**ANNUAL REPORT**
**COMPREHENSIVE RESEARCH ON RICE**
June 1, 2007 - December 31, 2007

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

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**LEVEL OF 2OO6 FUNDING**: $7,200

**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.

## EXPERIMENTS CONDUCTED:

***Objective 1 – Enzymatic hydrolysis and fermentation of rice straw***

### 2007 Research Methods

Two cellulosic materials are being used in this study:

- Cellulosics isolated via autoclaving of municipal solid waste (MSW) obtained from a Reno, NV landfill.
- Rice straw (M202) conventionally collected and baled during the 2005 harvest.

Both materials were ground in a Wiley mill to pass a 40-mesh screen prior to use.

**Liquid hot water pretreatment of MSW fibers and rice straw.** 25 OD g of ground MSW or rice straw 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. Mixing commenced and the reactor was allowed to ramp as fast as possible to 120 $^0$C, which typically took place in approximately 30 minutes. Temperatures used in this study were 195 $^0$C, 205 $^0$C, 215 $^0$C, and 225 $^0$C. After the time at temperature, 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.

**Enzymatic Hydrolysis.** 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 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, 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 added at a 1:4 cellulase/cellobiase ratio.

In order to monitor the hydrolysis, 200 μL 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 and the progress of the enzymatic hydrolysis was monitored by HPLC.

**2007 Results**

The work detailed in the proposal is a portion of a larger project to show that a mixed cellulosic biorefinery based on MSW as the primary feedstock is an economically feasible venture. For this reason, the initial steps taken in this project have been to justify the economics of an MSW-based biorefinery and to build the demonstration plant at the Salinas County Crazy Horse Landfill.

Results have shown that utilizing autoclaved MSW with no pretreatment is an economically viable option even though only 40-50 % glucose conversion can be achieved. (**Figure 1**) Liquid hot water pretreatment at temperatures of 215-225 $^{0}$C however can enhance the % conversion of glucose to ~80 % yield and increase the volume of ethanol with only modest increases in energy requirements. Research also showed that glucose from unwashed MSW can be converted to ethanol at 80 % of theoretical yields indicating that inhibition of yeast activity is not an obstacle.


**Figure 1.** Net present value of a 3000 TPD MSW-based biorefinery with a 10 % discount rate. This economic model forecasts a process with no additional pretreatment (41 % hydrolysis rate).

The above work fulfills Goals A and B under Objective 1 and will be summarized in a series of upcoming papers. More importantly for the current work however, it opens potential avenues toward incorporating seasonal agricultural wastes such as rice straw into a biorefinery that can produce green biofuels.

Based upon the MSW results and the fact that the demonstration facility is in the final phases of permitting, we have begun to turn our focus to ag-waste materials that can be potentially converted to ethanol via liquid hot water pretreatment. Untreated, ground rice straw can be hydrolyzed to glucose at a 12 % theoretical conversion rate (**Figure 2**). Even though any unreacted solids can be gasified for energy/fuel production purposes, it is necessary to improve the ethanol yield to make rice straw a worthwhile feedstock for a mixed feedstock biorefinery.

With our knowledge of liquid hot water pretreatment of MSW, we have begun exploratory work on improving the hydrolyzability of rice straw utilizing this technique. Pretreatment temperature and residence time at pretreatment temperature were varied and the % pulp yields and glucose contents of the resultant pulps are listed in **Table 1**.

**Table 1.** Pretreatment conditions, % yields, and glucose contents of resultant rice straw samples.

| | Residence temp. ($^0$C) | Residence time (min) | % Yield | % Glucose | % Xylose |
|---|---|---|---|---|---|
| **Untreated** | **--** | **--** | **--** | **39.8** | **18.3** |
| **RS-1** | **195** | **30** | **52.7** | **58** | **0** |
| **RS-2** | **205** | **30** | **54.7** | **75.8** | **0** |
| **RS-3** | **215** | **30** | **54.0** | **74.8** | **0** |
| **RS-4** | **225** | **30** | **62.0** | **67.6** | **0** |
| **RS-5** | **195** | **60** | **52.1** | **69.4** | **0** |

All total yields were in the range of 52 -64 % of the original rice straw and the glucose contents ranged from 58-75 %. In each case the glucose content was much higher than the untreated rice straw glucose content of 39.8 %. Liquid hot water pretreatment removes primarily the hemicellulose component of the cell wall, enhancing the other components. Hemicellulose is interspersed between cellulose microfibrils in the cell wall, therefore removal of these components provides pores of accessibility to the cellulose not previously available. Although a small amount of amorphous cellulose may be solubilized, the cellulose remains intact and fully hydrated.

An additional advantage to the liquid hot water pretreatment is that a phenomenon known as autohydrolysis occurs at the high temperatures utilized in these experiments. Xylan in the rice straw cell wall is partially acetylated and saponification of these acetyl groups with elevated temperature produces small amounts of acetic acid. This acetic acid catalyzes random chain scissions in the cellulose microfibrils producing two shorter chains with two new end groups.

Cellulases are endoglucanases meaning that they degrade the cellulose polymer by removing cellobiose end units, hence creating additional end groups provides more substrate on which these enzymes can work. As a result the rate of hydrolysis is increased. Additionally as the cellulose chains are shortened, accessibility of enzyme is continually improved.
The impact of these pretreatments is illustrated in **Figure 1**, where it can be seen that the extent of enzymatic hydrolysis is improved in each case with % conversion of available glucose ranging between 48-60 %. This is a marked improvement over the untreated rice straw conversion of 12 %. In the framework of the flexible biorefinery which will undoubtedly require a gasifier to produce process energy from the unreacted solids, such an increase is sufficient to include rice straw as a substrate for the mixed feedstock biorefinery.

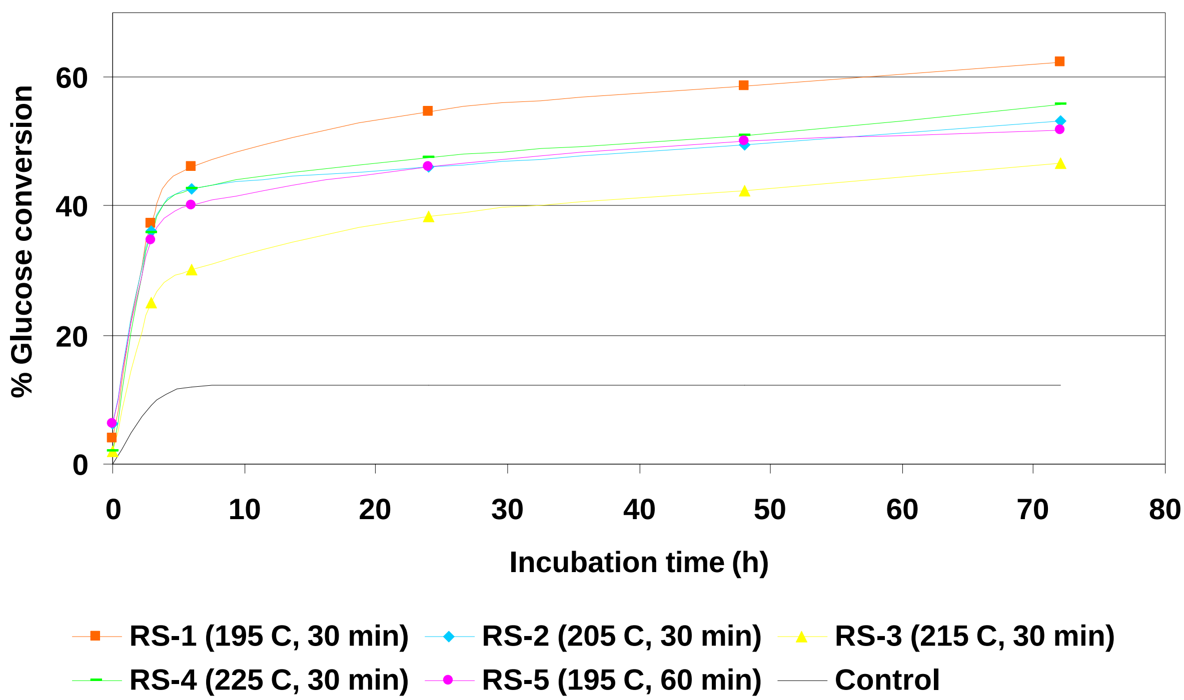


**Figure 2.** Glucose yields from enzymatic hydrolysis of control and pretreated rice straw samples.

## Future Work

Future work for ***Objective 1*** includes optimization of the liquid hot water pretreatment of rice straw for the purposes of improving the substrate for enzymatic hydrolysis. Major factors in optimization include the solubilization of carbohydrate polymers in the brown liquor and the stability of the resulting sugar, the levels of inhibitors produced by autohydrolysis, the extent of enzymatic hydrolyzability, and the ease of fermentation of the glucose derived from rice straw.

Work with ***Objective 2*** has not yet begun but will involve examining gelatinization of broken rice kernels both by the classical technique and also by steam autoclaving. Enzymatic hydrolysis and fermentation conditions will be optimized. Finally it will be proven that broken rice kernel flour can be commingled with MSW and enzymatically hydrolyzed simultaneously.