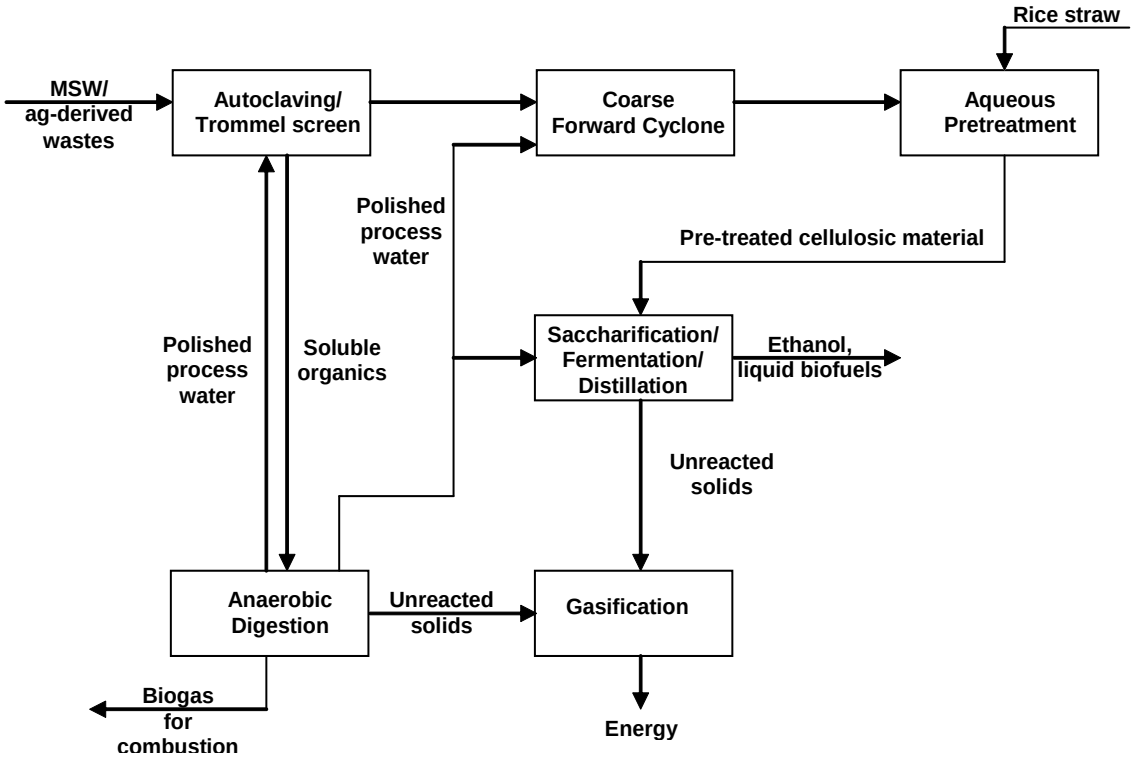

Figure 2. Simplified flow scheme for the integrated biorefinery including incorporation of rice straw after aqueous pretreatment.

While maximizing ethanol production is always of interest, inclusion of a gasifier to produce electrical energy and process steam eliminates the reliance of the ethanol facility on fossil fuel and provides a revenue from sale of electricity to the grid. Table 4 shows the breakdown of the available energy from 3000 tpd wet MSW. As can be seen, approximately 50,000 gal/d of ethanol can be produced from MSW yet the bulk of the Btu available still go to production of electricity. This is primarily due to the fact that the lignin (25 % by weight of biomass) is inert with respect to enzymatic hydrolysis and the rejects from screening MSW (1" accepts and overs) are extremely high in Btu content.

Table 4. Available energy capacity of integrated biorefinery based upon wet MSW on a 3000 tpd basis.

Energy Outputs	EtOH mt/day	EtOH gal/day	gal/mt wet MSW	gal/mt OD MSW	gal/mt OD pulp	gal/yr	MMbtu/mt wet MSW	MMbtu/mt OD MSW	MMbtu/day	MMbtu/yr
Ethanol	**143.4**	**50,548**	**16.8**	**23.3**	**57.1**	**18,449,900**	**1.28**	**1.77**	**3,845**	**1,401,100**
Gasification										
Insoluble Residue							1.06	1.46	3,172	1,157,700
1" Accepts							1.57	2.17	4,723	1,723,900
Overs							1.75	2.42	5,255	1,918,100
Total							**4.73**	**6.54**	**14,207**	**4,799,700**
Anaerobic Digestion							**1.72**	**2.37**	**5,132**	**1,873,100**
Total Energy Content							**6.73**	**10.68**	**24,471**	**8,073,900**

The total output of 20,000 MMBtu are more than is required to heat the daily process and power all equipment resulting in a significant revenue for sale of electricity. Calculated at $0.07/kWh, the revenue from electricity from MSW would be $31MM/yr, and the revenue from ethanol is roughly equivalent. (Table 5) The total revenues from processing 3000 tpd are approximately $100MM/yr. As can also be seen, the economic indicators for this type of facility are quite positive.

Table 5. Revenue flow and economic analysis for the 3000 tpd biorefinery.

Revenues	**$/ yr**
Electricity, $/yr	$ 31,586,604
Ethanol, $/yr	$ 29,975,999
Tipping fees for diversion, $/yr	$ 36,274,364
Recyclables, $/yr	$ 6,598,834
Total, $/yr	**$ 104,435,802**
PROFITS, $/yr	**$ 83,801,640**
Economic Indicators	
NPV @ 10 % discount rate, $	**$ 104,815,859**
IRR, %	**16.2**
Annualized ROI, %	**16.8**
Payback Period, yrs	**5.8**

A preliminary breakdown of producing ethanol from rice straw shows that in a best case scenario based upon experimental data shows that in addition to roughly 37,000 gal/d of ethanol, approximately 3800 MMBtu additional energy are available from gasification and anaerobic digestion on a 1000 tpd basis from rice straw. (Table 6) It should be noted that the numbers being compared here are roughly equivalent; ~1000 tpd of dry MSW pulp is available from 3000 tpd wet MSW. It can be seen in total that the MSW provides much more energy, however that is

in part because in order to isolate 1000 tpd of MSW pulp there is ~1000 tpd rejects. In the delivery of 1000 tpd basis of cellulosics for enzyme hydrolysis from MSW therefore nearly 20,000 MMBtu of additional energy is produced. The ethanol available from rice straw is comparable to that from MSW, 37,000 versus 50,000 gal/d, respectively, however rice straw as a standalone will not produce enough additional energy to heat the process. It is therefore apparent that rice straw would be better utilized if it were incorporated as additional capacity to the baseline MSW for ethanol production. The ethanol producer in this case would need to anticipate incorporation of ag-waste and add capacity when sizing capital equipment or have additional capacity already built-in. Another alternative could be supplementing feedstock flow should the time come that source separation limits the availability coming into the materials recovery facility (MRF).

Table 6. Available energy capacity from rice straw.

Energy Outputs		MMBtu/ mt OD straw	MMBtu/ Day
Ethanol		3.18	3,200
mt/day	208		
gal/day	37,560		
gal/mt OD straw	37.6		
Gasification		2.42	2,400
Anaerobic Digestion		1.40	1,400
Total Energy Content		7.00	7,000

Table 7 shows the revenue (not accounting for any operating costs) that can be generated daily from the rice straw after the different pretreatments. Revenue is based upon a price of $1.88/gal which was the current market price as of November 16, 2008. [10] On a 1000 tpd basis, the net value of the ethanol varies from $14,000/day from the control to $70,000/day in the case of the best scenario (RS-8). With the exception of RS-9, which is valued at $26,700, the other

pretreatments varied between $49,000-64,000/day. Of course on a 1000 tpd basis, the $/mt OD straw is the daily revenue divided by 1000. At $49-64/mt OD straw producing ethanol from the straw should be a break even or profitable venture when delivering rice straw locally and indicates that it should be considered an adequate supplement to the feedstock flow. A simplistic estimation of the cost of delivery of rice straw with the caveat that the delivery distance is likely to be less than 30 miles is presented below:

At 1000 tpd➔ 50 trucks per day required
20 mt/truck
$ 30 /bale (1100 lbs/bale) [11]
$ 66,000/day (cost of straw)
- $ 15,000/day credit ($15/mt credit)
$ 51,000/day➔$ 51/mt raw material cost

At $64/mt revenue that leaves $13/mt to either contribute to profit or operating costs.

Table 7. Potential revenue from production of ethanol from rice straw.

	Residence temp. (^{0}C)	Residence time (min)	Severity Factor (R_o)	Ethanol yield (gal/d)	$/day*	$/mt OD straw
Untreated	--	--	--	7,747	14,600	14.56
RS-6	190	10	3.65	33,868	63,700	63.67
RS-1	195	30	4.27	34,307	64,500	64.50
RS-5	195	60	4.58	30,432	57,200	57.21
RS-2	205	30	4.57	30,465	57,300	57.27
RS-7	210	15	4.41	26,202	49,200	49.26
RS-8	210	20	4.54	37,560	70,600	70.61
RS-3	215	30	4.86	26,263	49,400	49.37
RS-4	225	30	5.16	33,494	63,000	62.97
RS-9	230	20	5.13	14,199	26,700	26.69

* Revenue based upon market price of $ 1.88/gal EtOH. [10]

Reducing the reliance on the rice straw to cover the capital and operating expenditures makes it easier to justify incorporating rice straw into the feedstock for the integrated biorefinery. It was assumed that no additional revenues were produced from combustion of rice straw residuals left over after fermentation. In reality, there is enough energy potential to produce about 0.4 MW_e based upon a 36 % conversion efficiency from heat to electricity. [12]

Hydrolysis and fermentation of broken rice kernels. At the time that the proposal was made back in early 2007, corn prices were at all time highs. It was of interest to at least some of the members of the Rice Research Board at the previous meeting in December 2006 to consider whether broken rice kernels were a viable option for the production of ethanol. Upon presentation to the Board it was made clear that while we could still perform this work, it was of more interest to learn about our rice straw research. Due to the relative lack of interest of the Board in broken rice fermentation and discoveries made in the laboratory, the research on broken rice kernel hydrolysis was minimized. The objectives as outlined in the proposal are listed below:

Objective 2 – Enzymatic hydrolysis and fermentation of broken rice kernels

D. Gelatinize broken rice kernels by traditional methods and by autoclaving.
E. Optimize enzymatic hydrolysis and fermentation conditions.
F. Commingle rice flour with MSW during hydrolysis and prove that co-hydrolysis is possible.

Several things were determined in relation to the proposal and the focus of the research was altered. One aspect of the research was to cook the whole rice and submit it to hydrolysis and fermentation. The process did not occur within a reasonable time period. In reflection, this research failed in part because starch hydrolysis is different than cellulose hydrolysis.

Hydrolysis of starch is achieved in three semi-simultaneous or continuous steps: gelatinization, liquefaction, and saccharification. Gelatinization involves the destruction of the crystalline structure which allows swelling of the starch granules. Swelling is limited in whole or broken rice because the organizational structure of the grain prevents complete swelling of the starch granules. As a result accessibility of enzyme to substrate is limited, liquefaction is retarded, and hence saccharification is not achieved. We had hypothesized that because the kernels were broken potentially this would provide an avenue of accessibility. This may hold true but the rate is still limited. After this initial experimentation it was found that it was necessary to grind the broken rice kernels and gelatinize by the traditional techniques.

The rice kernels were therefore ground using a UDY mill to pass a 20-mesh. Hydrolysis was set up to be performed at 5, 10, 15, and 20 % solids using a laboratory beaker setup equipped with an overhead mixer. The required amount of water was heated to 90 ^{0}C and then the rice flour was added with high rate mixing. The viscosity of the flour suspensions of course increase with solids content and mixing was difficult to achieve at 10 % solids. Above this threshold mixing was impossible and was abandoned. As a result due to our limitations, only 5 % and 10 % solids could be examined.

α-amylase was added and the solution was maintained at the same temperature for one hour. During this time gelatinization and then liquefaction were observed to be occurring systematically in solution. Viscosity increased dramatically with gelatinization and then decreased as the solution noticeably thinned. Amyloglucosidase was then added to complete the saccharification. Figure 3 shows the enzymatic hydrolysis curves for the 5 and 10 % solids

experiments. The theoretical glucose content in rice is ~80 % by weight and the data indicates essentially complete conversion of the 5 % solution after 17 hours. During the same time frame, only 80 % of the theoretical glucose is converted at the 10 % solids concentration in our beaker experiment. The reactions were stopped at 17 hours although it is probable with longer retention time the 10 % experiment would also achieve theoretical conversion.

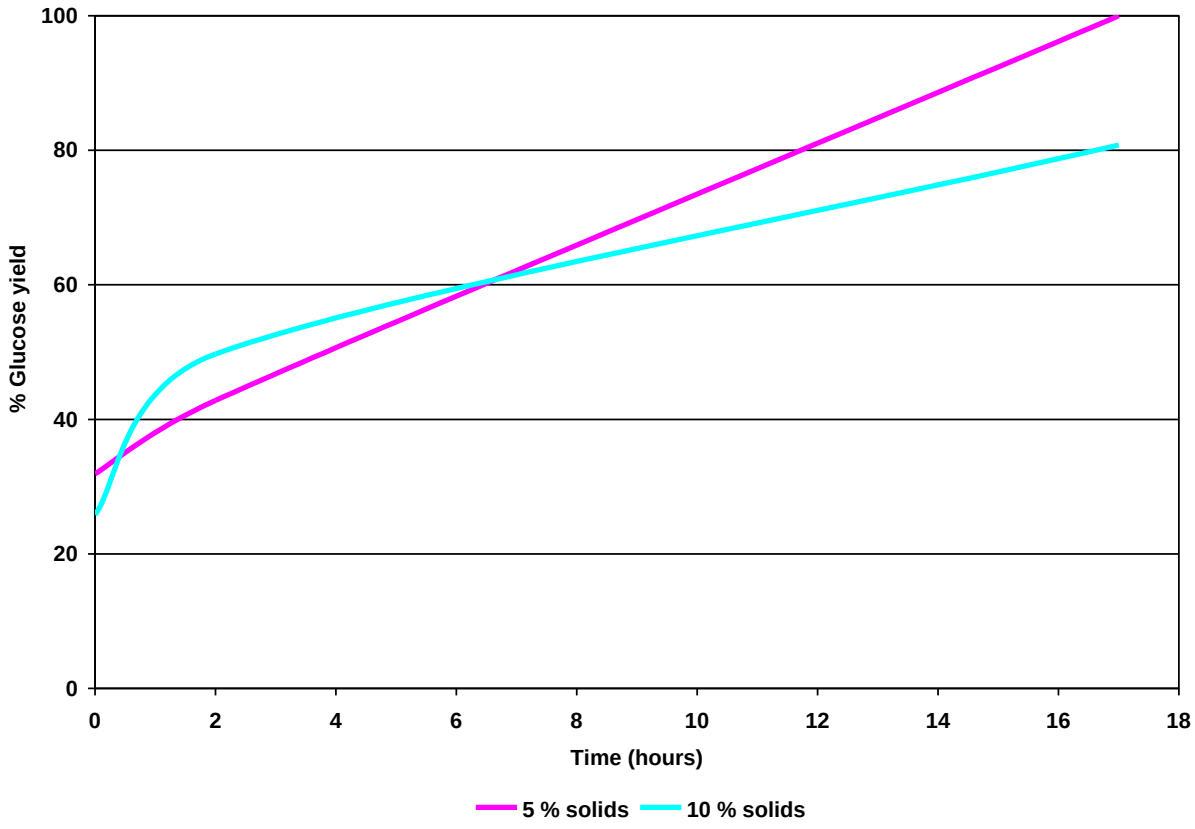

Figure 3. Yields based on theoretical glucose from saccharification of broken rice kernels.

The initial rates of conversion are much different for the two experiments. The 10 % solids experiment exhibits a shape typical of enzymatic reactions while the 5 % solids experiment exhibits a nearly straight line curve from time 0 to 17 hours. This is atypical of enzymatic reactions however the curve is an average of three experiments and further perusal of the data

and elimination of any outlying points may clear up concerns about curve shape. Another interesting feature is that the time 0 glucose conversion for the 5 % curve (31.9 %) is higher than the 10 % (25.8 %) and the curves intersect twice, at 1 hour and between 6-7 hours. It is unclear why the higher solids curve exhibits a higher yield at the early portions of hydrolysis, but it may be a phenomenon related to mixing.

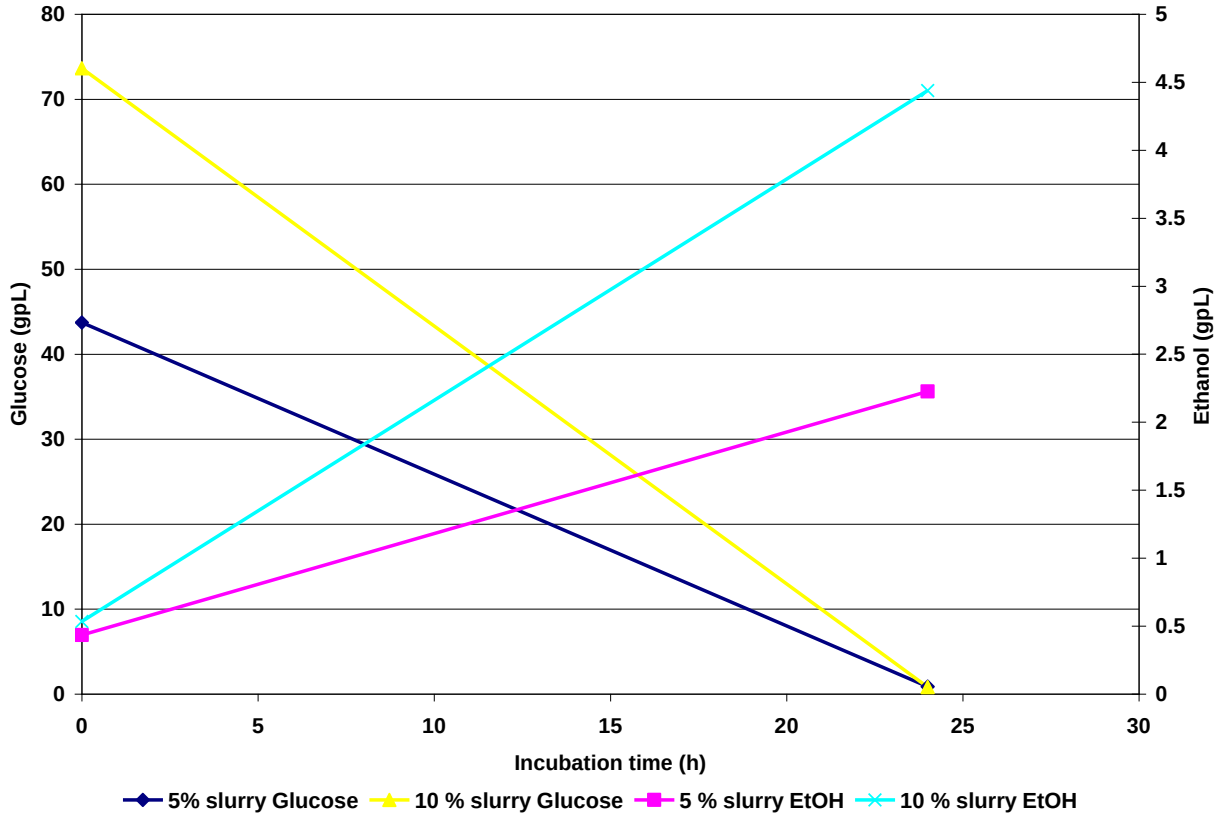

Figure 4. Conversion of glucose liberated from broken rice kernels to ethanol.

The 10 % experiment yielded a glucose concentration of 73.6 gpL while the 5 % experiment showed a 43.7 gpL concentration which are in line with what one could expect based on these solids contents and conversion rates. Although sampling was not performed between 0 and 24 hours, the glucose was rapidly consumed by the yeast and was at 0 gpL at the time of sampling. The ethanol concentration was 4.5 gpL in the 10 % experiment and half of that in the 5 %

experiment as would intuitively be expected. The concentration however is reduced roughly tenfold from what would be expected based upon the glucose contents. Although a calculation error has not been fleshed out, it is strongly possible that the discrepancy is related to human error.

One problem facing the cellulose-to-ethanol industry is that obtaining high concentrations of ethanol in solution is difficult. USDA as well as a couple of other laboratories have proposed recycling of hydrolyzate to increase the ethanol in the beer but we have not stringently tested this hypothesis as of yet. Distillation cost is one of the major obstacles of biomass based biofuels. As described here it is easy to achieve high ethanol contents from starch and if such an environment were to persist in the future , it would be easy to integrate a starch to ethanol production line into the integrated biorefinery should the available capacity be available in the distillation area.

It was proposed that broken rice kernels could be added directly to the autoclave in a biorefinery type scenario, which is always possible. It was determined however that the autoclave is so effective at solubilizing food waste that the starch simply goes into solution and is washed out to serve as substrate for the anaerobic digestion. (Figure 2) While this is a great option and one that could be utilized to provide quick bioenergy in the form of methane, it is not in the scope of this work and will not be detailed.

It was also suggested that rice flour and MSW could be commingled and hydrolyzed in the same fermentor. Through the more thorough development of the integrated biorefinery, it was

determined that the more appropriate course of action would be saccharification in a separate fermentor. It is relatively simple and a common operational technique to sterilize a fermentor between batches to keep down the growth of unwanted microorganisms. As a result the more likely alternative would be to incorporate a jet cooker into the process operation positionally in place of the aqueous pretreatment (Figure 2) and gelatinize the rice starch in the same manner that it is currently performed industrially. The additional incurred cost would be related only to purchase of the jet cooker and would be need-driven. Steam supply is already available to supply the jet cooker. Therefore if no measurable profit or a large enough feedstock supply would be available to provide a reasonable payoff period then the project as a standalone would not be approved.

CONCLUSIONS

The MSW-based integrated biorefinery devised at the USDA is an economically viable concept for producing ethanol from cellulosic materials in the present. Because the pretreatment techniques utilized are aqueous in nature, seasonal ag-wastes and those that are produced at lower density (tons/acre) than could support a standalone biorefinery are options for enhancing the supply of glucose to fermentation.

Rice straw was subjected to hot water pretreatment at varying residence temperatures and times with yields typically from 50-60 % on dry material and hydrolysis yields approaching 70 % of the theoretical glucose. Ultimately in an industrial setting, rice straw could be incorporated as additional feedstock at the time of harvest. It was determined that 1000 tpd of rice straw would provide an additional 30,000-35,000 gallons of ethanol per day at break even or slightly profitable conditions.

Broken rice kernels were also hydrolyzed and as expected it was shown that they can be converted to ethanol. However at this time it is not anticipated that broken rice kernels will be a viable feedstock for the integrated biorefinery. The different schemes to introduce completely different feedstocks to the same biorefinery underscores the potential flexibility of the integrated biorefinery.

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