BPI

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**A Low Energy Continuous Reactor Separator
for the Production of Ethanol
from Starch, Molasses and Cellulose**

**4th Quarterly Report to the
Energy Related Inventions Program**

January 16-March 15, 1995

Project DE-FG01-94CE15594

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Dr. Dale visited the Iowa pilot plant site in early February , taking a seed tank of flocculant yeast for the CSRS, and again in early March, taking a second batch of yeast.

On February 22, Dr. Dale gave a project update to on the CSRS for the Great Lakes Governor's Council in Chicago. A copy of this report is given as Appendix 1. He discussed the progress of the CSRS reactor and the application of this technology to cellulose during the next years effort.

An abstract for the Biomass Conference of the Americas was accepted for our project.

MASTER

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24,000 Liter Continuous Stirred Reactor-Separator (CSRS) Pilot Plant

During the second week of February, 1) seals on the shafts were changed from a Teflon block seal to a mechanical plastic lip seal. 2) a settling device for the overflow from stage 1 was installed by Dr. Dale, 3) a system allowing a 1 to 3% air addition below the agitator was added to allow for trace oxygen addition to the stages with rotometers installed on the control panel by Mr. Lehman in late Feb. The reactor ran quite well, but the yeast were not in the flocculant form desired by Dr. Dale.

The reactor was restarted with lower levels of magnesium, and higher levels of calcium to promote formation of yeast pellets/flocs. This proved successful in the second start-up of the system on March 9th. The reactor ran well, with near complete utilization of sugar at feed rates of 2 to 4 GPM during February and the first two weeks of March. However, leakage from the shaft seal area of tank 1 and 2 became slowly worse during March. The reactor was turned off on March 20 for replacing and rebuilding the shaft seals.

The absorber was completed by Lamont Fab. Co. on March 7th. and was taken over to Hopkinton in late March for installation. Lamont Fab. Co also worked on leveling the bottoms of stages 1 2 and 3 to allow for an exact fit of the shaft seals. This work was to be completed on Sat. March 31.

50 Liter Pilot Plant Studies

During February, two solvents were tested, the first, which gave high ethanol distribution coefficient in the lab, gave much lower coefficient when a 5 gallon batch was made. A second solvent system was then tested and is currently undergoing pilot scale performance tests by M. Moelhman.

Laboratory Studies

a) Ion effects on flocculation- During February and March, the effects of calcium and magnesium on flocculation performance was evaluated by Dr. Lei. Low levels of calcium seem sufficient to cause flocculation while operations with high magnesium and low/no calcium can lead to loss or non-exhibition of flocculant behavior by the adapted yeast strain. A 1 liter bio-reactor has been operated continuously during January, February, and March with the effect of nutrient level on fermentation performance evaluated by Dr. Lei.

b) Nutrient effect on highly concentrated flocculent cells- During March, Dr. Lei of our group completed an experiment where nutrient levels were successively reduced by 50% starting with a full strength media. Performance remained quite good until nutrient levels were at 12% and 0% levels. At these low levels, the density of the floc decreased, and cell loss from the stirred reactor increased substantially. The stirred fermenter was operated continuously for a period of over 6 weeks during this series of experiments

- c) Solvent selection for SAED system- M. Moelman ran a series of experiments where a solvent with a very high selectivity for ethanol over water, but low distribution levels for both water and ethanol was mixed at different molar concentrations with a solvent showing high distribution (0.4) for ethanol but low selectivity. A fairly linear relationship of the distribution and selectivity was noted with mole fraction. An optimal mix was chosen for pilot plant testing.
- d) Biomass conversion to ethanol in some SSF experiments were performed by Mr. Zhao, and a report on this work was completed by Dale for Xylan and NREL who asked our lab to test the Xylan cellulose pretreatment system.

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Appendix 1

A Project Status Report for Great Lakes Governor's
Technical

Advisory/Steering Committee on:

***I. A Pilot Continuous Stirred Reactor/Separator for
Ethanol from
Corn Grits/Starch and Biomass Streams***

A Project Review of Phase 2 work:

***II. A Pilot Continuous Reactor/Separator for Ethanol from
Cellulosics***

February 22, 1995

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Project 1994 Co-Sponsors

Great Lakes Governors Council

Permeate Refining Inc- Hopkinton IA- Site Host

DOE-NIST Energy Related Inventions Program

Purdue University- Dept. of Agricultural Engineering

DOE- Industrial Waste Programs

Xylan/Xymax Inc.

National Renewable Energy Laboratory

I. Ethanol from Corn Grits/Starch in a CSRS

A. Background- Ethanol Production Economics can be improved in several basic ways:

1. reduce costs for substrate- move from corn to biomass carbohydrate source
2. eliminate the cooking process for corn/starch
3. increase efficiency of conversion of substrate to ethanol
4. increase the active cell density in the bioreactor
5. increase the fermentation rate in the bioreactor by reducing the ethanol concentration
6. reducing the amount of 'bottoms water' leaving the process
7. reducing the amount of energy required to distill and dehydrate the ethanol product
8. reduce the labor requirements for an ethanol process by moving to a reliable continuous process
9. reduce the capital requirements for an ethanol plant...improve economics of scale to allow smaller more numerous ethanol facilities

B. Phase 1 results.....our objectives focus on corn/starch to ethanol with a pilot ethanol Continuous Stirred Reactor Separator built along with lab scale studies/ process development

2. No-Cook Conversion of starch/grits to Ethanol with Simultaneous Saccharification and Fermentation (SSF)- During 1994, we have make excellent progress in the SSF no-cook conversion of raw starch to ethanol. Highlights of our work include :

a) batch conversion of raw starch to ethanol with up to 98.5% efficiency of starch utilization...As shown in Figure 1 and 2 cooked and raw starch were converted with almost identical efficiencies.

b) long term operation (37 days) of a 4-liter CSRS lab scale with a 10 to 30% raw starch feed. We will operate the 24,000 liter CSRS on raw starch for a period of 10 to 14 days. Figure 3 shows results from the lab CSRS.

3. Increasing the efficiency of ethanol conversion from starch/biomass- We have not done a lot of work on efficiency during the 1994 phase of this project. Efficiency, defined as X p/s or grams ethanol per gram substrate has a theoretical maximum value of 0.51. Actual values range from 0.42 to as high as 0.47. These values can be improved by 1) reducing the amount of substrate converted to side products (cell mass, glycerol, or lactic acid), and 2) utilizing all of the substrate, i.e. leaving no unconverted dextrans, starch granules or perhaps even trying to utilize the corn fiber as a sugar source as per the New Energy/NREL project. We feel that we should be able to demonstrate high efficiencies by maintaining a very high cell density within the reactor and reducing the need for cell growth as well as reducing the level of fermentation nutrients needed to complete the fermentation.

Figure 1. Batch Fermentation Comparison of Cooked versus Raw Starch

Figure 2. Batch Co-culture of *S. Cerevisiae* & *A. Niger* for Ethanol from Raw Starch

Fig. 2 Conversion of Starch to Ethanol in CSRS

4. increase the active cell density in the bioreactor- We have been developing a strain of flocculent yeast which has the ability to form clumps ranging from 0.25 mm to 3-5 mm ID. These clumps tend to settle rapidly, with a settling time of 15 to 30 seconds in a 1 liter flask. With this strain, very high cell densities can be maintained in the bioreactor. In repeated batch and stirred reactors, we reached 20-40 g/l, while in an upflow tower reactor, a density of 80 g/l was reached. To some degree, fermentation rates are a linear function of cell density, in batch fermentations, 15 g/l is often reached. If a live cell density of 40 g/l can be maintained in the CSRS, we calculate complete conversion of a 30% sugar feed in a residence time of under 12 hours.

5. increase the fermentation rate in the bioreactor by reducing the ethanol concentration - A major feature of the CSRS is simultaneous ethanol removal from the reactor. In a standard batch or continuous cascade fermentation, towards the end of the fermentation, the yeast are working at high ethanol concentrations. This has the effect of severe inhibition of fermentation rates (90-99% loss in specific productivity), as well as an increase in the death rates for the cells. By incorporating separation in the CSRS, the allowable ethanol concentration can be a design variable. In our design work, we have preliminarily set the design ethanol levels shown in Figure 4, with a maximum ethanol concentration of 6 to 7% on stage 2. At this ethanol level, fermentation rates are about 30 to 40% of maximum.

6. reducing the amount of 'bottoms water' leaving the process - There are two basic methods to reduce/eliminate the effluent stream from the system 1) recycle the bottoms water as shown in Figure 5, and 2) feed highly concentrated feeds streams to the reactor.

1) Recycle- During our work on this project, the effects of an 80% recycle of bottoms water to the CSRS reactor feed was

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Figure 4. Design flows and concentrations in the CSRS.

Figure 5. The CRS concept with simultaneous starch hydrolysis in a 'no-cook' process.

Figure 6. SUBSTRATES CONCENTRATE WITH STAGES
SUCROSE FERMENTATION
80% recycle of 'Bottoms water'

tested over a period of 120 days. We noted a build-up in glycerol/lactic acid as shown in Figure 6, but the reactor continued to run well over the period of the experiment. We feel that this demonstrates the ability of the yeast and our system to run at 80% water recycle, as well as demonstrating the feasibility of recycling water as suggested in Figure 5.

2) Highly Concentrated Feeds- The CSRS should be able to handle highly concentrated feeds, as ethanol removal from the bioreactor eliminates the usual 20-24% sugar feed constraint. However, osmotic inhibition of cell growth is stronger than inhibition of productivity. A recent paper by Dale et al. (1994) suggests that both sugar and ethanol inhibition is largely an osmotic phenomena. Tests in our lab of feeding highly concentrated molasses to the CSRS indicated a loss in performance rates due to decrease in live cell mass on the stages. The use of osmotolerant strains rather than the flocculent strains might allow the feeding of very concentrated feeds, but cell recycling using either membrane or centrifuges would be required.

7. reducing the amount of energy required to distill and dehydrate the ethanol product- In the CSRS, we are developing a solvent absorption-extractive distillation process which allows the low energy recovery of an anhydrous ethanol product. An organic solvent- selected for the properties of 1) low volatility, 2) non-toxic to yeast, 3) high affinity for ethanol, and 4) low affinity for water is used in the process shown in Figure 7. Several solvents have been found to be suitable, and we are continuing selecting, developing, and testing some solvents with the desired properties. Our project goal is to install the SAED system at PRI during the next 2 months. Most of the required equipment has been purchased, and preliminary design work finished.

8. reduce the labor requirements to run an ethanol process by moving to a reliable continuous process- The CSRS system is

Figure 3: The Solvent Absorption-Extractive Distillation (SAED) process for ethanol recovery from gas streams.

Figure 8. Energy Req. for Anhydrous Ethanol

quite resistant to contamination or infection. In the lab we have run various batch and continuous tests using our flocculent yeast strain for over a total of 250 days of continuous operation in various experiments, often with no medium sterilization, with very few system upsets over all these tests. We are quite confident of the robustness of the proposed system.

9. reduce the capital requirements for an ethanol operation - A major goal of this project is to develop low cost systems for ethanol production. Our company would like to be able to offer grain handlers and feed lot operations a higher value product that can be operated on a fairly small scale. We are targeting the range of 350,000-5 million gal/yr as being a size that is currently uneconomic for batch ethanol operations due to the need for evaporation and drying of the stillage, large scale storage of dried distiller's grain, and expensive and complex distillation and dehydration technologies currently used. Our CSRS preliminary economics for a stand-alone plant w/o distiller's wet grain drying are calculated to drop from \$1.50/annual gallon at the 500,000 gal/yr scale to \$0.75/annual gallon at the 5 million gal/yr scale.

B. Project Progress Report- Corn/Starch to Ethanol

I. Applied Research- Pilot CSRS/SAED system

1. Detailed Design, Prints of CSRS.....completed 5/30/94.
2. Fabricate CSRS Reactor....completed 9/15/94
3. Fabricate Solvent Absorber/duct work...completed 2/20/95
4. Preliminary Fermentation Performance Tests of CSRS... currently in progress
5. Installation of SAED System.....sched. for 4/15/95
6. Operation of CSRS/SAED- 1 month continuous operationsched. for 6/15/95

II. Basic Research

1. No-Cook Starch Conversion to Ethanol/ Batch-Continuous.....completed 7/30/94
2. Extrusion cook tests...economics questionable
3. Biomass conversion to ethanol.....in progress
 - a) xylose conversion to ethanol....competed 7/30/94
 - b) extrusion SSF of straw/ switchgrass....in progress
 - c) improvement of extrusion (Xylan) pretreatmentin progress

Figure 2 - Stage 1 interior

CSRG

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II. Ethanol from Biomass using a Continuous Reactor Separator- This work is to apply the CSRS Simultaneous Saccharification and Fermentation to Biomass....both hemicellulose and cellulose. The basic concept to be tested, developed and demonstrated is shown in Figure 8. The project will combine the extrusion delignification process of Xylan/Xymax Inc. which solublizes the lignin fraction of the biomass and the hemicellulose. We propose to first ferment the hemicellulose

Phase II- Project Partners/Co-Sponsors Include:

Great Lakes Governor's Biomass Council
Permeate Refining Inc. (Site Host)
Xylan/Xymax Inc.
Bio-Process Innovations Inc.
Purdue University-Ag. Eng. Dept.
Dept. of Energy- Industrial Waste Programs
Dept. of Energy- Energy Related Inventions Program
Merrill Iron and Steel

I. Applied Research- Pilot Scale Biomass to Ethanol Bioreactor Demonstration

1. Design of Demo/Pilot Scale Biomass CSRS for Corn Stalks to Ethanol- Month 1-8
2. Build/Modify CSRS for Biomass Ferm.- Month 6-14
3. Operate CSRS on Pretreated Corn Stalks- Month 15-18

II. Basic Research- Development of Procedures for Biomass Utilization

1. Evaluate Enzyme (Cellulase/hemicellulase) Production by Various Fungi sources- Test Co-culture of cellulase organisms with fermentation yeasts- Month 1-15
2. Operate 4 to 6 liter lab scale CSRS using two stage SSF fermentation.....xylose in stage 1 and 2, cellulose in stages 3 and 4.- Month 6-16
3. Process Modeling/Optimization/Economics for Biomass Ethanol Production- Month 1-18