# DOE Inventions & Innovations Program- Final Report

## Project Title: High Speed/ Low Effluent Process for Ethanol

**Covering Period:** June, 2003 through April 30, 2006
**Date of Report:** May 30, 2006

**Recipient:** Bio-Process Innovation, Inc. ( www.bio-process.com )
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**Award Number:** DE-FG36-03GO13006

**Working Partners:** Xethanol, TEMA

**Cost-Sharing Partners:** BPI, TEMA, Xethanol, ADM

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**Executive Summary:** In this project, BPI demonstrated a new ethanol fermentation technology, termed the High Speed/ Low Effluent (HS/LE) process on both lab and large pilot scale as it would apply to wet mill and/or dry mill corn ethanol production. The HS/LE process allows very rapid fermentations, with 18 to 22% sugar syrups converted to 9 to 11% ethanol 'beers' in 6 to 12 hours using either a 'consecutive batch' or 'continuous cascade' implementation. This represents a 5 to 8X increase in fermentation speeds over conventional 72 hour batch fermentations which are the norm in the fuel ethanol industry today. The 'consecutive batch' technology was demonstrated on a large pilot scale (4,800 L) in a dry mill corn ethanol plant near Cedar Rapids, IA (Xethanol Biofuels). The pilot demonstrated that 12 hour fermentations can be accomplished on an industrial scale in a non-sterile industrial environment.

Other objectives met in this project included development of a Low Energy (LE) Distillation process which reduces the energy requirements for distillation from about 14,000 BTU/gal steam ($0.126/gal with natural gas @ $9.00 MCF) to as low as 0.40 KW/gal electrical requirements ($0.022/gal with electricity @ $0.055/KWH). BPI also worked on the development of processes that would allow application of the HS/LE fermentation process to dry mill ethanol plants. A High-Value Corn ethanol plant concept was developed to produce 1) corn germ/oil, 2) corn bran, 3) ethanol, 4) zein protein, and 5) nutritional protein, giving multiple higher value products from the incoming corn stream.

BPI is planning to implement and commercialize these technologies in the USA, and in cane producing areas around the world. Current projects in various stages of planning and execution include a 5,000 Liter/day cane juice ethanol demonstration project in Columbia, a 125,000 LPD molasses ethanol 'expansion project' in Pakistan, and a 30 million gal/yr dry mill ethanol facility near BPI's offices in Indiana. We further plan to promote the process to current wet mill corn processors (ADM, Cargill, and Staley's)

**Background:**

The High Speed/ Low Effluent (HS/LE) fermentation process allows very fast (6-12 hours) and complete fermentation of sugars by means of a self-aggregating yeast strain developed by BPI. This yeast allows a stable, high density yeast population to be maintained in the bio-reactors. In this project, lab and pilot scale studies were completed to permitthis technology to be confidently introduced into the US corn ethanol industry. There are three basic sections of this project: 1) application of the fermentation technology to the wet mill corn syrup/ethanol industry, 2) application to the dry mill corn ethanol industry, and 3) combining the fermentation technology with low energy distillation technology.

In applying the HS/LE fermentation technology to wet mill corn syrup (Task 1), there were three major questions to be addressed. 1) Ensure complete conversion of dextrins to glucose in the short fermentation period: We must entirely saccharify the dextrins to glucose before the completion of the fermentation to ensure complete utilization of the starch/dextrins in the feed (Task 1.1), 2) Determine limits of 'back-set: We completed an effort to determine the limits of stillage recycle (back-set) with the goal of demonstrating successful back set at a high percentage of the feed make-up. This saves capital and energy associated with evaporation of the stillage (Task 1.2). 3) Demonstrate scalability of process to pilot and commercial scale: A major goal of this project was to scale-up and demonstrate the fermentation system at the 1,200 gallon scale.(Task 1.3).

Application of the HS/LE to dry mill syrup (Task 2) requires a fairly clear/clean syrup to be produced from the dry mill process. This goal could be attained by removing the corn fiber either before or after the cook process. Completed trials by project partner TEMA on a dry mill corn mash with a rinsing centrifuge indicated that finely ground corn tended to clog the screens of the rinsing centrifuge so that drainage rates of the rinse liquid through the retained solids slowed over time. Other solid separation technologies will be evaluated and tested in the future.

**Energy-** Energy requirements in distillation can be reduced by integrating energy requirements throughout the ethanol plant (i.e. re-using steam from the evaporators), or by recompressing the overhead ethanol vapors and using these hot vapors to drive the reboiler. This MVR distillation concept can reduce energy usage for the distillation significantly (50% to as much as 75% reduction in utility costs), and even more energy savings and capital savings can be obtained when accompanied with design modifications of the distillation column itself. The design and modeling of the BPI Low Energy MVR system, along with the incorporation of energy saving technologies into corn ethanol plant, and modeling of the entire corn ethanol facility was completed as Task 3 of this project.

## List of Acronyms

| | |
|---|---|
| BPI | Bio-Process Innovation (contractor) |
| Brix | Sugar Concentration (%) generally applied to sucrose |
| CIP | Clean in Place |
| DDS | Dry Disintegration System- a new technology under development for corn dry fractionation by Xethanol |
| DM | Dry Mill |
| DG | Distillers' Grains |
| DDG | Dry Distillers' Grains |
| HV | High Value |
| HS/LE | High Speed / Low Effluent |
| HPDG | High Protein Distillers' Grains |
| KL | Kilo Liter (1000 liters) |
| LE Dist. | Low Energy Distillation |
| OD | Optical Density- a measure of clarity of a solution |
| MVR | Mechanical Vapor Recompression |
| RI | Refractive Index |
| RTD | Residence Time Distribution |
| SEMO | A company in the dry fractionation corn industry |
| TEMA | A company making rinsing centrifuges based in Cincinnati |

## Project Description:

### 1. Project Goals

### Task 1.0 Application of the HS/LE Process to Wet Mill Corn Syrups

### Task 1.1 Saccharification

The ability to complete conversion of dextrin polymers (2 through 8 or more glucose monomers per unit) to glucose- termed saccharification- either prior to or during fermentation (simultaneous saccharification and fermentation' (SSF)) using commercially available dextrin product (GPC M200) was studied. We determined that saccharification proceeds rapidly enough to not be a limiting factor in HS/LE performance. A report on this work is attached as Appendix 1.1

### Task 1.2 Effects of stillage recycle on HS/LE performance

The effect of recycling stillage was evaluated in a long term series of experiments. We did not notice any negative effects in fermentation rates at 50%, 65% or 75%backset levels, but at the 75% levels of backset incomplete sugar utilization was noted. A report on this work is attached as Appendix 1.2

**Task 1.3. Design/ Construction, and Operation of Pilot Plant for Wet Mill Syrup-**

In cooperation with Xethanol Biofuels and Xethanol, we designed and built two different pilot plants. Version 1 was designed and sited in Xethanol's small ethanol plant located in Hopkinton, IA during Q4 through Q7 of the project. Ethanol operations at this plant were stopped in February, 2005 (Q7), just as we were nearing completion of construction of our Version 1 pilot plant. In ensuing discussions with Xethanol, it was decided to site a second version of the pilot plant, (Version 2) in their plant in Blairstown, near Cedar Rapids, IA. Version 2 was then designed with the selection of vessels by Jim Stewart, GM, with a 4,800 Liter vessel selected as the HS/LE reactor. Dr. Dale completed a Process Flow Diagram (PFD), followed by a Piping and Instrumentation Diagram (P&ID). During the last 6 months of 2005, Xethanol pipefitters, electricians, and welders assembled and completed modifications to the vessels (including a large set of sight glasses), set the vessels, set pumps, piping, steam, water and electrical connections based on the P&ID. The pilot plant was completed in early February, 2006. Preliminary tests resulted in the failure of the drive one agitator in T-301, which was then repaired and replaced.

A shake-down trial in March '06 was followed by performance trials in April. These trials were quite successful, with performance of the non-sterile, industrial scale HS/LE reactor closely matching performance of lab scale trials. A report on this work is attached as Appendix 1.3

A 'Material Transfer Agreement' was developed with ADM. BPI then provided the ADM research center with HS/ LE yeast. ADM completed some preliminary small scale trials of a continuous 3 stage CSTR implementation of the BPI technology on corn syrups from their Decatur IL facility. Yeast clogging of the tubing connecting the reactors caused operational problems. They may or may not pursue further trials based on conversations Dr. Dale has had with Dr. Charles Abbas, Director of Yeast and Renewable Research for ADM.

**Task 2.0 Application of HS/LE to Dry Mill Syrups**

Traditional dry mill corn ethanol production consists of grinding the corn, then adding liquid (a mix of water and thin stillage backset) to the milled corn to produce a mash. The mash is then liquefied (cooked w/ alpha-amylase and jet cooked @ 240 F followed by hold @ 195 F- where the starch granules are converted to dextrin polymers) and saccharified (addition of gluco-amylase after the mash is cooled to 140 F- where the dextrin polymers are converted to the glucose monomer). The whole mash is then taken to fermenters and the glucose converted to ethanol. To apply the HS/LE to dry mill ethanol, the fiber needs to be taken out before the fermentation. Our original design (**P1**) was to separate and rinse the insoluble corn solids/fiber after the cook using a rinsing centrifuge. We identified a project cooperator, TEMA Industries, a company that builds and markets rinsing centrifuges. The performance of the rinsing centrifuge on corn mash was determined in trials completed by TEMA (Task 2.1 -year 2).

**Task 2.1 Effect of Insoluble Solids-**

During Q5, we began some preliminary lab and pilot scale tests with centrifuges to determine the necessary processing equipment to achieve the required clarity in the dry mill dextrins fed to the HS/LE fermenter from a solids washing centrifuge as per BPI's DM-2 process. During Q6 we determined at what concentration Non-Soluble Solids (NSS) interfere with the long term performance of the HS/LE process: 1) Dry milled 'cooked' mash (converted to dextrins/glucose) was screened and rinsed using a 500 micron screen to separate the soluble dextrins from the corn fibers/NSS. The levels of NSS in the liquid fraction were measured and correlated with the optical density (OD) of the dextrin syrup. Fermentation trials in 250 ml shake flasks were performed to evaluate the effects of the NSS on the HS/LE yeast strain. During Q6, BPI began a series of lab scale centrifugations of whole mash to determine the centrifugal force needed to clarify the syrup, as well as the quality/clarity of the supernate from the mash. During Q9 we continued this separation work on Process P5, the BPI 'High value' process (see below) determining what degree of syrup clarity (Optical Density or OD) was required for good yeast pellet formation (Appendix 2.1).

**Task 2.2 Solids Separations Technology**

A variety of separation technologies were evaluated as a part of this I&I project. We have been evaluating several processes in addition to the **P1** process (rinsing centrifuge separation of corn fibers/insoluble solids after cook of whole corn). Dr. Dale worked with two industrial cooperators, TEMA centrifuges and SEMO Milling to evaluate cost and performance of various improvements in corn ethanol processing.

**P2**- Dry grinding and separation of the corn fiber prior to cook. Xethanol Corp. has been in discussions with a company called Dry Disaggregation System (DDS USA), owners of a system which may allow a high starch fraction to be produced from cracked corn. Some preliminary trials in Hopkinton, IA were not particularly successful, but apparently the technology has been successful in separating wheat starch from gluten and hull in Europe. In recent conversations, Xethanol indicates that it is planning to run corn starch/ fiber separation trials. BPI may test the clarity of the dry mill syrups obtained from this DDS starch stream in post-DOE project, on-going development efforts.

**P3**- During Q4 of the project, at the Corn Utilization and Technology conference, Dale met U of Illinois researchers (M. Tumbleson, Vijay Singh ) who gave a talk and posters on the 'Quick Germ/ Quick Fiber' process. This shows promise in being able to speed up a wet mill process. This technology is a modified 'wet mill' process using enzymes w/ reduced SO2 for steeping.

**P4**- Another separation process termed 'bio-milling' is being developed by Biorefining Inc. of Minneapolis, MN. Dr. Dale met with Thom Menie, VP marketing for Biorefining in June of '04 at the Fuel Ethanol Workshop in Madison, WI, and has had follow-up conversations with Doug Van Thorre, President, and Wes Haines, CEO. Flow diagrams

of these P1, P2, P3 and P4 methods of producing a 'modified dry mill ethanol syrup' are included in Appendix 2.2

**P5**- A dry fractionation processing of the corn produces three streams: corn bran (the outer covering of the corn kernel, grits (the endosperm), and germ. This separation, termed 'dry milling' has been utilized in the grain industry since the 1920's. The application of this separation prior to fermenting only the grits is being suggested by a number of researchers. Our **P5 process** utilizes dry fractionation of the corn kernel, followed by a cook (liquefaction/saccharification) of the grits fraction, a separation of the fiber/protein (high protein Distillers Grains) from the corn syrup, and HS/LE fermentation of the corn syrup.

**P6-** During Q6 through Q8, BPI began designing and evaluating a process we have termed the 'High Value Process' for corn processing to ethanol. This process gives a variety of co-products from an ethanol facility rather than just ethanol and distillers grains as per current technology. A block flow diagram of the process is shown in Figure 3. As per this figure, the process implements 'dry fractionation' as per **P5**, but then further recovers a zein protein stream, and a 'nutritional' protein stream. The **P6** is designed to produce a variety of 'higher value' products.

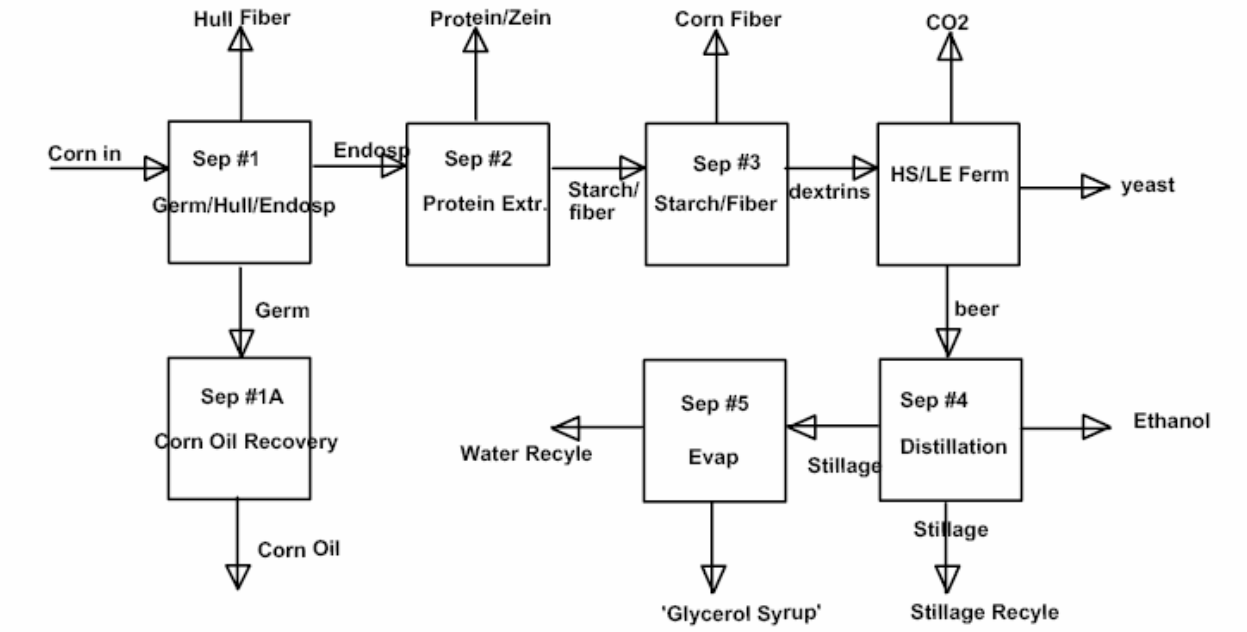



Figure 3. BPI's concept for the "P6", 'High Value' Corn Ethanol Dry Mill Facility.

An overview of the application of the HS/LE process to dry mill syrups is given in Appendix 2.2

## Task 2.3 Pilot Plant Demo w/ Dry Mill Syrup

The application of the pilot plant to dry mill syrups was not completed in this project due to time constraints. Xethanol is developing a 'dry fractionation' process (P5) utilizing a new milling/separation technology called the DDS. They hope to run the pilot with syrup from the converted starch fraction from the DDS system. BPI is planning a full scale 20 million gal/yr demo of a dry mill facility using conventional dry fractionation.

## Task 3.0 Low Energy Distillation for Fuel Ethanol

### 3.1-3.2 Low Energy Distillation- Modeling and Optimization, Column Design

The largest energy use in a corn ethanol plant is the distillation column. Energy use for distillation is between 14,000 and 18,000 BTU/gallon. The largest need for cooling in the plant is the column condenser where the high proof ethanol (190 proof) is condensed with 1 part taken as product and 2.5 to 3.5 parts taken back to the column as reflux. Mechanical Vapor Recompression allows coupling of the heating and cooling requirements of the column. Energy use is changed from steam (natural gas) to electrical. Electrical requirements vary with the design parameters of the column. We determined that electrical requirements could be as low as .36 KW/gal asdescribed in Appendix 3.1-2. The use of the LE distillation and BPI HV P1 (dry fractionation) technology largely eliminates steam use for distillation, and reduces the amount of gas needed for DDG drying by about 50% by removing the hull and germ prior to cooking. Thus natural gas usage is cut from 34,000 BTU/gal to under 9,000 BTU/gal. If Distillers Grains (DG's) were fed locally to cattle without being dried, more gas savings could be obtained, but these sorts of savings were not included in the Table above in order to keep the comparison as fair as possible to competing systems.

A detailed report- Appendix 3.1 and 3.2- on our process design efforts on four cases is appended for a base 15 million gal/yr sized ethanol facility. A Net Present Value (NPV) analysis was performed to allow capital and energy costs to be brought back to a single 'present value' of the costs for purchasing and running an ethanol distillation column, producing 188 proof ethanol from a 12% (v/v) feed beer stream. As per this economic evaluation, the 'best' implementation of the BPI Low Energy Distillation column can reduce the NPV of costs (initial capital and energy summed over a 12 year operations span) from $15,053,000 to $4,795,000 for Case 3, a case designed to minimize energy costs for the BPI Low Energy Distillation System. During Q4, we added a fifth case. This fifth case would apply to whole mash, and is directly applicable to current dry mill facilities. A NPV for Case 5 showed total costs only slightly higher than Case 3. This represents an enormous savings to the ethanol producer- a nearly 70% savings over 12 years. Capital costs for the BPI Low Energy Distillation system (Case 3 & 5 ) were estimated at $2.6 million, only 30% higher than the base case gas fired steam driven distillation column which is standard in the industry today, and does not include savings associated with reduced boiler size. These very favorable results should provide a great impetus to implement the technology.

### 3.3 Total Corn Plant Modeling.

BPI completed a detailed model of a variety of corn ethanol plants. The model includes 1) flows and mass balances, 2) energy inputs, 3) equipment list and size, 4) economic inputs for equipment, energy, plant labor, enzymes & chemicals as well as providing for inflationary costs. Printouts of the model of a 'generic dry mill ethanol' plant and the model for P6, BPI's High Value corn dry mill ethanol concept are given in Appendix 3.3

### 4.0 Publications/ Patents:

1) BPI was issued patent # 7,070,967 (B2) on the HS/LE process on July 4, 2006.

2) A powerpoint presentation entitled "High Speed Low Effluent Fermentation Process for Dry Mill Corn" was developed and given to ICM, a major dry mill ethanol plant design/build company based in Wichita KS and also mailed to Xethanol, a company starting to run ethanol plants in Iowa. Xethanol is also developing a dry starch/fiber separation technology.

3) A modified version of this file was sent to Xethanol executives in December-2003.

4) A poster Presentation on the HS/LE process for Corn Ethanol (appended w/ Q3) was presented on June 7-9, 2004 at the Corn Utilization Conference (Indianapolis, IN)

5) A poster presentation on the HS/LE process, along with our concept for HV processing was given on May 1-4, 2005 at the Biotechnology for Fuels and Chemicals Symposium in Denver, CO.

6) A poster describing our project results and concepts for a 'Next Generation' corn ethanol plant was presented at the National Corn Growers Convention in Houston in late June, 2006.

These files are attached as Appendix 4.1, 4.2, and 4.3

### 5.0 Commercialization Efforts

BPI has a number of commercialization efforts underway:

1) Cane Juice Ethanol. A 5000 Liter per Day demonstration plant is under construction in Columbia S.A. Partners in this trial include BPI, Contactos Mundiales, and Orgánicos de Valle LTDA. A photo of the HS/LE reactor built for this plant is shown below in Figure 5.1

Figure 5.1 HS/LE reactors for Cane Juice Ethanol Production (Columbia, SA)

2) Molasses Ethanol. A distillery in Pakistan is planning to double their fuel ethanol production from 125 KL/day to 250 KL/day using the HS/LE reactor, and is evaluating the possibility of the LE distillation as well. A photo of Dr. Dale at this plant is shown below in Figure 5.2
3) USA Dry Mill Corn Ethanol. BPI is working towards siting a 30 Million Gallon/ Yr fuel ethanol plant near Greenfield IN using process P5- Dry Fractionation. This plant will demonstrate the HS/LE process and hopefully the LE Distillation as well.
4) USA Wet Mill Ethanol. BPI is in contact with ADM and plans to have discussions with the other 3 major wet millers in the USA to promote the HS/LE process. ADM ran a preliminary trial of the technology in August of 2006.

Figure 5.2 Pakistani Molasses Ethanol Expansion Project

## APPENDIX A. Task Sched. HS/LE Corn Eth.

| Task Number | Task Description | Task Completion Date | | | | Progress Notes |
|---|---|---|---|---|---|---|
| | | Original Planned | Revised Planned | Actual | Percent Complete | |
| **1** | **Wet Mill HS/LE** | | | | | |
| 1.1 | Saccharification | 11/03 | 12/03 | 12/03 | 100% | Dextrins convert easily |
| 1.2 | Stillage Recycle | 11/03 | 12/03 | 12/03 | 100% | Complete long term trial @ 60% set-back |
| 1.3 | Pilot Plant | 6/04 | 6/05 | 4/06 | 100% | Pilot sited, plumbed and electrical/instru. Completed |
| **2** | **Dry Mill HS/LE** | | | | | |
| 2.1 | Effect of Insol. Solids | 5/04 | 12/04 | 12/04 | 100% | Began trials w/ Dry mill syrups |
| 2.2 | Solids Sep'n Tech. | 8/04 | 6/05 | 12/05 | 100% | Met w/ TEMA, began Exp. design |
| 2.3 | Pilot scale trial for dry mill syrup | | | | | Not completed due to time constraints; trials pending |
| **3** | **Low Energy Dist.** | | | | | |
| 3.1 | Low Eng. Distillation Modeling/Optimiz. | 2/04 | 3/04 | 6/04 | 100% | Completed stage model of column, prelim econ. |
| 3.2 | Detailed Eng.of Distillation Column | 5/04 | 6/04 | 9/05 | 100% | Completed evaluation of Reboiler designs |
| 3.3 | Total Corn Plant Modeling | 9/05 | 9/05 | 12/05 | 100% | Built detailed spreadsheet of plant |
| **4.0** | Project Management/ Commercialization | 5/05 | 9/05 | 4/06 | 100% | Completed final report/ Begin commerc. efforts |

**Appendix A**

## Appendix B

## Final Spending Schedule

HS/LE Production of Ethanol

**Final Spending Schedule** **Project Period:** 7/1/2003 to 4/30/2006

| Task | Approved Budget | Final Project Expenditures |
|---|---|---|
| Task 1 Wet Mill HS/LE | | |
| 1.1 Saccharification | 23,511 | 23,535 |
| 1.2 Stillage Recycle | 23,511 | 23,485 |
| 1.3/2.3 Wet Mill/Dry Mill Pilot | 389,250 | 665,825* |
| Task 2. Dry Mill HS/LE | | |
| 2.1 Effect of Insol Solids | 33,292 | 33,500 |
| 2.2 Solids Sep'n Tech | 23,511 | 21,373 |
| Task 3. Low Energy Distillation | | |
| 3.1 Modeling/ Optimization | 29,389 | 31,683 |
| 3.2 Detailed Engineering | 17,634 | 17,590 |
| 3.3 Integrated Plant Design | 23,511 | 35,700 |
| Task 4. Project Management | 47,023 | 77,170* |
| **Total** | 610,634 | 927,764* |
| | | |
| DOE Share | 200,000 | 200,000 |
| Cost Share | 410,634 | 727,764* |

* The higher costs for the pilot reflect that the pilot plant site was re-located after being near completion at site 1, and the pilot plant re-designed, and built with different stainless steel vessels at site 2. The higher costs for project management reflect the fact that the project time line increased from 24 months to 34 months to complete the project. All these increased costs were borne by the project participants.

*see note above

# Appendix C

## Energy Savings Metrics

The following Energy Savings Metrics table is completed based on a specific unit of production, one gallon of anhydrous fuel ethanol.

**One Unit of Proposed Technology:**

A corn ethanol plant utilizing the BPI HS/LE reactor, dry fractionation of the corn, and LE Distillation system sized at the 15 million gallons per year (15MGPY) scale.

**One Unit of Current Technology:**

The current 'State of the Art' ethanol plant offered by ICM utilizes 34,500 BTU's/gal natural gas, and 0.75 KWH/gal to produce anhydrous ethanol and Dry Distiller's Grains w/ Solubles (DDGS) as per (www.icminc.com/pdf/PerformanceGuarantees.pdf)

**Energy Savings Metrics**

| Type of Energy Used | A | B | C=A-B | D | E=CxD |
|---|---|---|---|---|---|
| | Current Technology (Btu / gal) | Proposed Technology (Btu / gal) | Energy Savings (Btu / gal) | Estimated Number of Units in U.S. by 2010 gal/yr | Energy Savings by 2010 (Btu / yr) |
| Oil / Gasoline | | | | | |
| Natural Gas | 34,500 | 8,400 | 26,100 | 2 billion | 52.2 trillion |
| Coal | | | | | |
| Electricity (@ 10,500 Btu / kWh) | 7,875 | 15,330 | (7,455) | 2 billion | (14.9 trillion) |
| Other Energy 1 (Explain) | | | | | |
| Other Energy 2 (Explain) | | | | | |
| Other Energy …n (Explain) | | | | | |
| Total Per Unit | 42,375 | 23,730 | 18,645 | 2 billion | 37.3 trillion |

## Discussion of Energy Savings:

The BPI HS/LE process offers the ability to reduce energy costs per gallon by almost 50%. There is a national priority, and a recently signed ENERGY POLICY ACT OF 2005, that calls for moving from our current national ethanol production level of about 4 billion gallons to at least 7.4 billion gallons by 2012 (as per Table below from RFA web site (http://www.ethanolrfa.org/policy/regulations/federal/standard/)

| Year | Renewable Fuels (billions of gallons) |
|---|---|
| 2006 | 4.0 |
| 2007 | 4.7 |
| 2008 | 5.4 |
| 2009 | 6.1 |
| 2010 | 6.8 |
| 2011 | 7.4 |
| 2012 | 7.5 |

The energy savings suggested in the Table above would represent a 50% market penetration/implementation in new construction as the US moves from 4 to 8 billion gallons/yr. If the technology were back- fitted into existing facilities, total energy savings suggested above could be doubled. The BPI technology largely eliminates steam use for distillation, and reduces gas need for DDG drying by about 50% via removal of hull and germ prior to cooking. Thus natural gas usage is cut from 34,000 BTU/gal to under 9,000 BTU/gal. If Distillers Grains (DG's) are fed locally wet, more gas savings could be obtained, but extraneous savings were not included in the Table above so as to keep the comparison as fair as possible.

Electrical needs increase as both the compressor for the distillation and the de-fractionation of the grain require electrical inputs. However, even at the suggested 10,500 BTU/KWH conversion there are enormous savings to be obtained.

# Appendix 1.1 Saccharification of Wet Mill Dextrins

Background:

Starch is a long chained polymer of glucose monomers. The starch polymers are tightly bound up in granules within the grain hull. Milling the grain breaks the grain into hull fibers, germ, and starch granules. The starch polymers must be 'unfolded' from the tightly bound granule by a cooking process which opens up the starch molecules. Without the addition of cleaving enzymes, the uncoiled starch polymer forms a very viscous gel. If cleaving enzymes are present, these enzymes (high temperature bacterial alpha-amylase) hydrolyze some of the glucose linkages- reducing the size of the starch polymer molecule from 1000's of glucose monomers to a mix of short chain 'dextrin' polymers with a range of 5 to 30 glucose monomers. This process is called 'liquefaction' as the starch is 1) converted from a non-soluble granule to an open- gel forming- large polymer, and then 2) the long chain polymer is cleaved into shorter water soluble dextrins.

In order to ensure complete fermentation of all the starch derived dextrins, the short chain dextrins must be converted to glucose monomers prior to or during the fermentation as the yeast can not metabolize dextrins. Glucoamylase (GA) is the enzyme used to break dextrin polymers down to the glucose monomers. A commercial glucoamylase sold by Enzyme Development Co. was used in this work.

General Method: Bio-Reactor

The HS/LE bio-reactor was built using a 1000 mL column, yeast cell bed of between 120 – 220 mL BPSC-15, stir plate and magnetic stir bar. The yeast cells were grown first anareobically from a cell culture in a test tube containing 25% glucose and 3,3,3 g/L YMP solution. Cells were then transferred to a 250 mL flask and grown aerobically in a similar solution. 15 mL of cells were grown in this fashion and added to the column reactor. At first a 17.5% glucose solution was added to the bio-reactor and a filtered bubbler was included. This step was repeated (trials A – C) and the cell bed reached 120 mL. Consecutive batch fermentations were then used for each trial. For each batch 600 mL of 25% glucose solution was added. The glucose solution also included yeast extract, malt extract and peptone (YMP), Sodium Metabisulfite (MBS), $K_2HPO_4$, $NH_4SO_4$, and $NH_2OH$. pH was kept between 2.6 and 4.3 through the addition of ammonia. Brix was measured periodically using both refractive index (RI) and hydrometer. In addition to this bio-reactor a Multigen bio-reactor (described later) was utilized in some experiments.

### 1.1.1 Simultaneous Sacchrification and Fermentation versus Gluco Pre-Conversion in HS/LE efficiency (Deliverable: Report on Pre-sacch. versus Simultaneous Sacch/ Ferm of Commercial Dextrins)

The ability of free GA to convert commercial corn syrup dextrins quickly enough to allow a complete fermentation in 6 to 10 hours was tested in a magnetically stirred bio-reactor. In the continuous batch bio-reactor, batches were run with the

dextrins enzymatically Glucose Pre-Converted (GPC) and converted during the fermentation process (SSF). For the GPC 100% of the producer recommended level of glucoamylase was added to an autoclaved 25% dextrin (3,3,3 g/L YMP) solution at a temperature of 60$^{o}$ C for two hours. The GPC solution was then added to the bio-reactor with a cell bed of 200 mL. In the SSF batch, an autoclaved 25% dextrin (3,3,3 g/L YMP) solution was added to the reactor with a cell bed of 200 mL. The SSF dextrin solution had a slightly higher initial hydrometer brix of 26.9 compared with the GPC brix of 25.6.

**Figure 1:**

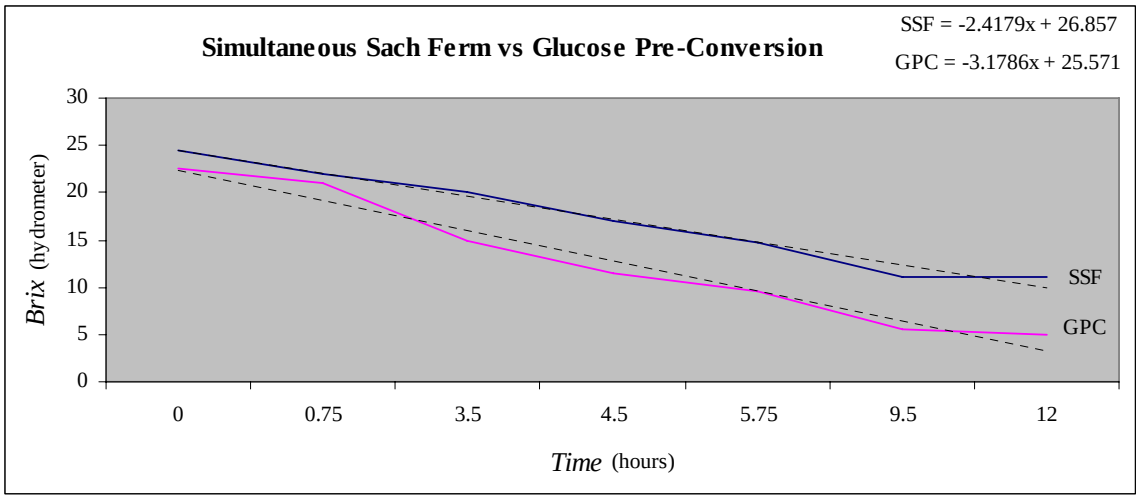



**Table 1:**
SSF Vs. GPC Results

| Batch | Time (hours) | Temp (C) | PH | Brix Hydr. | Brix RI |
|---|---|---|---|---|---|
| SSF 25% Dextrins | 0 | 28 | 4.4 | 24.5 | 25 |
| | 2.5 | 27 | 3.5 | 22 | 22.5 |
| | 4.25 | 27 | 3 | 20 | |
| | 5.25 | 27 | | 17 | |
| | 6.75 | 27 | 3.7 | 14.8 | 18.7 |
| | 8.75 | 28 | 4 | 11 | 17.5 |
| | 10 | 28 | 4.2 | 11 | 16 |
| GPC 25% Dextrins | 0 | 27 | 4.3 | 22.5 | 25 |
| | 0.75 | 27 | 4.2 | 21 | 24 |
| | 3.5 | 28 | 3.8 | 15 | 20 |
| | 4.5 | 28 | 3.2 | 11.5 | |
| | 5.75 | 28 | 4.4 | 9.5 | |
| | 9.5 | 28 | 4 | 5.5 | |
| | 12 | 28 | 3.8 | 5 | 13.5 |

It was found that the GPC batch fermented more quickly than the SSF batch. The average decrease in hydrometer brix per hour was 3.17 for GPC and 2.42 for SSF. However, when gauging efficiency it is important to factor into the equation the time spent carrying out the GPC. It is less efficient in terms of procedure and the addition of the two hours of GPC to the fermentation time. For fermentations running from 8-12 hours the GPC is a more efficient (in terms of time) method of converting dextrins into glucose.

**Simultaneous Saccharification and Fermentation (SSF) w/ BPSC-15**
The ability of free GA to convert commercial corn syrup dextrins quickly enough to allow a complete fermentation in 6 to 10 hours was tested in a stirred reactor. In one trial, 25% dextrins, nutrients, and 0.35 ml GA was added to a working reactor with 150 ml settled yeast volume, and 700 ml total working volume. A series of trials were performed. When GA was added at 1x the recommended dosage (1 ml/ Kg starch or 0.1%) to 650 ml of 25% dextrin solution (M-100) with nutrients we noted slightly incomplete dextrin conversion when the reactor was operated at 22$^{o}$C- approximately 8 g/L DP4+ dextrins remained along with 20 g/L glucose at 11.5 hours. When the reactor temperature was raised to 27-28$^{o}$ C, and the GA dose increased by 2X, all dextrins were converted at 8.5 hours into the fermentation although the glucose was not fully utilized even after 12 hours in one trial. In conclusion, SSF works quite well with commercial (acid hydrolysed) corn dextrins, although somewhat higher levels (1.5 to 2X GA levels) might be required to ensure the conversion of all the dextrins to glucose in the short fermentation cycles.

**Pre-saccharification with SSF**- A short – 15 minute- high temperature saccharification at the optimal temperature for the GA (55-60$^{o}$ C ) using 1 to 1.5X of the recommended dosage of GA followed by cooling and addition to the reactor was found to give complete saccharification of the dextrins by the end of the fermentations (8 to 12 hours). This treatment has the further advantage of being a quick ‘pasteurization’ of the feed. We used this treatment in our further work with stillage set-back.

**1.1.1a Optimal Glucoamylase in Sacchrification for HS/LE Fermentation**

The optimal amount of glucoamylase (GA) used in SSF was tested by adding two different level (100% and 200%) of producer recommended levels of the enzyme in a bioreactor. The GA was added to batches of dextrins (GPC M400) made up at 22-24% solids. Twelve trials were completed using SSF glucose conversion techniques. Three trials (E, I, and L ) used 100% (.16 mL) of gluco-amylase and eleven trials used 200% (.32 mL) of the recommended levels. Average hourly decreases in hydrometer brix were calculated through minimizing least squares residuals.

**Table 2:** Decrease in Average Hydrometer Brix Over Time

| Sample | % Recommended GA | Decrease in Brix (Hy) Per Hour |
|---|---|---|
| C | 200 | 1.3 |
| D | 200 | 1.1 |
| E * | 100 | 1.8 |
| F | 200 | 1.5 |
| G | 200 | 1.5 |
| H | 200 | 1.6 |
| I * | 100 | 0.6 |
| J | 200 | 1.4 |
| K * | 100 | 1.7 |

The average decrease in hydrometer brix by hour for all trials was 1.35 brix per hour. The average decrease for 200% gluco-amylase was 1.4 brix per hour. The average decrease for 100% gluco amalayse was 1.36 brix per hour. However, the change in brix for the 100% glucoamylase was both the largest and the smallest change per hour (1.8, 1.7 and .6). The slower average decrease in brix is probably underestimated by the outlier of .6 brix per hour. This slowest conversion (.6 brix per hour) was probably due to another factor. It is concluded that using 200% of recommended enzyme levels confers little to no benefits in terms of speeding saccharification and in fact may decrease the rate of saccharification in SSF.

### 1.1.2 Immobilized enzyme trials: (Deliverable: Report on Immobilized Gluco-amylase processing of dextrins to glucose.)

Immobilizaton of the enzyme gluco-amylase was tested using two different mediums: terri cloth and carbon substrates. The purpose of this experiment was to test whether the immobilized enzyme could be used repeatedly thereby decreasing the amount of enzyme needed to convert dextrins into glucose.

### 1.1.3-a Terry Cloth Immobilization

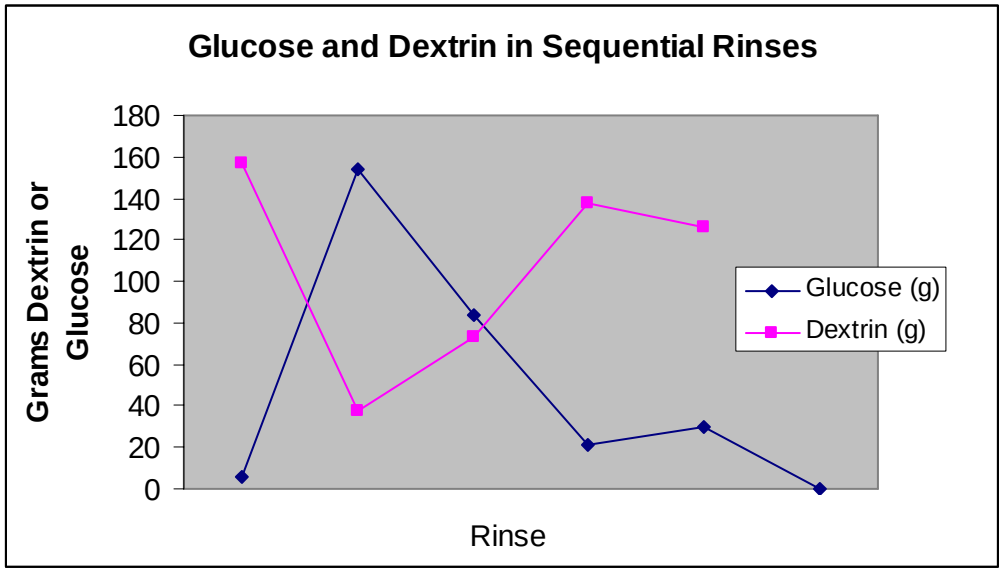
A terry cloth (8.5 cm x 6.5 cm) was saturated with 5 mL of gluco amalayse. This was allowed to dry then cut into fourths so that 1.25 mL of GA was on each piece. The GA saturated rag was then placed into 100 mL of a 20% (200 g/L) dexrin solution for 1 hour at a temperature of 130 – 140 degrees F with stirring. A sample of the dextrin solution was taken after 15 minutes and placed in boiling water to stop further conversion. The dextrin solution was then replaced with fresh 20% dextrin solution and the same steps were repeated. The samples were then run through a HPLC to the degree of conversion. This was done for a total of four rinses.

**Table 3:**
Enzyme Immobilization (Terry Cloth)

| Rinse | % Dextrins | % Glucose | g's Dextrin | g's Glucose | Glucose/Dextrin |
|---|---|---|---|---|---|
| Initial | 78.9 | 3 | 157 | 6 | 0.04 |
| 1 | 18.9 | 76.8 | 38 | 154 | 4.05 |
| 2 | 36.3 | 42.2 | 73 | 84 | 1.15 |
| 3 | 69.8 | 10.7 | 138 | 21.4 | 0.16 |
| 4 | 63.2 | 15 | 126 | 30 | 0.24 |

Most of the GA enzyme was washed away after the first rinse. Almost all enzyme was rinsed by the third rinse. It is peculiar that the fourth rinse had a better conversion rate than the third. The conclusion is that GA does not bind well enough by simple adsorption to terry cloth to be economically feasible.

**Figure 2:**
GA Enzyme on Terri Cloth




### 1.1.4-b Activated Carbon Substrate

Terry cloth was found to not bind GA very well after the first rinse. Activated carbon was then tested as a substrate for binding gluco-amylase. Five grams of carbon pellets were first dried for one hour at a temperature of 300 F. They were then allowed to cool and soaked in five mL GA for an hour. Excess GA was poured off after the hour. 600 mL of a 25% (250 g/L) dextrin solution was made and the pH was adjusted to 4.5. The carbon pellets were then placed into a flask with 100 mL of the dexrin solution. This was held at 130 F for one hour with gentle magnetic stirring. A sample of the dextrin/glucose solution was taken after the hour and placed in boiling water to stop further

sacchrification. The dextrin solution was then replaced with fresh 25% dextrin solution. This was repeated for a total of 6 rinses. The samples were then run through a HPLC to determine the amount of dextrin that was converted to glucose by the gluco-amylase. The initial dry weight of the activated carbon was 3.7 g, and the wet weight of the carbon transferred from flask to flask was measured at 8.2 g.

**Table 4:**

Conversion Rate of GA Activated Carbon

| Rinse | % Dextrin | % Glucose |
|---|---|---|
| Initial | 100 | 0 |
| 1 | 2 | 98 |
| 2 | 5 | 95 |
| 3 | 7 | 93 |
| 4 | 24 | 76 |
| 5 | 42 | 58 |
| 6 | 67 | 33 |

It was found that GA was adsorbed fairly well by the activated carbon. The immobilized GA activated carbon worked best in the first three rinses. At the end of the third rinse the GA had converted 93% of the dextrin into sugar. The percentage of dextrin converted into glucose decreased after the third rinse. The carbon was able to bind GA quite well in comparison to the terry cloth substrate. The use of GA activated carbon could be used to decrease GA expenditure.

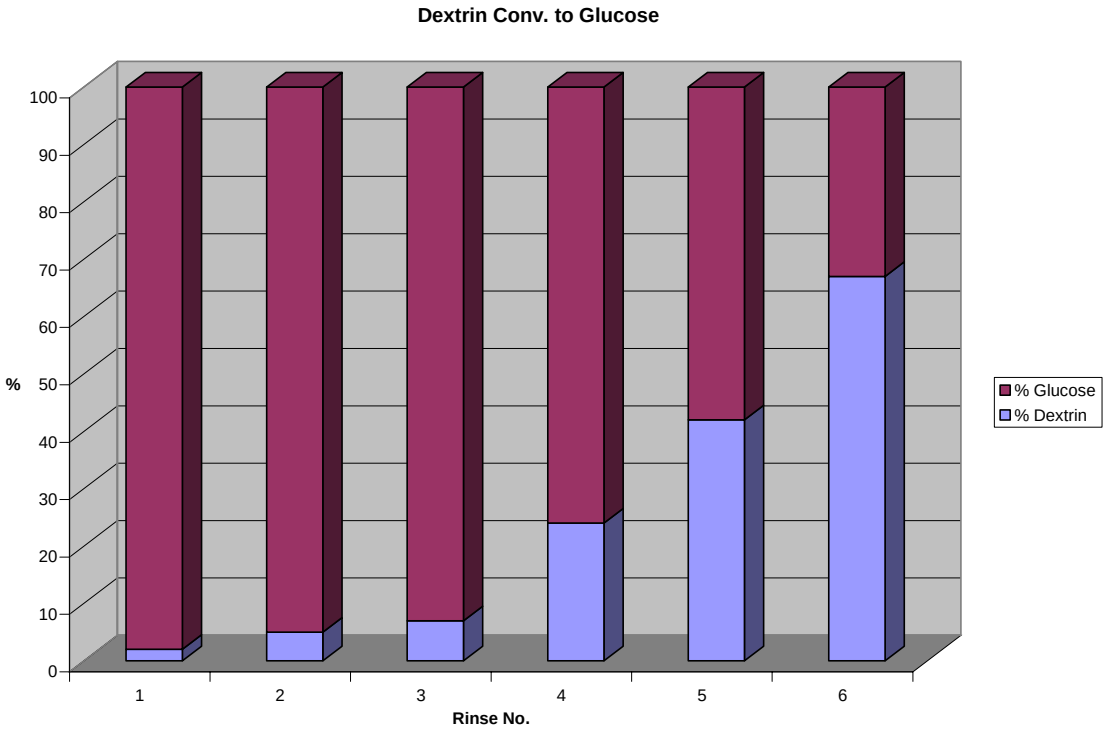



Figure 3. Dextrin conversion to Glucose by adsorbed GA on activated carbon.

Thus, simple adsorption into activated carbon works well, and improvements by chemical linking would further reduce or eliminate the leaching of the enzyme from the carrier. The ability of immobilized glucoamylase to convert dextrins has been demonstrated by a number of workers. Linking procedures are suggested by Lantero et al (1995), and Krishnan et al, (*Biores. Tech*. 75:99, 2000) used GA linked diatomaceous earth. Krishana et al. (2000) suggests that a column of this nature should be regenerated after about 3 months of operation.

### 1.1.3 *Process modeling and economics-Process Comparision*. (**Deliverable: Report on Immobilized versus Free Glucoamyase saccharification of of Commercial Dextrins)**

Both use of free enzyme and immobilized enzyme were demonstrated to work well with the HS/LE process. An economic comparison of the process is shown below.

1) Use of Free GA: In our work to date, a simple saccharification process consisting of a one hour hold at 140 F with a 1 X dosage of GA, followed by the HS/LE process. An industrial implementation of this process is shown in Figure 4 below. The use of free GA was shown to work well in our long term trials. Saccharification will then complete during the fermentation at 85F. This process is thus a combination of pre- and simultaneous saccharification of the dextrins.

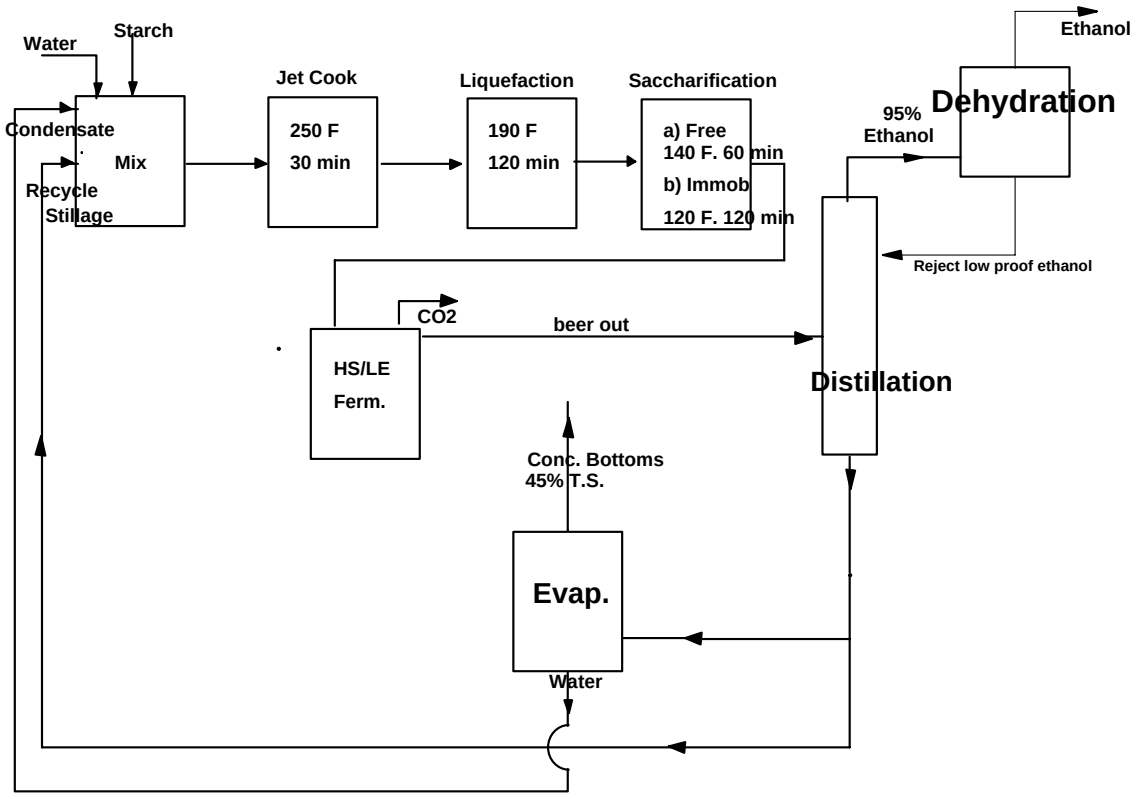



Figure 4. Block Flow Diagram for Starch Conversion to Ethanol. Free vs. Immobilized GA Saccharification.

The basic design of the two saccharification processes is as follows:

a) Free GA- GA is metered into the stream of dextrins leaving the liquefaction tank. This stream is cooled from 190 to 140 F. prior to injecting the GA enzyme. For a 10 million gal/yr facility, this flow would be 160 GPM of 25% dextrins. The stream with the enzyme is then taken to a baffled tank with a 60 to 90 minute residence time- a 15,000 gallon tank for this example. Costs for GA enzyme and the holding tank are shown in Table xx for several scales

b) Immobilized GA- The dextrin stream leaving the liquefaction tank is cooled to 120 F. and introduced into a series of 3 columns in which a bed of immobilized GA is maintained. A fourth column is introduced into service at the beginning of each month, while the column in service longest (3 months) is taken off stream to be rejuvenated with fresh immobilized GA. Thus the immobilized GA system consists of a total of 4 columns (3 in use, and one in standby/repacking mode) with a residence time of 40 minutes per column. Capital costs for the columns and packing are estimated as shown in Table 5.

Table 5. Economic Comparison of Free vs. Immobilized GA

**Saccharication Evaluation**

| | | |
|---|---|---|
| Cost of GA | 2.9 | $/L |
| Cost of GA Immob. Matrix | 8.5 | $/L |
| % solids | 0.25 | |
| Conversion | 0.47 | g eth/g starch |
| Marshal Swift Chem Cost Index | 1120 | |

| **Scale of Operations** (million gal/yr) | **5** | **10** | **20** | **40** |
|---|---|---|---|---|
| Flow rate (GPM) | 66 | 132 | 263 | 526 |
| 1) Free GA system | | | | |
| Capital Cost ( X $1000) | | | | |
| column size (gal) | 5920 | 11840 | 23680 | 47360 |
| column cost | $50.41 | $74.83 | $111.09 | $164.92 |
| piping/install | $17.64 | $26.19 | $38.88 | $57.72 |
| Enzyme Cost/yr (X $1000) | $28.19 | $56.37 | $112.75 | $225.49 |
| $/gal ethanol | $0.01 | $0.01 | $0.01 | $0.01 |
| | | | | |
| 2) Immobilized GA system | | | | |
| Capital Cost (x$1000) | | | | |
| centrifuge | $33.65 | $53.54 | $85.18 | $135.53 |
| immob.column size (gal) | 2,631 | 5,262 | 10,524 | 21,049 |
| Column cost | $127.01 | $188.55 | $279.90 | $415.52 |
| piping/install | $44.45 | $65.99 | $97.97 | $145.43 |

| | | | | |
|---|---|---|---|---|
| Initial Immob Enzy | $339.94 | $679.88 | $1,359.75 | $2,719.51 |
| Enzyme Cost/yr (x $1000) | $9.16 | $18.31 | $36.62 | $73.25 |
| Payback on Immob (Years) | 25.1 | 23.3 | 22.0 | 21.0 |

Conclusions

By moving to an immobilized enzyme column, a processor can save approximately 70% of his annual outlay on GA. However, this is a rather small cost, while the cost of installing the immobilized enzyme system is fairly high. Payback time to repay the capital costs associated with the immobilized GA system are estimated to be over 20 years as seen in Table 5. Most chemical industries do not invest in capital improvements unless there is a payback of under 10 years.

## Appendix 1.2. Effects of High Degree of Backset on HS/LE Performance (**Deliverable: Report on Effects of Stillage recycle with wet mill dextrose)**

The ability of the BPI HS/LE system to handle a high recycle rate has been established on molasses, but not on corn based glucose fermentations. In this portion of the project, BPI completed lab scale tests at various levels of 'stillage' recycle, using dextrins (GPC M300), wet mill glucose syrup (Pennford Products), and sucrose.

**Background**

In a typical dry mill ethanol plant, the corn mash is made-up by mixing the ground corn meal to 30-35% solids with a mix of 'thin stillage' or backset and water (fresh water and evaporator condensate). The more thin stillage used in the backset for mash make-up, the less stillage needs to be taken to the evaporator. With the HS/LE system, high backset ratios are possible. The intent of this section is to determine whether performance deterioration is noted with the use of high levels of stillage backset in the feed make-up.

Feeds were made up with either dry dextrin, concentrated wet mill syrup, or dry cane sugar with the desired degree of backset added. The final feeds are pasteurized at 140 to 150 F for 20 to 30 minutes. (This period is also used for saccharification of dextrins with the dextrin product). The backset was prepared by stripping the ethanol from a previous batch of 'beer' by atmospheric boiling of the completed beer. 30% of the total beer volume was boiled off. Ethanol levels were reduced to less than 5 g/L (0.5%), and any residual sugars beer are returned with the stillage. Brix (RI) is measured to determine the reaction rate, and samples were taken when the reaction was stopped, prior to the addition of fresh feed to the reactor. Samples were analyzed by HPLC for glucose, ethanol, dextrins, and level of glycerol and lactic acid. Glycerol is the major non-volatile by-product of the ethanol fermentation, and the glycerol levels can be expected to increase as the fraction of backset increases.

### 1.2.1 Effects of 50% Backset on HS/LE in Multigen Bio-Reactor

Using stillage, (the beer product stripped of ethanol) in making up fresh feed can reduce operating costs for ethanol production. The addition of stillage to the feed is known as backset. The HS/LE process was tested for the effects of a 50% backset use in feed preparation (50% DI $H_2O$) on the HS/LE fermentation process. This was used in conjunction with simultaneous saccharification and fermentation.

In this experiment a stirred 2 L bio-reactor (Multigen) was utilized. This is a more automated reactor than used in previous experiments as pH and temperature can be regulated through probes. When the pH dropped below 3.0 ammonia was pumped into the reactor until the ph reached 4.0. The temperature was maintained at 28 $^{o}$ C. Due to the size and number of probes in the Multigen reactor a hydrometer could not be used to monitor the course of the reaction, instead brix was measured by refractive index from samples pulled from the reactor. Autoclaved glucose solution was added to the reactor through an inlet and beer removed through an outlet using a siphon.

The reactor was disinfected using both an 80% alcohol solution and a 3% hydrogen peroxide solution. BPSC-15 yeast were grown up aerobically from a slant and added to the reactor. 1 liter of a 2 % (w/v) glucose solution with 5, 5, 5 g/L YMP was added to the reactor along with 100 mL of yeast cells. The low glucose solution was used to build up the yeast cell bed to 200 mL. The reactor was next run with 20% dextrin solution (3, 3, 3 g/L YMP) for four consecutive reaction to ensure there were no bacterial infections in the reactor.

The reactor was then run for eight consecutive batches (Trial # 0-7) using no backset and only DI $H_2O$ to make-up the feed solution. The reactor was then run for fifteen batches (Trial # 8-23) with a 50% backset, 50% DI $H_2O$ 200 g/L dextrin solution (3, 3, 3 YMP). The RI brix of the reactor was taken at the introduction of the solution and periodically during the fermentation process. The fermentation processes were run on a daily (24 hour) basis. Batches 0 through 17 were measured on an eight hour schedule, while batches 17 through 23 were measured on a twelve hour schedule. For purposes of process analysis batches 0 through 17 will be compared. The decrease in average brix per hour was calculated to indicate the speed of fermentation.

**Table 6:** Backset Fermentation (Trials 8-23) vs. DI Fermentation (Trials 0-7)

| Trial # | Date | Hours | Vt (mL) | Brix RI | Decrease in Brix (RI) from initial | | Average Decrease in Brix per Hour |
|---|---|---|---|---|---|---|---|
| 0 | 24-Aug | 0 | 1,250 | 17.2 | | | |
| | 25-Aug | 11 | | 7.8 | 9.4 | | 0.9 |
| 1 | | 0 | 1,000 | 17.2 | | | |
| | | 3 | | 14.5 | 2.7 | | |
| | | 7 | | 9.8 | 7.4 | | |
| | | 9.5 | | 7.5 | 9.7 | | 1 |
| 2 | | 0 | 1,070 | 17.2 | | | |
| | | 4.5 | | 10.1 | 7.1 | | |
| 3 | | 0 | 930 | 17.4 | | | |
| | 26-Aug | 8 | | 7.9 | 9.5 | | 1.2 |
| 4 | | 0 | 1,020 | 17 | | | |
| | | 5 | | 9 | 8 | | |
| | | 9 | | 7 | 10 | | 1.1 |
| 5 | | 0 | 1,030 | 15 | | | |
| | | 6 | | 10.2 | 4.8 | | 0.8 |
| 6 | | 0 | 890 | 15.7 | | | |
| | 27-Aug | 9 | | 5.8 | 9.9 | | 1.1 |
| 7 | | 0 | 1,080 | 15.5 | | | |
| | | 3.3 | | 11 | 4.5 | * | |
| 8* | | 0 | 1,090 | 17.5 | | | |
| | | 4.25 | | 11.4 | 6.1 | * | |
| 9 | | 0 | 850 | 18.8 | | | |
| | | 10 | | 8.5 | 10.3 | | 1 |
| 10 | | 0 | 1,010 | 18.5 | | | |
| | | 9 | | 11 | 7.5 | | 0.8 |
| 11 | | 0 | 950 | 16.5 | | | |
| | | 27 | | 13 | 3.5 | * | |
| 12 | 28-Aug | 0 | 1,000 | 18.2 | | | |
| | 29-Aug | 10 | | 7.6 | 10.6 | | 1.1 |
| 13 | | 0 | | 19.6 | | | |
| | | 7 | | 10.5 | 9.1 | | 1.3 |
| 14 | | 0 | | 19 | | | |
| | | 5.7 | | 13.5 | 5.5 | | 1 |
| 15 | | 0 | 1,100 | 19.5 | | | |
| | 30-Aug | 11 | | 8.6 | 10.9 | | 1 |
| 16 | | 0 | 1,050 | 17.5 | | | |
| | | 11 | | 8.6 | 8.9 | | 0.8 |
| 17* | | 0 | 1,300 | 19.2 | | | |
| | 31-Aug | 13 | | 9.8 | 9.6 | | 0.7 |
| 18 | | 0 | | 19.5 | | | |
| | | 4 | | 15.8 | 3.8 | | |
| | | 8 | | 13.5 | 6 | | |
| | | 11.5 | | 10.8 | 8.7 | | 0.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 19 | | 0 | 1,300 | 19.7 | | |
| | 1-Sep | 13 | | 9.8 | 9.9 | 0.8 |
| 20 | | 0 | 1,300 | 19.9 | | |
| | | 8 | | 12.9 | 7 | |
| | | 12 | | 10.1 | 9.8 | 0.8 |
| 21 | | 0 | | 20.1 | | |
| | 2-Sep | 12 | | 10.5 | 9.6 | 0.8 |
| 22 | | 0 | | 19 | | |
| | | 10.2 | | 11 | 8 | 0.8 |
| 23 | | 0 | | 19.2 | | |
| | 3-Aug | 13 | | 12 | 7.2 | 0.6 |

The decrease in brix per hour was slightly faster for the fermentation batches (0-7) that did not use the 50% backset in producing the next batches dextrin solution. However the sample time was increased from eight hours to twelve hours at trial 17. Decrease in RI brix is found to be quickest in the first four to five hours of fermentation. Taking these trials (and a few other time outliers 7, 8, 11) out of the analysis the average decrease in RI brix is found to be minimal. Non-backset solution fermentation was found to decrease an average of 1.1 brix per hour versus an average decrease of 1.0 RI brix for 50% backset solution. A factor that may have decreased the HS/LE backset performance in the latter trials (16- 23) besides time is the continued concentration of the backset solution.
In conclusion, a backset solution of: 50% stripped beer / 50% DI H2O was found to have little effect on HS/LE performance.

### 1.2.2 Performance on Wet Mill Syrup from Commercial Wet Miller- Penford Products

Substrate: Five gallons of 42% glucose syrup was shipped to BPI's lab in November 2003. The syrup is a commercial product available near the Xethanol ethanol plant in Hopkinton IA. The syrup was fairly clear with a small amount of small (1-2 mm diameter) starch pellets floating near the top. This syrup was strained through a terry cloth filter to remove the pellets, diluted 1:1with either distilled water or stillage backset (stillage being completed 'beer' from a previous fermentation which was then evaporated in an open vessel to 70% of initial volume to drive off the ethanol) to make a 20-22% glucose feed.

Nutrients: 1 g/L yeast extract, 2 g/L malt extract, 2 g/L $NH_4SO_4$, 1 g/L $KH_2PO_4$, 400 PPM MBS (275 ppm SO2- anti-bacterial)

Reactor: A 350 ml clear glass 'pop' bottler was marked with 20 ml graduations. The bottle was fitted with a magnetic stirrer, a foam plug covered with foil was used to cap the reactor, and then autoclaved at 121 C for 20 minutes.

Fermentations: It was decided to run 24 hour fermentations for simplicity of monitoring the long term performance. It was determined that approximately 10% cell volume (30 ml in a 300 ml working volume reactor) was the desired levels of cells to complete a

fermentation in this period of time. The sterile 350 ml reactor was seeded with an aerobically grown innoculum of BPSC-15, BPI's proprietary strain of yeast. The reactor was then placed in a temperature controlled incubator (set at 30C) on a magnetic stirrer. Fermentations were monitored by measuring refractive index (R.I. - brix readings on a hand held refractometer) and by taking samples for later analysis. The refractometer 'brix' reading are associated with weight percent sucrose solution, i.e. a 15 brix syrup has the same refractive index as a 15% (wt/wt) sucrose solution. Most sugars have similar R.I. so that a 15% glucose solution also reads 15 brix (R.I.) A fermentation's progress can be monitored via the brix (R.I.) readings as per Figure 1 below, based on a yield (Y p/s) of 0.47 g ethanol per g sugar and assuming 1) that the only components in the fermentation mix which affect refractive index are sugar and ethanol, 2) that the refractive index is an additive property of ethanol and sugar. In fact, brix reading are somewhat higher than calculated from these assumptions as shown in Figure 5, with a finishing R.I. brix usually determined as about 40 to 45% of initial brix.

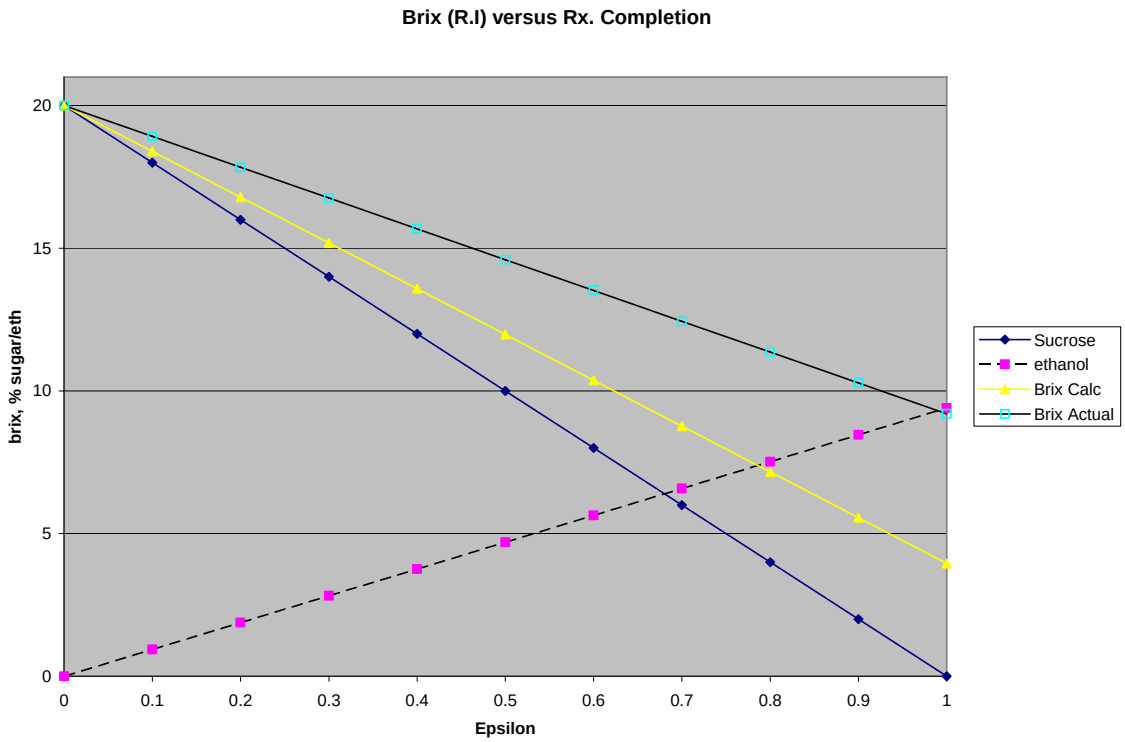



Figure 5. Brix RI versus Fermentation completion (ε)

Results

The fermentation of glucose syrup was started on 12/9/03. 20 ml of wet yeast from a previous experiment was added to the reactor. The reactor was placed in a temperature control cabinet at 24C. Initial brix (R.I.) was $20^{o}$. pH was noted to drop during the fermentations, so concentrated ammonium hydroxide (which also serves as a nitrogen source nutrient for the yeast) was added as need to try to keep the pH between

3.5 and 4.0 which is optimal for the fermentations. Table 7 gives the performance data from the reactor over a period of about 30 days

Table 7. Performance Data from Corn Syrup trials

| | | | | | |
|---|---|---|---|---|---|
| #1 | 12/10/03 | 11:05 | Bx 12.2 | pH 2.3 | add 0.47 ml ammon. |
| | | | pH 3.9 | | |
| | | 17:45 | 10 sec settling 30 ml cells, 30 sec-20 ml | | |
| | | 18:00 | Bx 8.4 | pH 3.1 | |
| #2 | 12/10/03 | 18:00 | Bx 18.1 | pH 4.0 | |
| | 12/11/03 | 12:55 | Bx 9.8 | pH 2.5 | add 0.47 ml ammon. |
| | | 18:10 | Bx 8.0 | pH 4.5 | |
| #3 | 12/11/03 | 18:15 | Bx 17.8 | pH 4.0 | |
| | 12/12/03 | 13.40 | Bx 13.4 | pH 2.5 | add 0.47 ml ammon. |
| | | 18:15 | Bx 11.0 | pH 3.4 | |
| #4 | 12/12/03 | 18:15 | Bx 18.2 | pH 4.0 | |
| | 12/13/03 | 10:30 | Bx 11.0 | pH 4.1 after adding 0.4 ml ammon. | |
| | | 15:00 | Bx 9.2 | pH 3.7 | |
| #5 | 12/13/03 | 15:00 | Bx 18.4 | pH 4.0 | |
| | 12/14/03 | 12:15 | Bx 8.2 | pH 3.9 after adding 0.4 ml ammon. | |
| #6 | 12/14/03 | 12:30 | Bx 18.2 | pH 4.0 | |
| | | 21:15 | Bx 14.2 | pH 2.9 | add 0.4 ml ammon. |
| | 12/15/03 | 15:50 | Bx 7.9 | pH 3.2 | |
| #7 | 12/15/03 | 16;00 | Bx 17.5 | pH 4.0 | |
| | 12/16/03 | 11:00 | Bx 13.0 | pH 2.6 | add 0.65 ml ammon |
| | | | | pH 4.5 | |
| | | 15:45 ` | Bx 11.5 | pH 3.4 | |
| | | 18:46 | Bx 11.5 | pH 3.3 | |
| #8 | 12/16/03 | 18:55 | Bx 18.3 | pH 4.0 | |
| | | 22:45 | Bx 16.5 | pH 3.1 | add 0.43 ml ammon. |
| | | | | pH 5.5 | |
| | 12/17/03 | 8:30 | Bx 12.0 | pH 3.3 | add 0.2 ml ammon. |
| | | 12:10 | Bx 10.4 | pH 3.7 | |
| #9 | 12/17/03 | 12:15 ` | Bx 18.5 | pH 4.0 | |
| | | 17:00 | Bx 16.8 | pH 2.9 | add 0.35 ml ammon |
| | | 22:00 | Bx 13.5 | pH 4.6 after add 0.35 ml | |
| | 12/18/03 | 9:30 | Bx 8.8 | pH 3.9 | |
| | | 11:30 | Bx 7.4 | | |

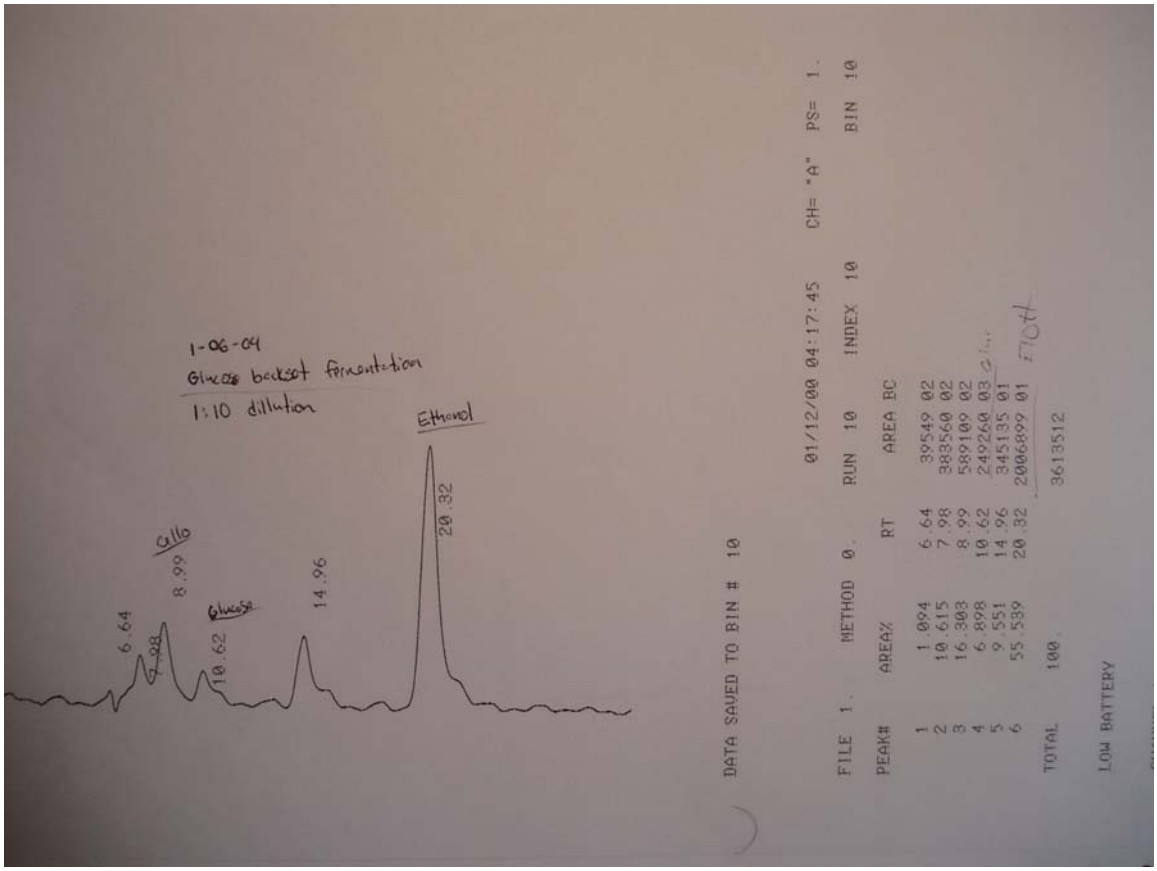
| | | | | | |
|---|---|---|---|---|---|
| #10 | 12/18/03 | 11:30 | Bx 19.0 | pH 4.0 | |
| | | 17:30 | | | add 0.35 ml ammon |
| | | 22:20 | Bx 12.4 | pH 3.7 | add 0.3 ml ammon |
| | 12/19/03 | 10:30 | Bx 8.8 | pH 3.9 | |
| #11 | 12/19/03 | 10:35 | Bx 18.8 | pH 4.0 | |
| | 12/20/03 | 9:47 | Bx 9.7 | pH 3.4 | |
| #12 | 12/21/03 | reactor recharged no data taken | | | |
| #13 | 12/22/03 | reactor recharged no data taken | | | |
| #14 | 12/23/03 | reactor recharged no data taken | | | |
| #15 | 12/24/03 | start use of backset: make up 2L new feed- 1 L glucose syrup | | | |
| | | 750 ml of 88% backset | | | |
| | | 250 ml H2O | | | |
| | | reactor recharged w/ sucrose (20%) and YPM and refrigerated | | | |
| #16 | 12/28 | reactor recharged and put back in incubator (29C) | | | |
| | 12/29 | 14:33 | Bx 11.5 | pH 2.7 | |
| #17 | 12/29 | 14:40 | Bx 21 | pH 4.5 | |
| | 12/30 | 14:00 | rxtr reset | no data | |
| #18 | 12/30 | 14:00 | Bx 20 | pH 4.5 | |
| | 12/31 | 9:50 | Bx 13.4 | pH 3.1 add 0.2 ml ammon. | |
| | | 15:56 | Bx 11.5 | pH 3.4 | |
| #19 | 12/31/03 | 16:00 | Bx 18.9 | pH 4.5 | |
| | | 24:00 | Bx 16.2 | pH 2.8 add 0.2 ml ammon | |
| | 1/01/04 | 17:45 | Bx 9.2 | pH 3.2 | |
| #20 | 1/01/04 | 17:50 | Bx 19.2 | pH 4.5 | |
| | | 23:00 | Bx 17.2 | pH 3.1 Rxtr left out on bench/no stirring..put back in..add ammon. | |
| | 1/02/04 | 16:30 | Bx 10.1 | pH 3.3 | |
| #21 | 1/02/04 | 16:40 | Bx 19.0 | pH 4.5 | |
| | 1/03/04 | 12:00 | Bx 13.0 | pH 3.5 | |
| | | 17:32 | Bx 11.0 | pH 3.3 add 0.2 ml ammon. | |
| | | 21:30 | Bx 9.5 | pH 3.7- pour off excess yeast ..bring back from 40 ml to 30 ml | |

| | | | | |
|---|---|---|---|---|
| #22 | 1/03/04 | 21:35 | Bx 19.1 | pH 4.5 |
| | 1/04/04 | 11:45 | Bx 14.0 | pH 3.3 |
| | | 21:15 | Bx 10.5 | pH 3.7 |

make-up new feed w/ 1100 ml backset, 850 ml fresh 42% syrup, 100 ml H20, nutrients
Bx feed 19.9

| | | | | |
|---|---|---|---|---|
| #23 | 1/04/04 | 21:20 | Bx 18.5 | pH 4.5 |
| | 1/05/04 | 11:30 | Bx 10.7 | pH 3.2 add 0.2 ml ammon. |
| | | 21:30 | Bx 8.2 | pH 3.6 |
| #24 | 1/05/04 | 21:35 | Bx 18.0 | pH 4.5 |
| | 1/06/04 | 11:15 | Bx 11.2 | pH 3.1 add 0.2 ml ammon |
| | | 21:45 | Bx 8.6 | pH 3.4 |
| #25 | 1/06/04 | 21:50 | Bx 18.2 | pH 4.5 |
| | 1/07/04 | 11:30 | Bx 13.8 | pH 4.0 after add 0.2 ml ammon |
| | | 21:40 | Bx 8.6 | pH 3.2 |
| #26 | 1/07/04 | 21:55 | Bx 18.4 | pH 4.5 |
| | 1/08/04 | 15:00 | Bx 9.2 | pH 3.5 after add 0.2 ml ammon |
| | | 22:00 | Bx 7.8 | pH 3.3 |

Figure 6. HPLC Chromatogram of completed fermentation # 24.



A HPLC chromatogram of the completed Trial #24 is shown above in Figure 6. As per this chromatogram, glucose was nearly completely utilized (about 6.6 g/L remaining) with glycerol (14.9 minutes) measured at 6.1 g/L and ethanol (20.3 minutes) measured at 133 g/L.

**Conclusions:**

These experiments showed :

1) that the commercial glucose syrup can be used successfully for the HS/LE fermentation process, as the yeast pellets remained in good condition over a period of 30 days operation and trials and,

2) that adding backset at 50 to 65% had no adverse effect on the speed of the fermentation, finishing in 18 to 24 hours with a 10% yeast volume.

3) We also noted that addition of the backset helped maintain the pH in the desired range (3.5-4.0). When the fermentation media was made up without backset, the pH dropped very quickly, reaching values as low as 2.5 and 2.6 as noted in Table 7. About 0.15% wt/wt concentrated ammonia was required to bring the pH back to 3.8-4.2 range. When the fermentation media was made-up with stillage, pH only dropped to 2.8 to 3.3, and only 0.07% wt/wt concentrated ammonium hydroxide was needed to maintain the pH. We are not certain if less acid-50% less based on the reduced amount of ammonia required- (probably succinic acid) was produced by the yeast when the backset was added, or whether the better buffering capacity of the media with the backset reduced the need for pH controlling ammonia.

**1.2.3 HS/LE Performance with Higher Levels of Stillage Setback.**

A second 350 ml reactor, similar to the reactor described in Section 1.1.1 was marked with graduations, fitted with a foam plug, and sterilized. 20 ml. Floc yeast was added to the reactor, and some initial performance trials started using a 20% sucrose solution with the same nutrients described in Section 1.1.1.

Table 8. Performance of HS/LE on Sucrose syrup w/ high backset

| | | | | |
|---|---|---|---|---|
| #1 | 12/11/03 | 18:15 | Bx 21 | pH 4.0 |
| | 12/12/03 | 13:30 | Bx 16.8 | pH 2.5 add 0.4 ml ammon. |
| | | 18.15 | Bx 15.2 | pH 3.8 |
| #2 | 12/12/03 | 18:30 | Bx 21 | pH 4.0 |
| | 12/13/03 | 10:30 | Bx 12.5 | pH 4.0 after adding 0.4 ml ammon. |
| | | 15:00 | Bx 10.0 | pH 3.7 |

| | | | | | |
|---|---|---|---|---|---|
| #3 | 12/13/03 | 15:10 | Bx 19.2 | pH 4.0 | |
| | 12/14 | 12:15 | Bx 9.9 | pH 4.0 after add 0.4 ml ammon. | |
| #4 | 12/14 | 12:30 | Bx 19.5 | pH 4.0 | |
| | | 21:15 | Bx 14.2 | pH 2.9 add 0.4 ml ammon | |
| | 12/15/03 | 15:50 | Bx 7.9 | pH 3.6 | |
| #5 | 12/15/03 | 16;00 | Bx 19.9 | pH 4.0 | |
| | 12/16/03 | 11:00 | Bx 16.8 | pH 2.6 | add 0.65 ml ammon |
| | | | | pH 4.5 | |
| | | 15:45 ` | Bx 14.5 | pH 2.9 | |
| | | 18:46 | Bx 14.2 | pH 3.3 | |
| #6 | 12/16/03 | 18:55 | Bx 18.3 | pH 4.0 | |
| | | 22:45 | Bx 17.5 | pH 3.1 | add 0.43 ml ammon. |
| | | | | pH 5.4 | |
| | 12/17/03 | 8:30 | Bx 12.2 | pH 3.3 | add 0.2 ml ammon. |
| | | 12:10 | Bx 10.6 | pH 3.7 cell volume up to 30 ml | |
| #7 | 12/17/03 | 12:15 ` | Bx 18.9 | pH 4.0 | |
| | | 17:00 | Bx 16.6 | pH 3.0 | add 0.35 ml ammon |
| | | 22:00 | Bx 13.8 | pH 4.6 after add 0.35 ml | |
| | 12/18/03 | 9:30 | Bx 8.8 | pH 3.9 | |
| | | 11:30 | Bx 7.4 | | |
| #8 | 12/18/03 | 11:30 | Bx 19.0 | pH 4.0 | |
| | | 17:30 | | | add 0.35 ml ammon |
| | | 22:20 | Bx 12.6 | pH 3.6 | add 0.3 ml ammon |
| | 12/19/03 | 10:30 | Bx 8.3 | pH 4.0 | |
| #9 | 12/19/03 | 10:35 | Bx 18.8 | pH 4.0 | |
| | 12/20/03 | 9:47 | Bx 10.0 | pH 3.4 | |
| #10 | 12/20/03 | 9:50 | Bx 19.3 | pH 4.0 | |
| | | 20:20 | Bx 18.1 | pH 4.0 after 0.35 ml ammon | |
| | 12/21/03 | 10:31 | Bx 10.4 | pH 3.2 | |
| #11 | 12/21/03 | 10:35 | Bx 19.4 | | |
| | 12/22/04 | 12:30 | reactor recharged no data taken | | |
| #12 | 12/23/03 | reactor recharged no data taken | | | |

#13 12/24/03 start use of backset: make up 2L new feed- 200g/L sucrose, 800 ml of 75% backet (750 ml of backset brought to 88% of initial volume to simulate stillage plus 250 ml H2O) Br feed 24.5 This corresponds to a 75% backset ratio.

reactor recharged w/ sucrose (20%) and YPM and refrigerated

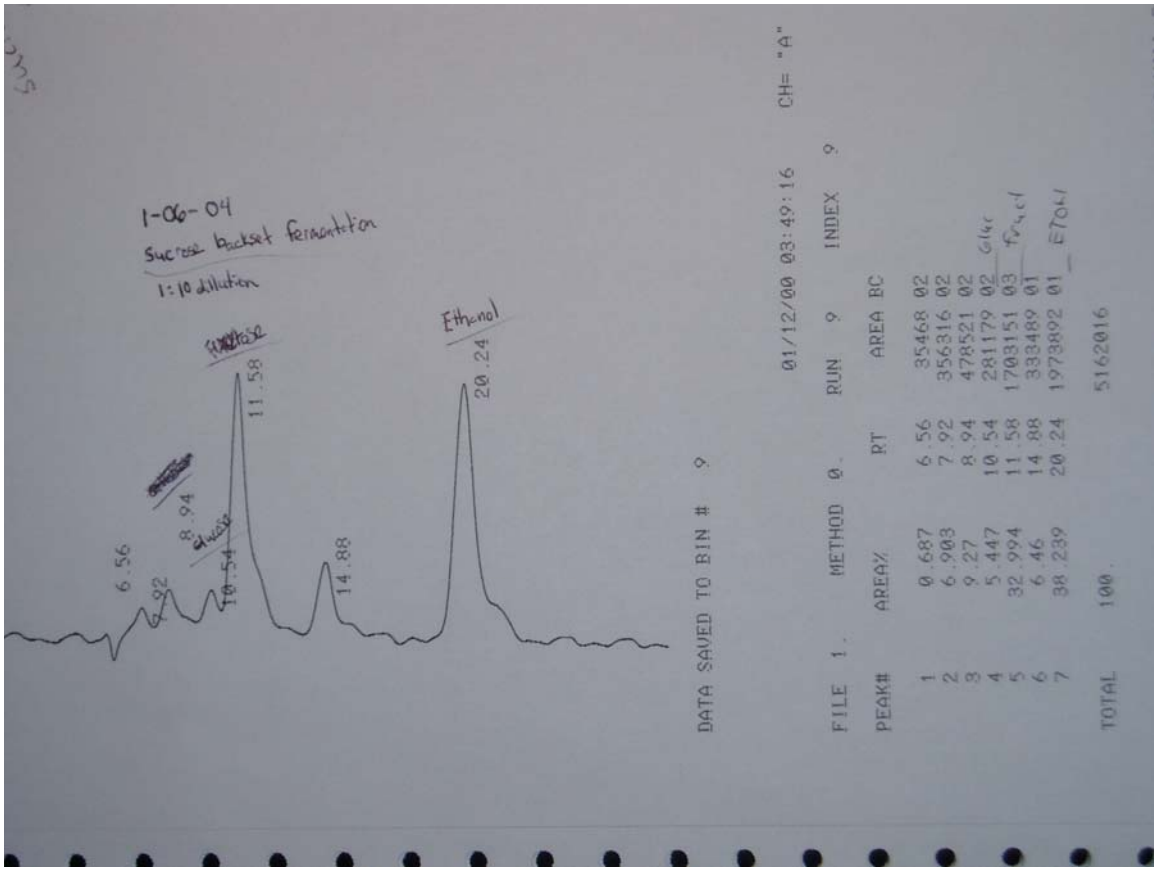
| | | | | |
|---|---|---|---|---|
| #14 | 12/28 | reactor recharged and put back in incubator (29C) | | |
| | 12/29 | 14:33 | Bx 14.8 | pH 3.0 |
| #15 | 12/29 | 14:40 | Bx 24 | pH 4.5 |
| | 12/30 | 14:00 | rxtr reset | no data |
| #16 | 12/30 | 14:00 | Bx 24 | pH 4.5 |
| | | 24:00 | Bx 21 | pH 3.1 add 0.2 ml ammon. |
| | 12/31 | 9:50 | Bx 15.4 | pH 3.3 add 0.2 ml ammon |
| | 12/31 | 15:55 | Bx 13.6 | pH 3.8 |
| #17 | 12/31/03 | 16:00 | Bx 24 | pH 4.5 |
| | | 24:00 | Bx 18.4 | pH 3.1 add 0.2 ml ammon |
| | 1/01/04 | 17:45 | Bx 12.3 | pH 3.6 |
| #18 | 1/01/04 | 17:50 | Bx 24 | pH 4.5 |
| | | 23:00 | Bx 19.2 | pH 3.3 Rxtr left out on bench/no stirring..put back in..add 0.2 ammon. |
| | 1/02/04 | 16:30 | Bx 13.2 | pH 3.5 |
| #19 | 1/02/04 | 16:40 | Bx 24 | pH 4.5 |
| | 1/03/04 | 12:00 | Bx 15.2 | pH 3.4 |
| | | 17:32 | Bx 12.8 | pH 3.3 add 0.2 ml ammon. |
| | | 21:30 | Bx 11.4 | pH 3.9- pour off excess yeast ..bring back from 40 ml to 30 ml |
| #20 | 1/03/04 | 21:35 | Bx 24 | pH 4.5 |
| | 1/04/04 | 11:45 | Bx 15.0 | pH 3.4 |
| | | 21:15 | Bx 11.9 | pH 3.7 |
| #21 | 1/04/04 | 21:20 | Bx 24 | pH 4.5 |
| | 1/05/04 | 11:30 | Bx 16.2 | pH 3.2 add 0.2 ml ammon. |
| | | 21:30 | Bx 12.8 | pH 3.5 |
| #22 | 1/05/04 | 21:35 | Bx 24 | pH 4.5 |
| | 1/06/04 | 11:15 | Bx 14.5 | pH 3.3 add 0.2 ml ammon |
| | | 21:45 | Bx 11.1 | pH 3.6 |
| #23 | 1/06/04 | 21:50 | Bx 24 | pH 4.5 |

| | | | | |
|---|---|---|---|---|
| | 1/07/04 | 11:30 | Bx 14.2 | pH 3.9 after add 0.2 ml ammon |
| | | 21:40 | Bx 12.2 | pH 3.5 |
| #24 | 1/07/04 | 21:55 | Bx 24 | pH 4.5 |
| | 1/08/04 | 15:00 | Bx 13.5 | pH 4.0 after add 0.2 ml ammon |
| | | 22:00 | Bx 12.5 | pH 3.8 |
| #25 | 1/08 | 22:00 | Bx 24 | pH 4.5 |
| | 1/09 | 11:45 | Bx 15.0 | pH 3.7 after add 0.2 ml ammon |
| | | 22:00 | Bx 11.8 | pH 3.7 |

Experiment stopped...

A HPLC chromatogram of the #22 fermentation broth taken before resetting the reactor for the next trial is shown in Figure 7.

Figure 7. HPLC Chromatogram of completed high backset sucrose fermentation # 22




as per this chromatogram, the sugar was incompletely utilized with a residual fructose concentration of 45 g/L shown. Ethanol concentration is measured at 131 g/L and glycerol (the peak at 14.88 minutes) about 5.9 g/L.

Conclusions:

The fermentation rates were not noticeably adversely affected by moving from 0 to 75% backset rates. We noted near complete fermentations in 24 hours with somewhat higher initial sugars (24-25%) with backset versus near complete fermentations in 24 hours at 20% initial sugar with no backset. (Trying to hold yeast volume at 10% throughout these trials.) As with the corn syrup trials at 50% backset, use of backset helped control the degree of pH swings. We noted that we only needed about 0.2 ml ammonia/per 300 ml fermentation (0.07% conc. Ammonia) to control pH when backset was used versus 0.4-0.7 ml (0.15-0.25 %) in sucrose fermentations made up with no backset. The sugar concentrations fed were higher, with incomplete utilizations leading to sugar being added back to the reactor with the backset. Ethanol concentrations reached (131 g/L) were about the same as the glucose reactor at 50% backset (133 g/L).

## APPENDIX 1.3 Pilot Scale High Speed/ Low Effluent Ethanol

### Background:

BPI has developed and filed for patent protection the ***High Speed/ Low Effluent (HS/LE)*** process for production of ethanol from dextrins/glucose. The process is based on a strain of yeast and operating procedures developed by BPI over the past few years. The yeast was developed to have an extreme 'floc durability' through 1) a strain selection process in which a number of highly flocculent yeast were compared, and 2) then beginning with one 'best' selected strain, improving the strain by a long process of 'natural selection'- selecting and re-selecting extremely durable floc yeast mutant pellets from reactors which were run for periods of months.

The resultant process allows complete fermentation of 150 to 220 g/L glucose syrups to ethanol in 4 to 8 hours, in either a continuous cascade or consecutive batch mode over extended periods of several to many months. In the Consecutive Batch (CB) mode of operation, the fermenter is available for immediate re-set after completion of fermentation and a settling period during which completed beer is decanted. This allows 2 (12 hour) to 3 (8 hour) batches of 10 to 14% (v/v) ethanol to be produced per reactor per day.

### Advantages of HS/LE Process

1. Increases productivity of fermenters by a factor of about 5X
2. Decreases effluent stillage by using a high degree of backset
3. Decreases nutrient needs/costs
4. Produces a clean, nearly sparkling clear, non-fouling 'beer' to take to the distillation column
5. Produces a clean high density yeast paste by-product with no need for centrifuges
6. Reduces waste water/ cleaning chemicals by eliminating need for CIP of fermenter(s) between batches.
7. Reduces operator/equipment needs as process is easily automated.
8. Eliminates need to purchase, and propagate yeast seed cultures for each batch.

**Consecutive Batch**

During 2001-2003, BPI ran a 2L stirred fermenter using dextrins converted to glucose at a concentration of 200 to 240 g/L. We ran run the system in the Consecutive Batch Mode at 3 cycles per day over a period of 3 months (206 cycles) and determined excellent results with fermentations going to near completion in as little as 5 hours. Over this period, we determined a 'minimal nutrient' make-up of for the glucose feed stock.

**Continuous Cascade Mode**

We have also run BPI's High Speed/ Low Effluent system in the continuous cascade (3 consecutive stirred reactors) mode. A 1 liter Multigen reactor was used. Batches of 5 gallons feed were made-up to run the 3 experiments described in Table 1. We caught the reactor effluent in a closed pot which we held at 65C. The effluent was then transferred to the feed tanks to simulate stage 2, and once again to simulate stage 3. The volume of dead cells in the bottom of the effluent pot was measured after each stage, and the dry wt. estimated. We used a proprietary nutrient formulation consisting of inorganic N, P and K supplemented with micro-nutrients/vitamins and corn steep liquor (CSL) Pekin, IL.

The results from these experiments are shown in the table below. We began the trials with a 3 hour residence time. We noted near complete sugar utilization in two stages (6 hours total) although there was still measurable glucose noted (4 g/L). Cutting the residence time to 2 hours/stg, 6 hours total, we noted that stage 3 had only 30 g/L glucose in the feed, which was reduced to a level we were not able to measure (under 0.5 g/L). Reducing the residence time again to 1.3 hours per stage (4 hours total), should have fed about 50 g/L glucose to Stage 3 based on my modeling of the system, instead we fed a 77 g/L glucose/ 68 g/L ethanol feed to stage 3, and ended up with a 97 g/L ethanol, 13 g/L glucose as a final product.

We had some problems with temperature control (overheating) in Stage 1, which then caused Stg 2 to not perform as well as it should have at the 1.3 hour RTD. We did note performance of stage 2 to improve over time after we improved the cooling system, with a final sample of the overflow showing glucose reduced to 55 g/L versus the avg. of 77 g/L.

Table 1. BPI HS/LE Fermentation of Corn Syrup

| | time (RTD) | Ethanol | Sugar | Productivity | g cells/L out |
|---|---|---|---|---|---|
| | Hr | g/L | g/L | g eth/L hr | |
| Feed | 3 hour/stg | | 220 | | |
| Stg 1 | 3 | 73 | 57 | 24.3 | n.m. |
| Stg 2 | 6 | 105 | 4 | 10.7 | n.m. |
| Feed | 2 hour/stg | | 230 | | |
| Stg 1 | 2 | 63.6 | 98 | 31.8 | 1.1 |
| Stg 2 | 4 | 95 | 30 | 15.7 | 0.4 |
| Stg 3 | 6 | 110 | 0.2 | 7.5 | 0.4 |
| Feed | 1.3 hour/stg | | 210 | | |
| Stg 1 | 1.33 | 40 | 128 | 30.1 | 1 |
| Stg 2 | 2.66 | 68 | 77 | 21.1 | 0.7 |
| Stg 3 | 4 | 97 | 13 | 21.8 | 0.4 |

Basically, we noted a productivity of around 30 grams ethanol per L hr for stage 1 when the ethanol level in the reactor was under 65 g/L. Stage 2 and 3 productivities ranged from 10 to 22 g/L depending on sugar availability and ethanol concentrations. The system can be run at 4 hour total RTD and give 12 to 13 % ethanol (v/v) with very low residual sugars.

**BPI Fermentation Technology (theoretical) background:**

**1) Inhibition of yeast growth and productivity by product** (ethanol), substrate (glucose) and other inhibitors (salts, glycerol, etc.)

Dale et al, (1994) developed an osmolality decribing both substrate and product inhibition of the ethanolic fermentation as:

$$\nu=\nu_m [1 - \varepsilon/k_{\varepsilon\nu m}] \qquad \text{Eq. 1}$$

$$\mu=\mu_m [1-\varepsilon/k_{\varepsilon\upsilon m}] \qquad \text{Eq. 2}$$

Growth is more strongly inhibited by osmolality than is productivity with $k_{\varepsilon\upsilon m}$ values of ranging from around 2 to 2.5 os/kg, while $k_{\varepsilon\nu m}$ runs 3.5 to 5.0 depending upon yeast species, osmo-tolerance, and ethanol tolerance. We have determined a value of 4.5 to 5.0 for $k_{\varepsilon\nu m}$ for our flocculent yeast BPSC-15. Osmolality of the solution can be determined as a simple additive function of the osmolality of the various components of the solution broth.

$$\varepsilon=\varepsilon_s + \varepsilon_{eth} + \varepsilon_{inhib} \qquad \text{Eq. 3}$$

This model allows an easy determination of the effects of stillage recycle based on the osmolality of the inerts being brought back around to the feed make-up. BPI has completed some work with recycle of molasses stillage which indicated a 27% decrease in average productivity rates for a molasses feed made up with 30% stillage. Our lab results closely followed this modeling, with Consecutive Batch Mode operation indicating an average fermentation completion in 8 hours versus 6 hours (33% decrease in average productivity).

We have done some preliminary modeling on the effects of stillage recycle for the corn syrup fermentation with the HS/LE process. These results indicate that at 70% recycle of stillage, glycerol and other non fermentables would be concentrated by a factor of 3.5X for an outlet glycerol concentration of 30 to 35 g/L (versus 9-10 g/L for no stillage recycle).

**2) Long term viability of immobilized cells**. Dale et al (1984) showed that for an immobilized cell population exposed to constant conditions of ethanol and sugar, that the steady state live cell fraction can be estimated based on a number of simplifying assumptions as:

$$X_{ssl} = [\mu/ (\mu + K_d)] \qquad \text{Eq 4}$$

Based on this analysis, we can see that if a cell population (i.e. one particular yeast pellet) is exposed to continuous conditions of zero growth, the steady state live cell density will be zero. Thus it is important for a pellet to occasionally see conditions allowing cell growth. Thus, stage 1 conditions should be maintained such that there is cell growth, with the overflow of younger cells refreshing the population of stages 2 and 3 where there is little cell growth due to the higher osmolality (largely due to ethanol).

**3) Determination of optimal/ minimal nutritional requirements for HS/LE process** on glucose syrups. In regular batch fermentation, yeast grow from a start-up inoculation level of around 0.5 g/L to a final concentration of 8 to 12 g/L. Thus, there must be enough nutrients in the media to provide for generation of 12 g/L yeast. With the BPI HS/LE process, we must only provide enough nutrients to allow for minimal growth, plus maintenance of the established yeast bed. This level of nutrients is less than that required to grow 12 g/L yeast. BPI has completed a nutritional study for the Consecutive Batch Mode of operation and determined a 'minimal' nutrient level which allowed good long term performance. Our goal was to see a steady production of about 1 to 3 g/L cells produced. This would allow a re-generation of a system with 50-120 g/L cells every 40 to 120 hours.

We used the following nutrients: *Nitrogen*: Ammonium sulfate , *Potassium*/ *phosphorus*: Potassium phosphate (monobasic), *Magnesium*: Magnesium sulfate, *Protein*: CSL from Pekin, *Trace elements & vitamins*
This work follows work completed a few years back (Chen, C., et al., 1993) on determining minimal nutritional needs for immobilized cells for a different strain of

yeast, which was also immobilized in a different manner than the floc BPSC 15 yeast of the HS/LE process.

**Pilot Scale Demonstration (1000-5000 gallon) Consecutive Batch**

The five major questions which we attempted to answer in this scale-up trial were:

1) *Can the HS/LE process be scaled up by 1000 to 2000 X (from the 2L lab scale to 2000 to 4500 L industrial scale).*

In this project, we selected a 6' ID by 9.5' tall stainless steel vessel for the HS/LE reactor. Working volume of the reactor was 4,500 L at 80% full.

2) *Will the process perform in a non-sterile, industrial, full scale environment?*

Typical corn ethanol plants have a near sterile feed (following the jet cooking of the mash at 230 F ) but all down stream processing is merely 'somewhat sanitary' with tanks cleaned with a CIP solution after a fermentation, but no real effort made to ensure piping and tanks are any more than 'somewhat sanitized', much less sterilized. Most ethanol plants live with some degree of lactic acid contamination of the fermentation systems, with a standard practice of adding antibiotics to the fermenters when the developed lactic acid levels reach some set-point level (typically over 5 g/L). To be easily integrated into current level of ethanol plant sanitation, the HS/LE process should ideally be able to handle this 'somewhat sanitary' level of operations.

3) *Can the HS/LE complete fermentations in 8 to 12 hours on an industrial scale as demonstrated on the lab scale?*

Our trials intend to test whether lab scale performance can be replicated on full non-sterile industrial scale.

4) *Can a full industrial scale system be designed to cool the HS/LE reactor (i.e. external cooling).*

The HS/LE process produces the same amount of heat as glucose is converted to ethanol and CO2 as does any other yeast or bacterial fermentation. However, the 5 X higher speed of the fermentation means that heat must be removed 5 times faster. Jacketing a fermenter is not a 'scaleable' design, as fermenters become larger, the surface to volume ratio decreases. External cooling loops are a scaleable design, and we implemented a external circulation/ shell and tube HX in our pilot design.

5) *Can the yeast pellets be pulled through a low speed centrifugal pump without damaging/losing their flocc nature?*

In our lab scale trials, we determined that exposure of the yeast pellets to high turbulence for extended periods of time caused the yeast to 'de-floc'. Interestingly, once the turbulence was decreased to minimal levels, the yeast would not form floccs again. In order to move large volumes of fermenter fluids through an external cooling loop, a centrifugal pump might be required. The effects on the yeast over time in pulling the yeast pellets through such a pump are not known.

**Project Performance**

During Q5, we completed our siting negotiations, receiving a letter of intent from PRI/ Xethanol as well as signing a siting agreement. Text of these letters/agreements were appended w/ Q5 quarterly report. A reactor vessel- a 4 stage Stirred Reactor Separator, owned by BPI was selected for use in the pilot demonstrations. The bottom stage- with a volume of approximately 5000 Liters, was selected as a reactor for the consecutive batch demonstration of the High Speed/ Low Effluent fermentation technology. Two 4,000 gallon vessels were to be used to store the wet mill syrup and/or clarified stillage. A piping design and control strategy for the reactor was developed. PRI General Manager Bob Lehman, and Xethanol engineer Eric Lee worked with Dr. Dale of BPI in completing a design scheme for installing all necessary piping/valves, controls and instrumentation for the wet mill syrup trials during Q5. During Q6 Bob Lehman completed: getting quotes for all necessary piping controls and instrumentation, ordering all stainless steel piping fittings, elbows and valves, specified ordered and received a Micro-motion flow/density meter to control sugar concentration fed to the pilot, and specified and ordered motor frequency drives for the stirring motor and the cooling circulation pump. During Q7, all the required piping, pumps and controls were installed. Leads from the controls were taken to a central DCS (Distributed Control System) and a program developed for filling, emptying, and controlling stirring speed and recirculation speed through the cooling system. During Q8, Xethanol stopped operations at the Hopkinton site, just as we were getting ready to run the pilot facility. Photos of the completed 'version 1' pilot plant were included with the Q8 report.

During Q9, Xethanol selected and moved three vessels which served as the basis for 'version 2' pilot plant to the Blairstown corn ethanol plant. Designs for piping and operating the pilot were completed by BPI. A schematic of the set-up is shown in Figure 3.1.1, and the vessels T-201 and T-301 shown in the following photos. Jim Stewart, General Manager of Xethanol Biofuels operations in Blairstown is monitored the plumbing and instrumenting of the version 2 pilot facility.

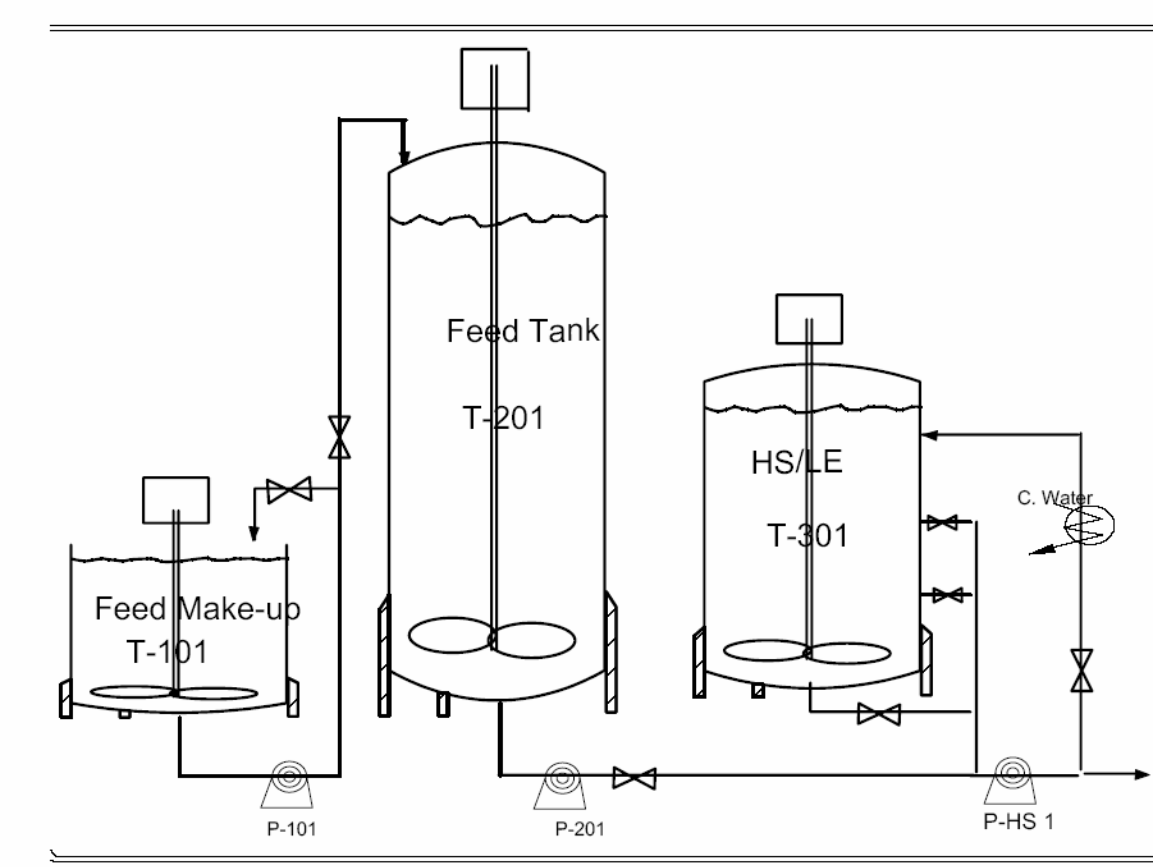



Figure 3.1.1 Basic layout of ‘version 2’ pilot plant

Figure 3.1.2 Tank T-201- 1, 250 Liter (8’ by 6’ diameter- under construction)

Figure 3.1.3 Vessel T-301 4,250 Liter HS/LE reactor (9'x 6' diameter-under construction)

During Q10, siting, plumbing, and most of the electrical was completed. Sight glasses, a low speed stirrer, and instrumentation were added to T-301, the HS/LE reactor as per the Figure 3.1.4 below.

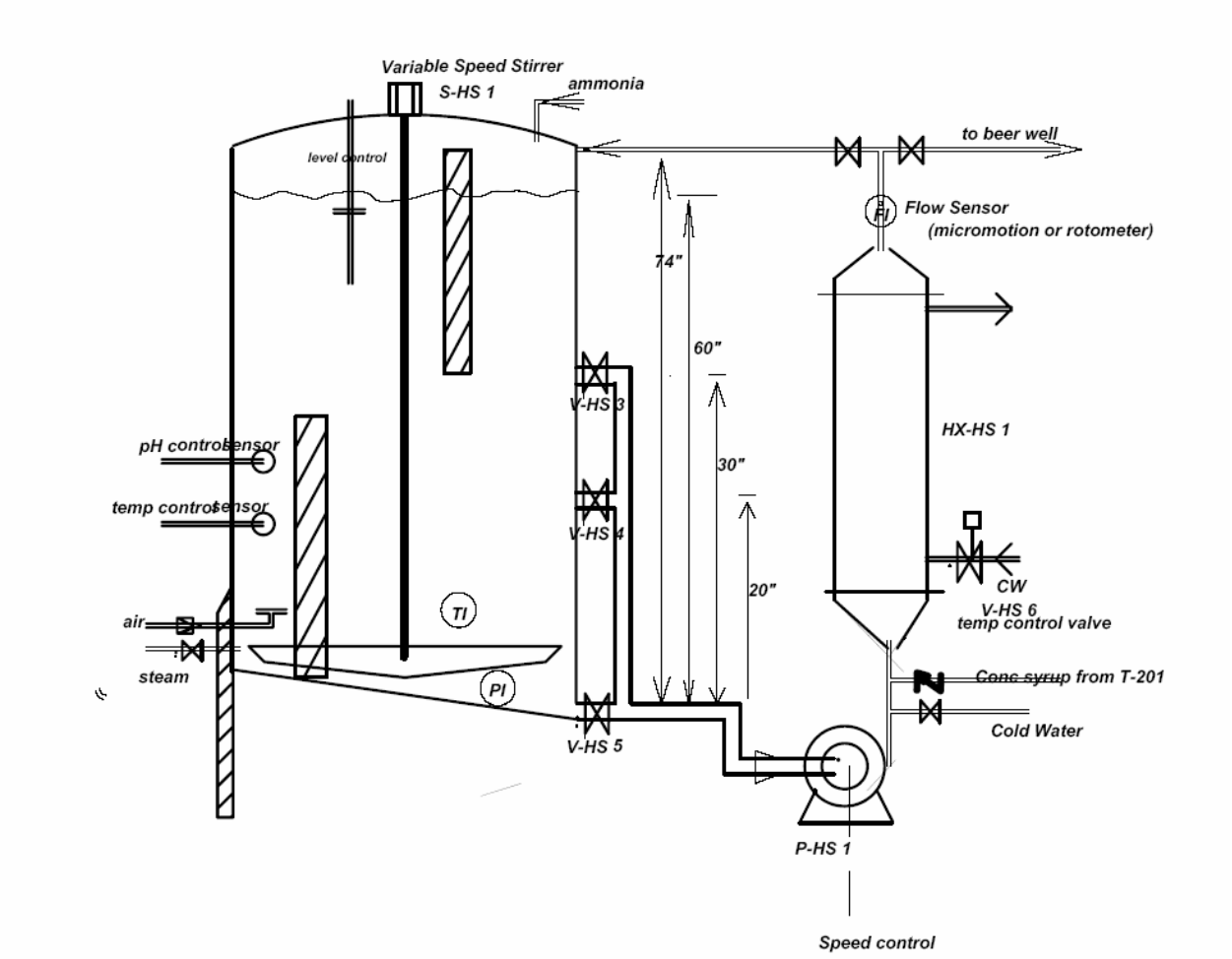
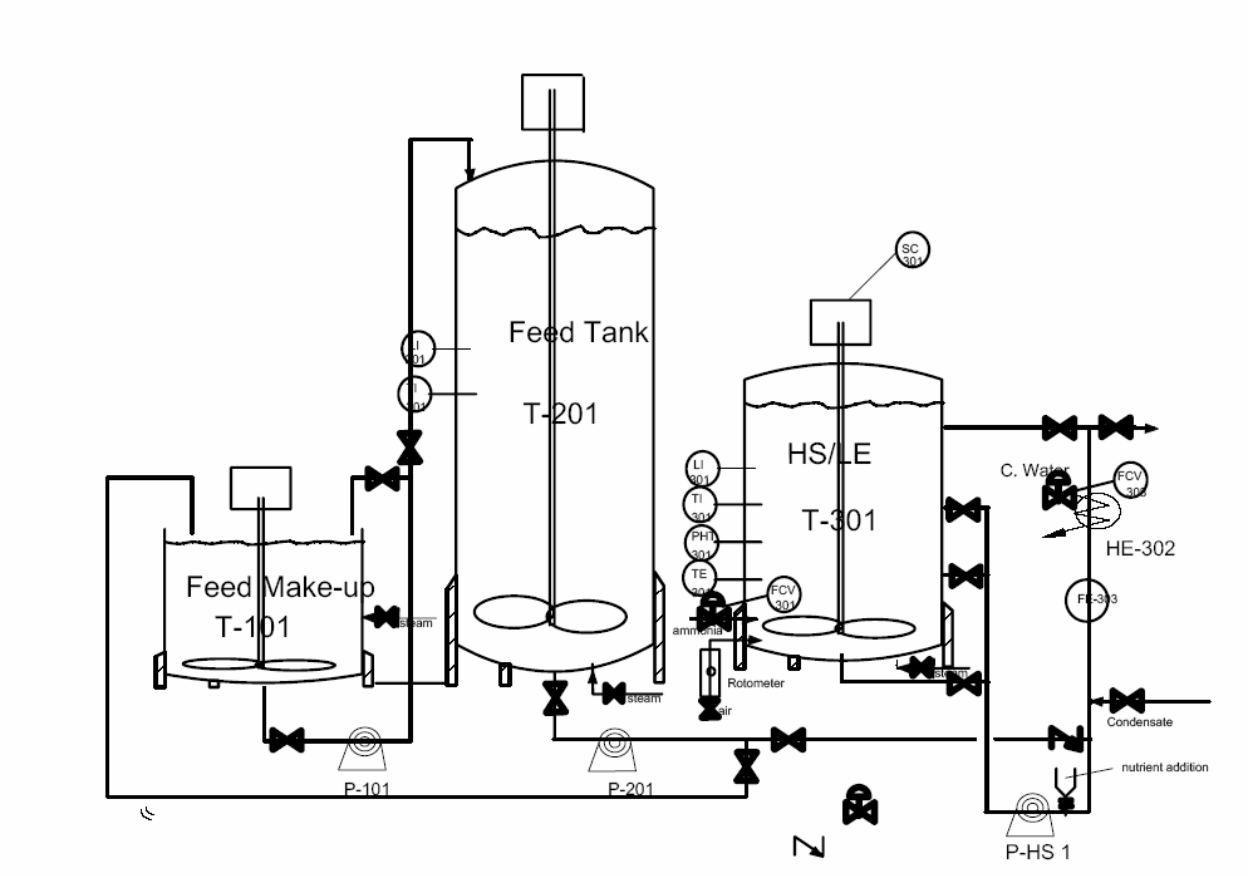



Figure 3.1.4. Piping and Instrumentation Diagram for the HS/LE Reactor (T-301).

Instrumentation, temperature, pH control, flow monitors were installed as per diagram 3.1.5 below.




3.1.5 Instrumentation Diagram for pilot.

Preliminary schedules set construction completion of the pilot plant for around 12/20/2005, but plant operations (a planned shut down from Nov 15 through Nov 30 required all the plant personnel to complete maintenance and improvements. By Jan. 3, 2006, Dr. Dale found most of the piping, sight glasses, controls installed, with only some electrical work remaining.

During Q12, the system of tanks, piping, valves and controls was completed by mid February by Xethanol Machinist Jamie Schwab, Pipefitter Craig Mixon, and Electrician Dan Wagaman. Preliminary shake down trials by Xethanol personnel led to the failure of Agitator 301. The motor was removed, repaired, and re-installed. Dale visited the plant on March 15, and found all systems installed and working. Jim Stewart, General Manager for Xethanol Biofuels, ordered in a set of nutrients for the pilot trials.

The completed 4,800 L (1,200 gallon) HS/LE pilot reactor (T-103), 5000 L (1,500) gallon syrup hold tank (T-102), and 1,500L (400 gallon) feed mix tank (T-

101) pilot system and control panel as fabricated by Xethanol personnel are shown in Figures 3.1.7 below

Figure 3.1.7 Completed 4500 L pilot HS/LE system

In the picture (Figure 3.1.7), Mix tank T-103 is on the far right, Syrup Feed Tank T-102 is in the middle, and the 4500 L HS/LE reactor (T-103) is on the left with the rectangular sight glasses which were fabricated by Xethanol pipefitter, Craig Mixon.


Figure 3.1.8. Control Panel for Pilot system

The electrical control panel allows the operators to start and stop the pumps and agitators, and has the read-out for the pH monitor/controller. This control panel was built, wired, and tested by Xethanol electrician Dan Wagaman.

On 3/15-3/17 Dale and Xethanol personnel completed a preliminary trial run of the pilot system. Xethanol provided twenty five 650# drums of 90% dextrin syrups as feed stock for the pilot trials (over 16,000#) as well as ten 50# bags of Ethanol Technology AYF 1000 yeast food/nutrient mix and one 500# drum of AYF 1700 liquid yeast extract concentrate.

The mixing/ syrup unloading, and fermentation system was tested using standard Altech brand yeast. The whole system performed well, with pumps, agitators, controls, and process temperature/ pH/ and flow meters all working as per design specifications. (The actual fermentation of the dextrins to ethanol was not too successful – the syrup was infected by a wild yeast strain as the syrups had been allowed to sit in the fermenters for extended periods.)

Figure 3.1.9. Dr. Dale at shake-down trial of 3/19. Cooling external Heat Exchanger HX 301(in yellow paint) is on the left, circulating pump P-301 can be seen below HS/LE Reactor T-301.

Procedures for more careful preparation/handling of the syrups were developed. Between 3/20 and 4/1, Dr. Dale and his lab manager, Brian Billings, completed a set of nutritional fermentation trials with the dextrin syrups in the BPI lab, testing 15 to 20 formulations of the nutrients obtained by Xethanol General Manager Jim Stewart. A 'growth' medium and 'maintenance' medium were determined. During this same period, Xethanol Biofuels worked on improving methods for getting the thick 90% solids dextrin syrup from the drums into the T-101 mix tank.

On Mon, 4/3, Dale met with Xethanol General Manager Jim Stewart, and a set of modifications was discussed and agreed upon. On Tues, 4/4, the HS/LE reactor was modified by Xethanol machinists to allow a single drum (650# of syrup) to be used to make a 1000 L batch for the pilot trials (i.e. use only the bottom third of the 4500 L reactor). Temperature, pH, and reactor draw lines were modified so that the standard batch for the trials would consist of a 'left' volume of about 250 L (65 gallons), and a working volume of about 1000 to 1200 L (250-350 gallons). These modifications were completed by noon, at which time the reactors were steam sanitized, and one drum of dextrin syrup added to mix tank T-101.

A procedure for adding the drum of 100 brix dextrin syrup to Mix tank T-101 was developed by General Manager Jim Stewart. The procedure involved heating the syrup with an electric immersion heater, then lifting and pouring the syrup into T-101 using a drum dumper and a fork lift as shown in Figure 3.1.10 and 11 on the following page.

Figures 3.1.10 and 3.1.11. Xethanol employees Jason Barth and Frank Ries prepare to load 650# of 90% dextrin syrup into Mix Tank T-101


**Reactor Start-up**

On 4/4/2006, nutrients were mixed up and insoluble solids strained out by Xethanol Lab Manager Dannyl Weaver based on the recipe given in Appendix 1. The syrup was adjusted to about 18° brix (approx 180 g/L dextrin solids, then transferred from T-102 (syrup hold tank) to the T-103 (HS/LE Reactor). The syrup was heated to 170 ° F at 19:30, and then circulated through the pump, piping, heat exchanger HX-103 to ensure the sanitation of the reactor system.

The recirculation pump P-103 was then started and valves to cooling water to HX-103 opened. By 21:40 the temperature had dropped to 120 F and by 22:30 to 106 F. At 23:30, the temperature was 94 F, and then the 5 gallon yeast inoculation vessel prepared by BPI was added at 24:00. pH and temperature were monitored at 2 hour intervals by the Xeth plant personnel. The temperature dropped slowly from 89 F to 82 F by noon on 4/5. pH was stable at 6.1, and was reduced at 14:00 to 4.7 by addition of sulfuric acid to reduce the possibility of bacterial growth.

A microscopic cell count at 14:00 by Dannyl Weaver showed good cell density, but the cells were largely 'singles' rather than clumps. At 16:00 the cells began clumping and visual observation of floccs noted. A sample showed about 10 ml of 'light, feathery floccs' per 250 ml. RI (refractive index) Brix was measured at 16 brix. A HPLC chromatogram showed 5% glucose and 5.4 g/L ethanol. The fermentation was allowed to go to near completion over about 60 hours. The HPLC data (as completed by Xethanol lab manager, Dan Weaver)

on glucose and ethanol concentrations over the initial fermentation are shown in Figure 3.1.12. Brix as measured by a refractometer is also plotted in the figure.

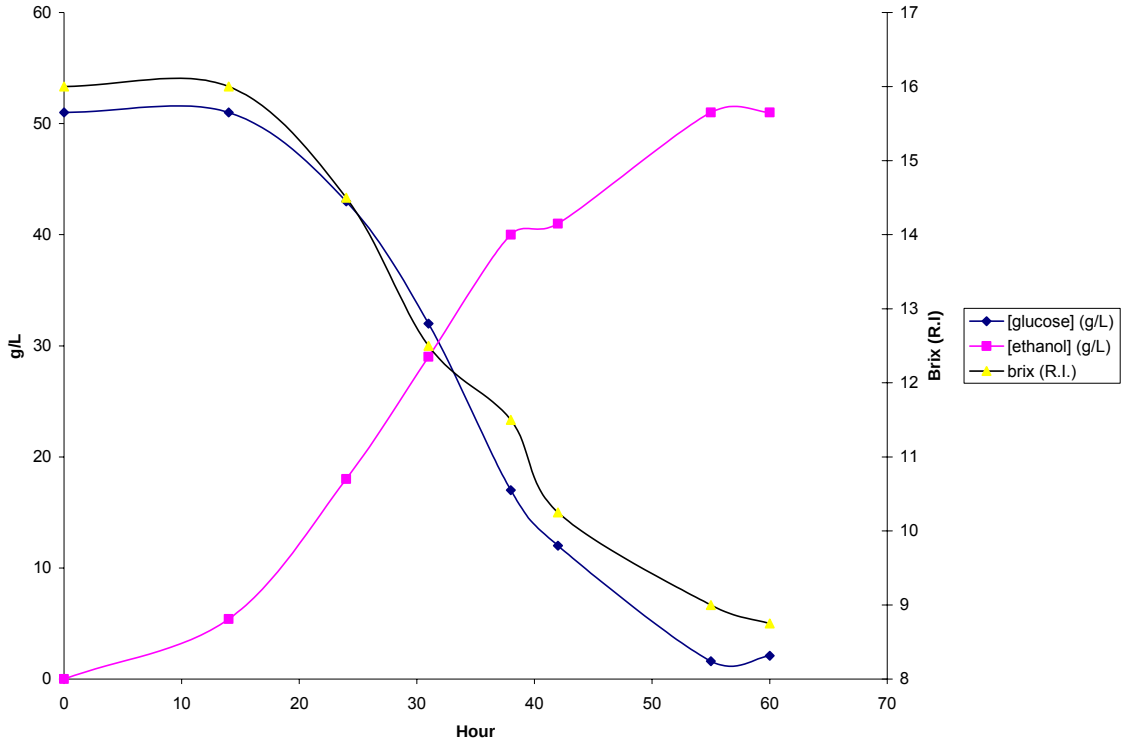



Figure 3.1.12. Performance of the first 'set' of the fermenter with the HS/LE yeast.

As per the figure, the fermentation went to completion in about 60 hours producing 52 g/L ethanol (about 7% v/v). HPLC data showed 24 g/L DP4, 3.4 g/L DP3 (triose), and 28 g/L DP2 (maltose) left, indicating incomplete performance of glucoamylase. This fermentation performance/time period is typical for batch ethanol fermentations.

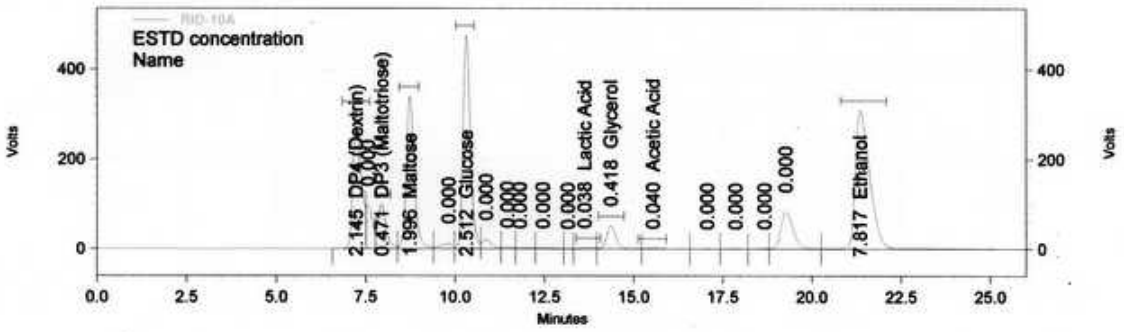
The second fermentation was started on 4/7 at 12:00 by draining the top 75% of the fermentation broth from the HS/LE reactor after a settling period of 30 minutes (with recirculation and stirring stopped). 500 Liters of fresh water (125 gallons) was then added, and then approximately 500 Liters of hot syrup (with nutrients added) slowly metered in starting at 13:30 and completed at 14:15. The final 'initial brix' (RI) at 14:15 was 16.5. This fermentation ran for 28 hours until reset on 4/8 at 16:00. Due to the initial set of yeast, fermentation time was almost halved. The basic re-set procedure for the reactor is given in Appendix 1.

The third fermentation began at 17:00 on 4/8 and ran til 17:25 on 4/9 (24.5 hours) achieving 78.2 g/L (9.8% v/v) ethanol. The final HPLC chromatogram is shown in Figure 3.1.13.

## *Area % Report*

**Method Name:** C:\EZStart\Projects\Default\Method\Ethanol 1.met
**Data:** C:\EZStart\Projects\Default\Data\Clark Dale Project - 3rd Prop Final - 4-9-06.dat
**User:** System
**Acquired:** 4/9/2006 5:15:23 PM
**Printed:** 4/9/2006 5:41:34 PM

≤ 24 hrs




**RID-10A Results**

| Retention Time | Name | Concentration (w/v%) |
|---|---|---|
| 7.233 | DP4 (Dextrin) | 2.145 |
| 7.942 | DP3 (Maltotriose) | 0.471 |
| 8.733 | Maltose | 1.996 |
| 10.317 | Glucose | 2.512 |
| 13.633 | Lactic Acid | 0.038 |
| 14.367 | Glycerol | 0.418 |
| 15.550 | Acetic Acid | 0.040 |
| 21.358 | Ethanol | 7.817 |
| Totals | | 15.437 |

Good flocs were noted, and a shorter settling time (5 minutes) was implemented in the reset procedure. Typical yeast floc pellets are shown in the Figure 3.1.14 below.


Figure 3.1.14 Photo showing typical yeast pellets. Pellet diameters ranged from 0.5 to 10 mm.

The fourth set began at 18:00 4/9 with an initial brix (RI) of 15.75. Brix over time is plotted in Figure 3.1.15.

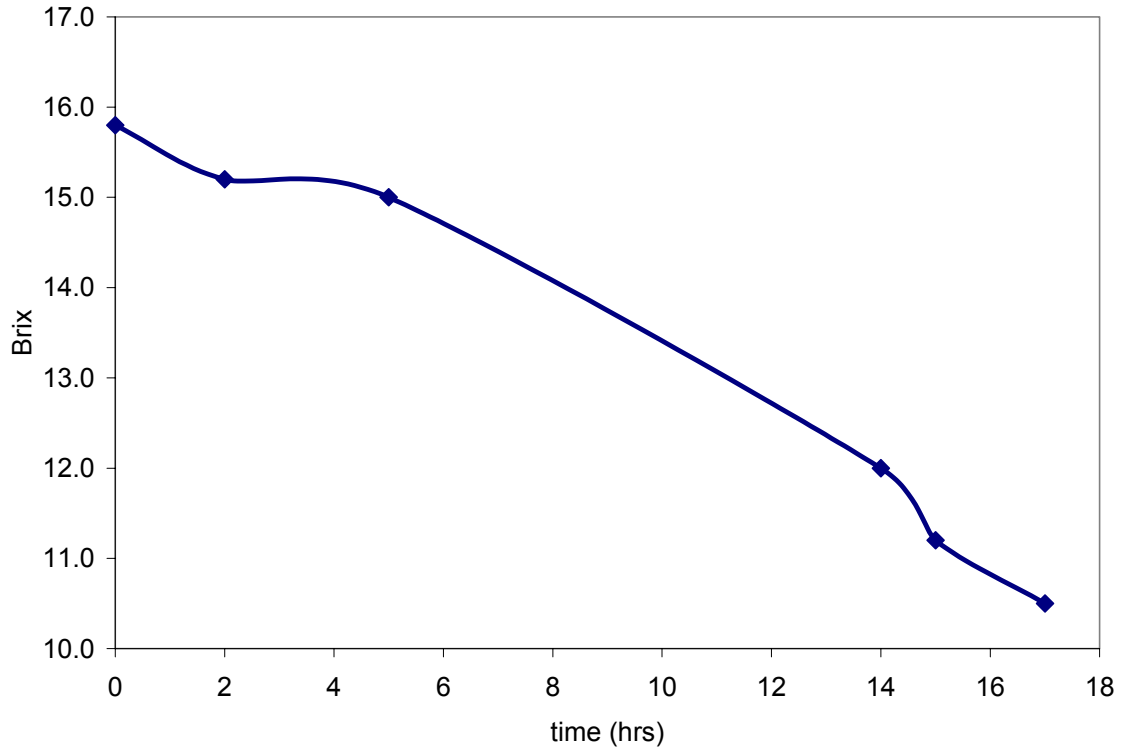



Figure 3.1.15 Brix vs time on Set #4

A final chromatogram at 14:00 on 4/10 showed near complete glucose utilization (less than ½% glucose) with an ethanol concentration of 60 g/L (7.5% v/v). The design fermentation time we planned to achieve in these trials was 12 hours, and in this fermentation we were beginning to get close to the goal performance rates.

The fifth set began at 14:00 4/10 with a brix (RI) of 16.25, and was near complete in under 18 hours at 10:30 4/11. At this point, after discussions with the plant manager, Travis Roster, we began 12 hour sets, which was the design performance for the reactor, even though settled cell density was not up to 25% in the HS/LE reactor. The sets were scheduled for 11:00 AM and 11:00 PM. Two Xethanol personnel, Jason Barth and Frank Ries, were assigned full time to the project. They began preparing the 40 brix syrup w/ nutrients at 8:30 AM for the 11:00 AM set, would reset the reactor w/ the fresh syrup at 11:00, and, once the 11:00 AM set was complete, prepare a second 110 gallon 40 brix syrup w/ nutrients in mix tank T-101 and move the syrup into T-102 for the 11:00 PM reactor re-set. The night shift (with or without Dr. Dale's assistance) would re-set the reactor using the syrup in T-102 at about 11:00 to 11:30 PM. We began adding more glucoamylase – 300 ml in the hot make-up syrup, and 100 ml in the fermenter after the set- after set 6 after determining the levels added in previous ferment were much too low.

This basic procedure was followed for Set #6 at 11:00 PM on 4/10 through the final Set #20 on 4/18 at 11:00 AM. Brix (RI) over time for the multiple sets is shown in Figure 3.1.16.

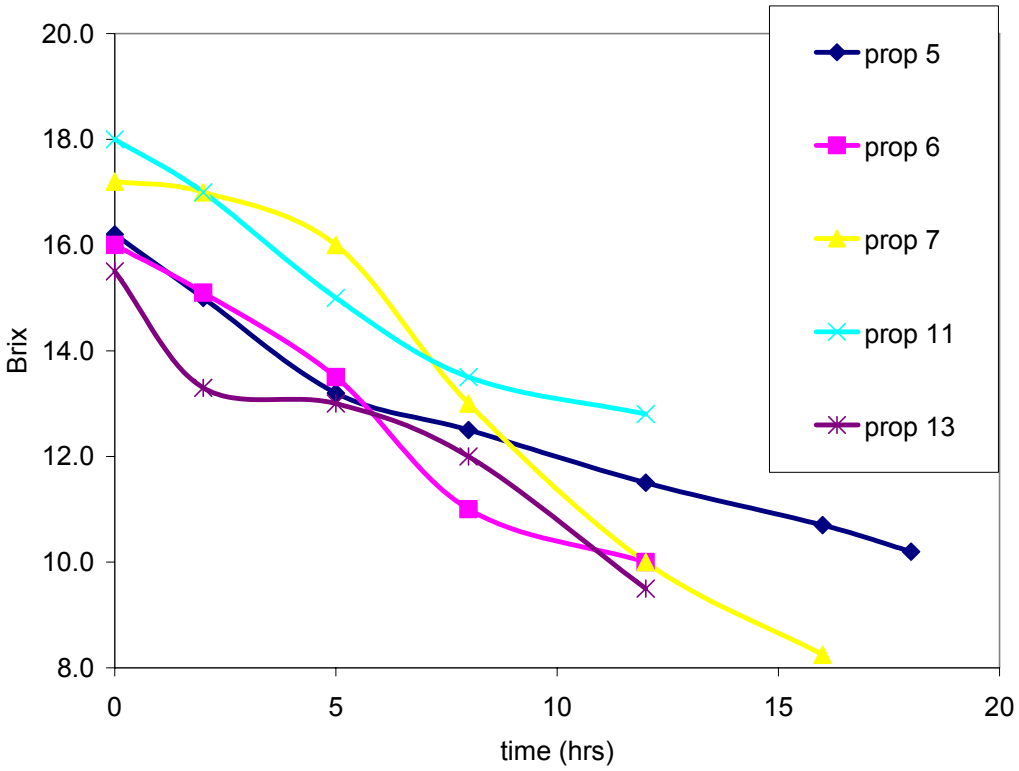



Figure 3.1.16. Performance of various sets over time.

Figure 3.1.17 (following page) shows the residual yeast left after 75% of the medium was pulled off before adding water and syrup as per the lab scale re-set procedures of Appendix 1.1

Figure 3.1.17. Settled yeast after draw-off of Set #17, before addition of fresh syrup to begin Set #18.

The stirring agitator of for the HS/LE reactor was a 3.5' (0.58 $D_v$ ) two bladed propeller spinning at about 12 RPM. This turned out to be too slow to effectively lift and suspend the yeast pellets later in the fermentation process as the density of the medium dropped.

The dimensionless stirring Reynolds number for an impeller in a vessel is defined as (Geankoplis, 1985):

$$N_{Re} = D_a^2 * N_s * \rho / \mu \qquad \text{Eq. 5}$$

Where: $D_a$ is the impeller diameter
$N_s$ is the Revolutions per second
$\rho$ is the liquid density
$\mu$ is the liquid viscosity

In our lab scale reactor we found a propeller speed of 300 RPM with a Ruston 6 bladed impeller was generally adequate to lift, suspend and give small (0.5 to 1 mm diameter) pellet size. (Size of pellets are observed to grow smaller as turbulence in the reactor increases). The lab scale 'sufficient turbulence' corresponds to impeller Reynolds, $N_{Re,}$ of 11,500. The larger, slower impeller in the pilot plant at 12 RPM can be calculated to give an impeller Reynolds of about 200,000. However, this propeller size, speed, and placement (perhaps too far from the bottom of the reactor) was too slow to suspend and break-up the yeast pellets towards the end of the fermentation cycle. In our further trials we intend to try two different impellers, 1) a Ruston (.5 $D_v$) at higher speeds, and a two bladed 'sweep' impellor (0.85 $D_v$) at lower speeds to observe the effect of agitation on the pellets and fermentation performance.

A recent article in *Chemical Engineering*, Himmelsbach et al (2006) discusses mixing and scale-up issues for stirred vessels. Where suspension of solids (such as yeast pellets in our fermentation vessel) is concerned, there are three basic regimes: a) "temporary local dispersion" or "off-bottom motion", where the solids settle on the bottom and there is a defined clear area above the solids, b) "off-bottom" suspension defined as where no particle comes to rest for more than one second on the bottom of the vessel, and c) "visually uniform suspension" where there is no large clear zones free of suspended particles/pellets. In our lab scale trials, we worked in zones a) "local dispersion" where the pellets were dispersed, but there was a large clear area above the pellets, and b) "off-bottom motion". Zweitering (1958) gave the following minimal shaft speed for which "off-bottom motion" occurs

$$N_s = S_{imp} * s_v^{0.1} *(g * (\rho_s - \rho_l)/ \rho_l) * d_p^{0.2} * c_w^{0.13} * D^{-0.85} \qquad \text{Eq 6}$$

*Where $S_{imp}$ is the Zweitering impeller constant (about 7 for Rushton type impellers), $s_v$ is the settling velocity of the pellet, $\rho_s$ is the density of the pellet, $d_p$ is the diameter of the pellet, $c_w$ is the concentration of the pellets in the fluid (kg/kg), and D is the diameter of the impeller.*

Using this Zweitering correlation, a minimum shaft speed of about 100 RPM can be determined based on our estimates for the physical properties of the pellets and the pilot plant parameters of Eq. 6.

In our pilot operations, as our stirring speed was too slow (10-12 RPM), to help break-up and suspend the yeast pellets. We used aeration and use of the bottom draw from the cone bottom of the HS/LE T-305 during fermentation sets 6

through 20 to try to maximize turbulence in the reactor to help suspend the yeast pellets. We noted that pellet size decreased and fermentation performance improved when the large (10-30 mm sized clumps) were pulled through the circulation pump. Due to not having a higher speed propeller and/or a speed controller on the agitator, the only scale-up issue not successfully addressed in these trials was determination of ideal agitation rates for best performance of the pellets/ fermentations on large scale fermenters.

**Summary and Conclusions**

The pilot trials were quite successful. The five major questions which we hoped to answer in this scale-up trial were:

1) Can the HS/LE process be successfully scaled up by 2000 X (from the 2L lab scale to 4500 L industrial scale).

*Yes. The 4,500 L reactor demonstrated good fermentation performance, closely matching performance on the lab scale.*

2) Will the process perform in a non-sterile, industrial, full scale environment?

*Yes. A 'somewhat sanitary' operation was adequate for excellent performance. The 40 brix feed syrup was 'pasteurized' by holding at 150 F. for 30 to 60 minutes prior to introducing into the reactor.*

3) Can the HS/LE complete fermentations in 8 to 12 hours on an industrial scale as demonstrated on the lab scale?

*Yes. We demonstrated near complete utilization of glucose and ethanol concentrations of 7 to 10% (v/v) in 20 consecutive 'sets' of the HS/LE reactor*

4) Can a full industrial scale system be designed to cool the HS/LE reactor (i.e. external cooling).

*Yes. The external cooling loop was quite capable of maintaining the temperature at between 80 and 90 F. A simple Self Actuating Control Valve (SATV) was used to regulate the cooling water flow to the shell side of the heat exchanger (HX-301), while the fermenter contents were circulated through the tube side. The SATV valve reacted rather slowly to higher temperatures, and allowed the temperature to fall somewhat below the set point temperature of 85 F. An electronic control system for the cooling water flow would have performed much better in holding the HS/LE system closer to the desired set point.*

5) Can the yeast pellets be pulled through a low speed centrifugal pump without damaging/losing their flocc nature?

*Yes. A 6" impeller/ centrifugal pump (P-301) was used to circulate the 'beer' in the HS/LE reactor through the 'cooling loop' consisting of drawing either from the bottom or side of the reactor, and passing through the pump, the tube side of the HX-301 heat exchanger, and then back into the reactor. The pump was set up with a variable speed 'frequency drive' which allowed the speed to be varied. We ran the pump at 30% speed (540 RPM) which circulated the reactor contents at about 20 to 30 GPM through the cooling loop.*

**References**

Dale, M.C, & M. Moelhman. 2006. A high speed, consecutive batch or continuous, low effluent process for the production of ethanol from molasses, starches, or sugars. US Patent.

Dale, M.C., A. Eagger, and M.R. Okos. 1994. "Osmolality Efffects on Free and Immobilized K. fragilis Growth and Productivity in Whey Permeate Concentrate." Process Biochem. 29:535-544

Dale, M.C., N. Perrin, and M. Okos. 1994. "Production of Ethanol from Concentrated Sucrose and Molasses Solution Using S. Pombe in an ICRS AICHE Symp. Series #300 Vol 90:56-62.

Lin, J., M.C. Dale, and M. Okos. 1991. "Osmotic ($a_w$) Effects on Growth and Ethanol Production of Free and Immobilized Z. mobilis." Process Biochem. 26:143.

Chen, C., M.C. Dale and M.R. Okos. 1990."The Long Term Effects of Ethanol on Immobilized Cell Reactor Performance." Biotechnology and Bioengineering. 36:975

Chen, C., M.C. Dale and M.R. Okos. 1990. "Minimal Nutritional Requirements for Immobilized Yeast." Biotechnology and Bioengineering. 36:993

Dale, M.C., C. Chen and M.R. Okos. 1990. "The Effects of Ethanol and Temperature on Immobilized Cell Reactors: Cell Growth and Death Rates as Critical Factors in Reactor Modeling and Design." Biotechnology and Bioengineering. 36:983

Geankopolis, C. 1985. Transport Processes and Unit Operations. Allyn & Bacon Press

Himmelsbach, W., D. Houlton, W. Keller, & M. Lovallo. 2006. Mixing Systems: Design and Scale-up. Chem. Eng. April Vol 113:4

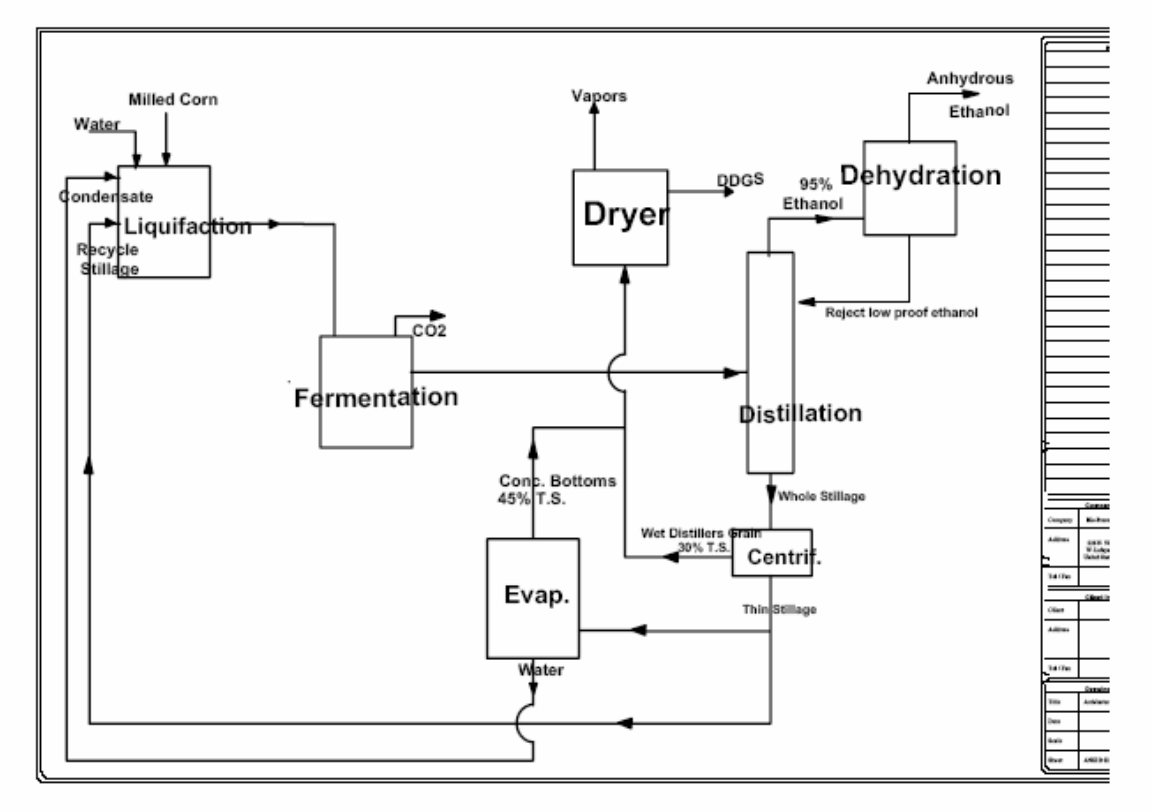
Pinho, F. and A. Cavadas. 2001. Power Consumption and Suspension Criteria for Two Phase Flow in a Stirred Vessel *Brasilian Congress of Mechanical Engineering*, 26-30 November 2001,

# Task 2.0 Dry Mill Syrup Production for the HS/LE Fermentations

## Background

The basic dry mill flow diagram for conventional ethanol plants is shown in Figure 2.0.1 As per this diagram, milled corn is mixed with water, 'thin stillage', and condensate and this fresh mash taken to the cook-liquifaction process. The liquefied mash is then fermented, distilled, and the whole stillage from the column taken to a centrifuge to give wet distillers grains and thin stillage. The thin stillage is split into a recycle stream and a stream taken to the evaporator. A complete model of this system was completed as part of this project.

Figure 2.0.1 Conventional dry mill flows




## Appendix 2.1 Clarification of Whole corn mash (Process P2) / and Extracted Grits (Process P5) via centrifugation

These studies aimed to determine the amount of centrifugation [measured in gravities (*g*)] required to sufficiently clarify corn liquefaction supernatant to allow unhindered growth and maintenance of flocculent yeast utilized in fermentation. In samples that demonstrated no growth effectively fermented the liquefaction product. Corn, finely milled and merely cracked, was processed using liquefaction. Samples were obtained for the two starting materials both

before and after distillase, or gluco-amylase, treatment. Samples of each class were directly analyzed for their response to centrifugation, and other samples of each class were filtered through a standard window screen mesh prior to analysis.

The fineness of the corn starting material did not seem to affect liquefaction, as both starting materials yielded 13.5% (wt.) glucose from a 20% (wt.) dry milled corn slurry, as determined using high-performance liquid chromatography (HPLC). Although it was hypothesized that cracked corn may have been easier to clarify because it began with larger solids that would pellet more easily during centrifugation, the same amount of off-white cloudy material, assumed to be largely protein, was observed in its liquefaction supernatant as that of finely milled corn, and removing this cloudy material was more difficult than removing the other solids, principally fibrous chunks.

It was demonstrated that a mere overnight resting period in 4 degrees C settled out most of the fibrous chunks in the liquefaction products from both starting materials. The relatively easy process of filtering the remaining supernatant through a standard window screen type mesh prior to centrifugation decreased the amount of material entering centrifugation, a possible economic improvement for the process, but did not further clarify the end result of centrifugation. Analysis of the samples obtained prior to distillase treatment showed results the same as those of the fully treated samples. Centrifugation prior to Distillase treatment did not improve supernatant clarity or diminish the amount of centrifugation required to clarify it.

Spin time was held constant in these experiments. All samples (50 ml tubes) were centrifuged for 10 min. at speeds ranging from 0-4,000 rpm, corresponding to 0-8000*g* in a 6 in. diameter IEC centrifuge. Centrifugation was carried out by placing 50 mL samples of digested material in 50 mL conical tubes. Following centrifugation the samples appeared as two layers, a fibrous pellet, which filled the bottom of the tube up to ~5 mL, and a yellowish supernatant. The supernatant was then assigned an optical density by light scattering at 620 nm (OD620) value using spectroscopy. A sixty-fold dilution was required to bring the samples into the sensitive range of the instrument for this application. Without centrifugation a corn digest diluted sixty-fold demonstrated an OD620 of 0.30. OD620 of the samples appreciably decreased with increasing gravities until a ceiling of 1309*g* (1600rpm, OD620=0.11) beyond which the supernatant was not noticeably clarified up to 2045*g* (2000 rpm, OD620=0.10) the upper limit of this experiment.

Following centrifugation samples were inoculated with pinhead sized flocculent yeast and incubated overnight at 30ºC in a shaking incubator, shaking at 200-250 rpm. Experiments were also done using stir bars to agitate during fermentation and the grinding effects of the stir bar was determined to interfere with the formation or disrupt the flocculent yeast pellets. Fermentation was repeated multiple times for each type of sample because of slight variations in the size and assumed health of the flocks used for inoculation. It was virtually impossible to completely standardize this aspect of the experiment. Furthermore, flocculent growth was merely qualitatively analyzed by visually examining the flocks before and after fermentation. A more quantitative method to determine growth was not devised

Samples treated with less than 184*g* (600 rpm, OD620=0.25) consistently failed to demonstrate proficient flocculent growth. Samples treated with 327*g* (800 rpm, OD620=0.20) showed questionable growth, after some fermentations flocks appeared larger and after others did not. Growth improved noticeably in samples treated with 1309*g* (1200 rpm, OD620=0.15), which demonstrated consistent flock growth. The best results obtained in this experiment were with samples treated with 2045*g* (2000 rpm, OD620=0.10) that repeatedly demonstrated growth

from pinhead sized (0.2 mm) flocks before fermentation to 2 to 5 mm sized flocks afterwards, and which we characterized as proficient growth.

Future experiments may include variations in the amount of time samples experience a given level of *g*, which could be valuable in determining a residence time for a continual process. Temperature might be another good test variable, affecting solubility of various components and, hence, the amount of *g* required to isolate them via centrifugation.

## Appendix 2.2 Application of the HS/LE to Corn Ethanol

In Figure 2.2.1 below, conventional process flows for dry grind ethanol are contrasted with the suggested process (P-1) utilizing a rinsing centrifuge to separate solids prior to fermentation.

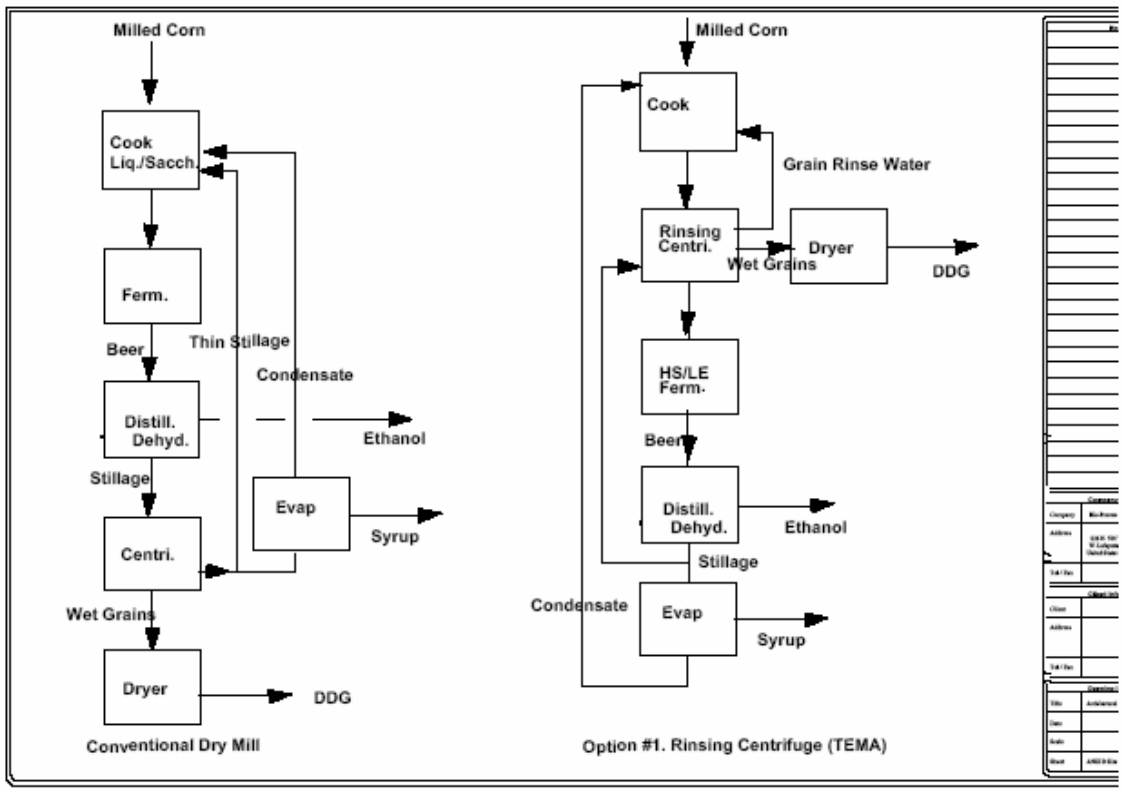



Figure 2.2.1. Comparison of process flows between conventional and pre-separation of solids using a rinsing centrifuge. This pre-separation process is termed 'P-1' and was our first process concept allowing use of the HS/LE in a dry grind ethanol factility

The P-1 process would give a clean clear syrup stream to take to the BPI HS/LE fermentation process. The Distiller's Dry Grain would not have gone through the fermentation or distillation system so would be a slightly higher quality product than is produced by the current/conventional process.

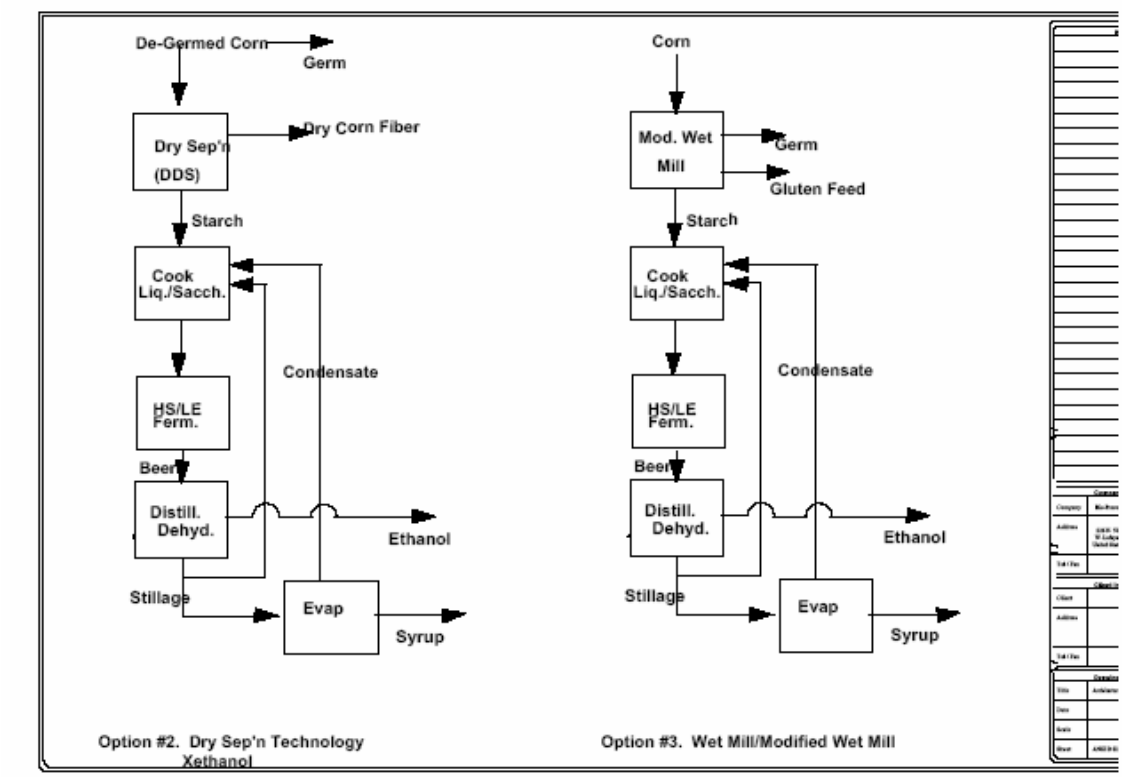



Figure 2.2.2. Process flows for Dry Separation of starch (P-2) and a 'modified wet mill' process (P-3) being developed by U of IL.

P-2 of Figure 2.2.1 would require a separation of the dry fiber and starch streams. Xethanol feels as though their DDS process might be able to accomplish this separation. P-3 is a 'modified wet mill' process being developed by U of Ill researchers and also Biorefining Inc. This sort of processing would allow recovery of higher value products- corn oil, corn protein, and the corn fiber rather than lumping these products into the 'Distiller's Dry Grains' The purified starch stream from the process would be cooked and fermented via the BPI process. An analysis of 'modified wet mill' processing by MBI, presented in May (Chattenooga Biotech/Bioeng Conf), indicated substantial improvement in corn ethanol plant operating economics with the production of these higher value streams.

The P-4 process is termed bio-milling and to the best of our knowledge is similar to the U of IL process and is being developed by Biorefining Inc. of Minneapolis, MN (www.biorefining.com) which separates the germ, bran, starch and protein prior to fermentation.

The P-5 process being developed by BPI consists of a dry fractionation (dry milling) which separates the incoming corn into three streams: bran, germ, and endosperm 'grits'. The grits are then cooked, the sugar rinsed from the grits which leaves a 'High Protein' distillers grain with up to 55% protein levels.

The P-6 process, also under development by BPI is termed the 'High Value' process consists of 1) a dry separation, and 2) zein followed by glutelin (water soluble) protein recovery from the grits fraction as per the flow chart of Figure 2.2.3

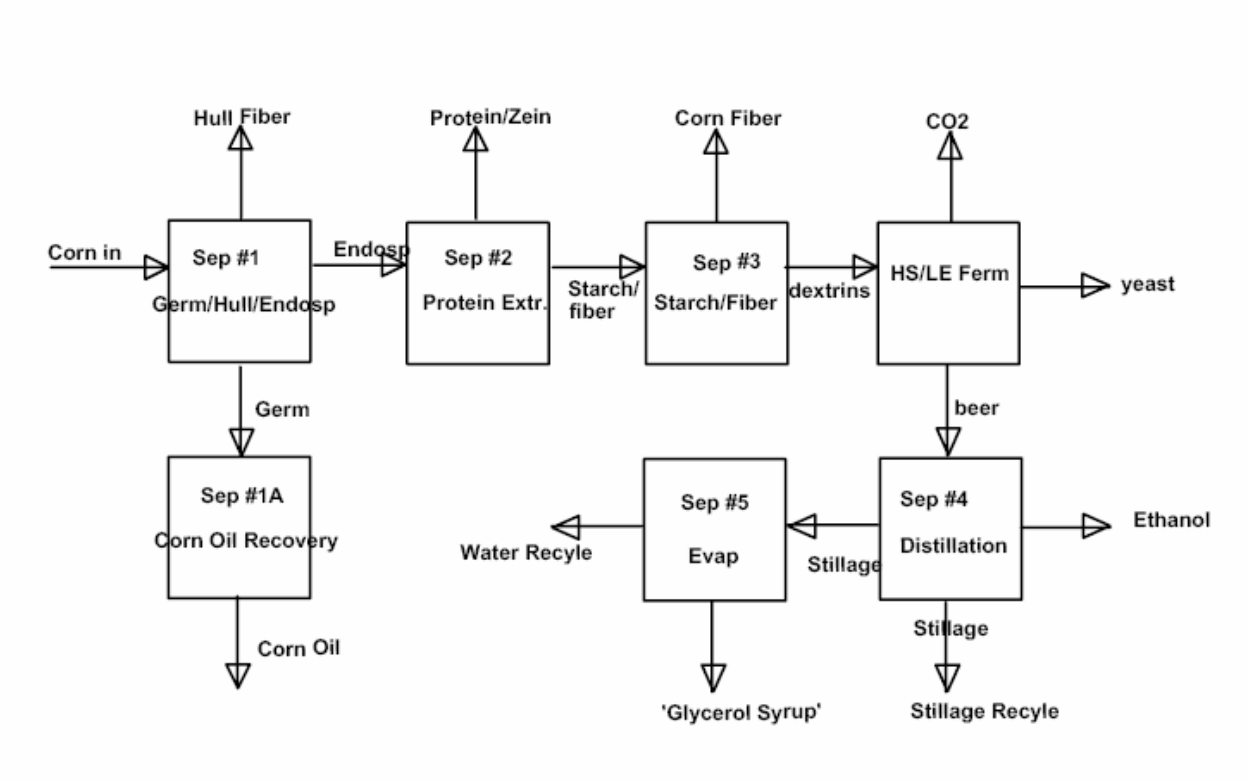



Figure 2.2.3. BPI's concept for the "P6", 'High Value' Corn Ethanol Dry Mill Facility.

# High Speed / Low Effluent Ethanol from Syrups and Dry Mill Corn

Fermentation Technology by

Bio-Process Innovations (USA)

(765) 746-2100 phone/fax

# BPI's HS / LE Process- Ethanol from Wet Mill Corn Syrup or Dry Mill Corn

- Speeds fermentation rates to under 8 hours for 12% v/v beers
- New technology under US and International Patent Status by BPI
- Continuous or High Speed Consecutive Batch Operation
- Proven on Lab and Pilot Scale

# Benefits of HS/LE Technology for Ethanol from Corn

- High Speed fermentation - 6 to 12 hour
- Decreased costs for yeast/ yeast recovery
- Production of a yeast ‘paste’ product
- Elimination of dilute rinse waste water/ caustics from tank cleaning
- High Levels of Stillage Backset-Decreased evaporation load
- Decreased size of fermenters

# Basis of Technology

- Self Flocculent Yeast
- Stable Long Term Operation
- Osmotolerant
- Contamination Resistant

Operate in either

Consecutive Batch

Cascade or Tower

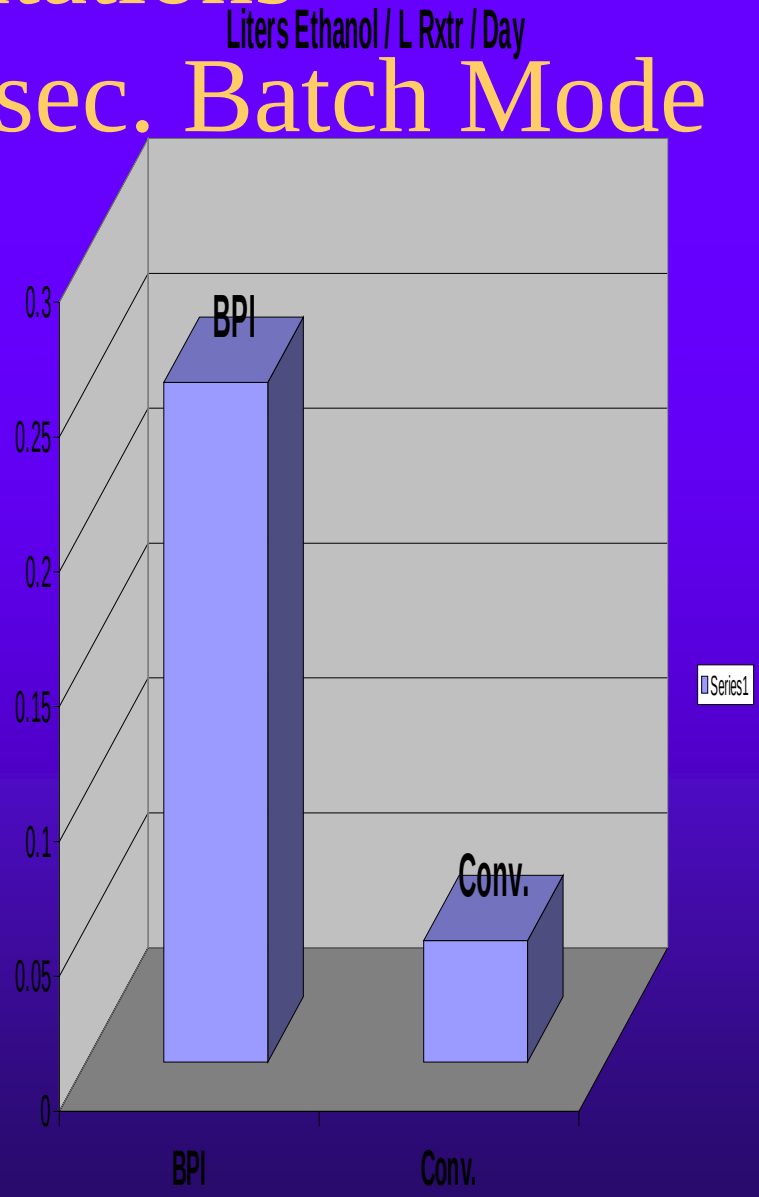
# History of Technology

- Developed over last 5 years for Molasses
- Tested on Wet Mill Corn syrups- last 3 years
- Currently negotiating siting molasses/ cane juice projects in Columbia, SA
- Recent recipient of DOE I&I grant to demo large pilot for Wet mill and Dry Mill Syrups- sited w/ Xethanol

# High Speed Fermentations
# 4.5 X Faster – Consec. Batch Mode

- BPI-12%- 8 hr ferm.
- A) Fill/Ferm –2 hr
- B) Ferment- 5 hour
- C) Drain- (75%) 1 hour
- 0.0113 gal eth/gal ferm/hr

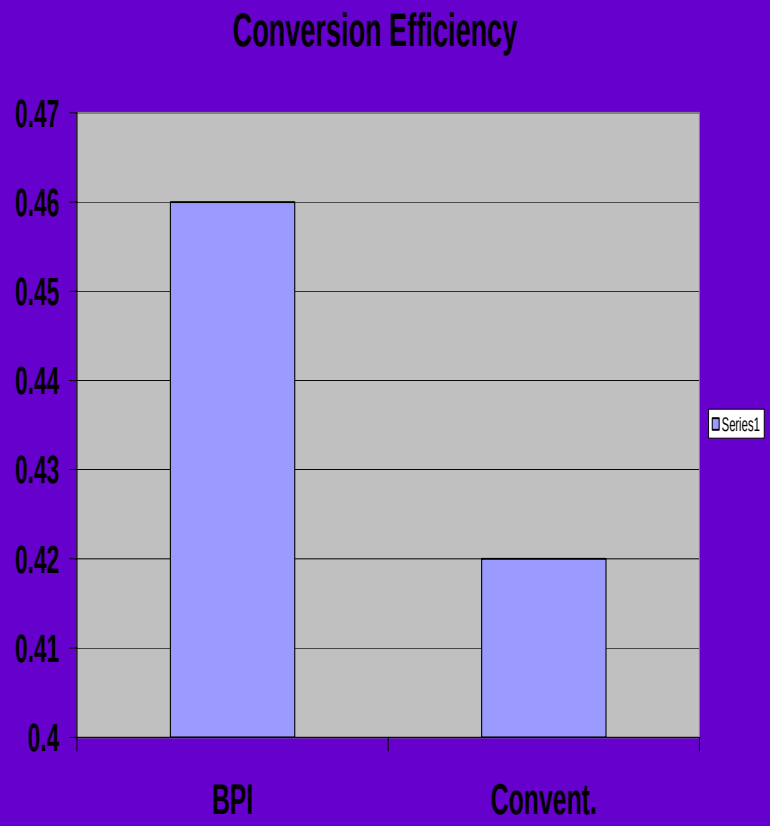
- Conv. Corn-14%- 56 hr
- A) Fill- 16 hour
- B) Ferment- 42 hour
- C) Drain-3 hour
- D)Clean & Rinse- 3 hour
- 0.0025 g eth/gal ferm/hr



# Decreased Costs for Yeast

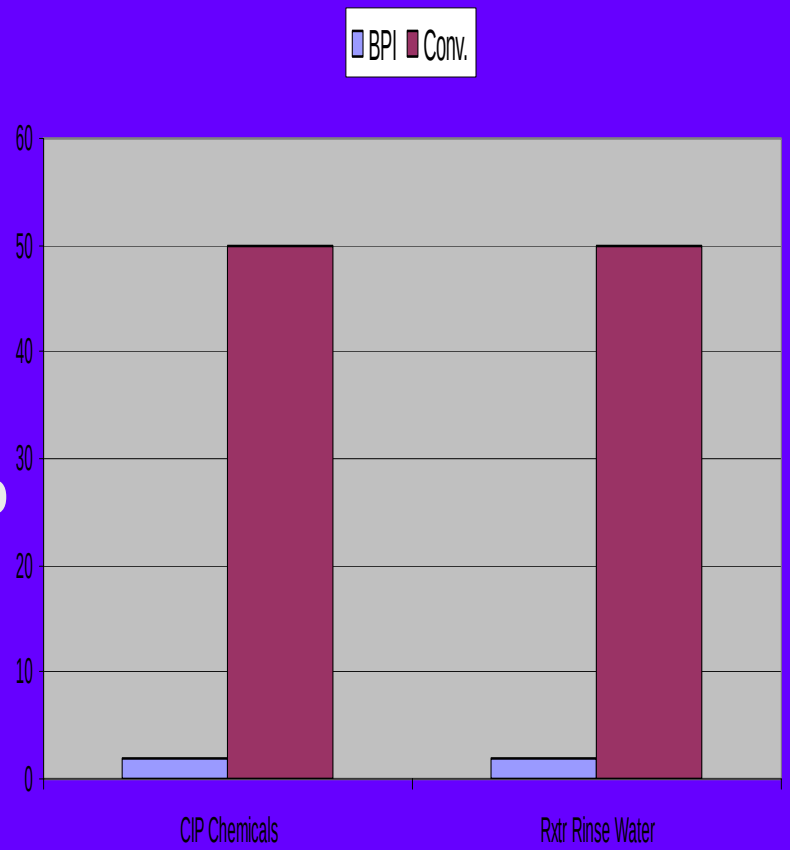
- No Centrifuges
- No Yeast Propagation
- No Purchase of Dry Yeast
- High Efficiency Conv. Of Sugars to Ethanol

Yeast Paste By-Product can be Dried and Sold



# Reduction of Rinse Water/ CIP Chemicals

- No cleaning of reactor between cycles
- No CIP chemicals
- No rinse water
- Can Operate for 200 to 500 cycles before restart
- Very Resistant to Contamination



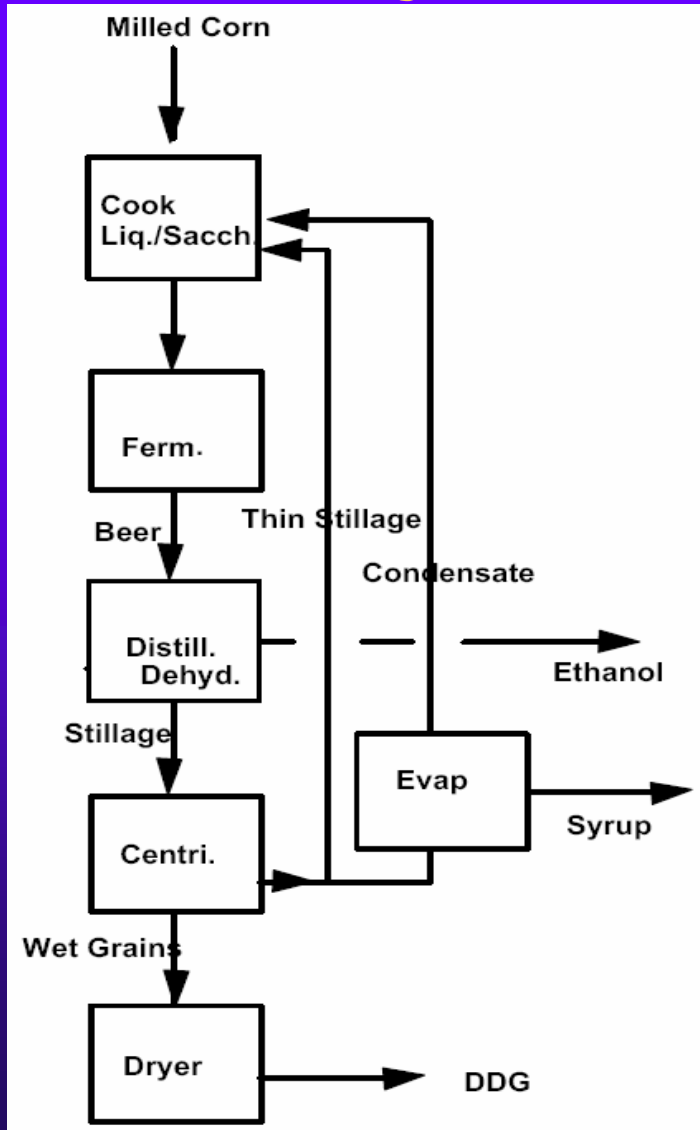
# Application of HS/LE

- Molasses
- Wet Mill Corn Syrup
- Dry Mill Corn Syrup after Fiber Removal (requires ‘clear’ substrate)

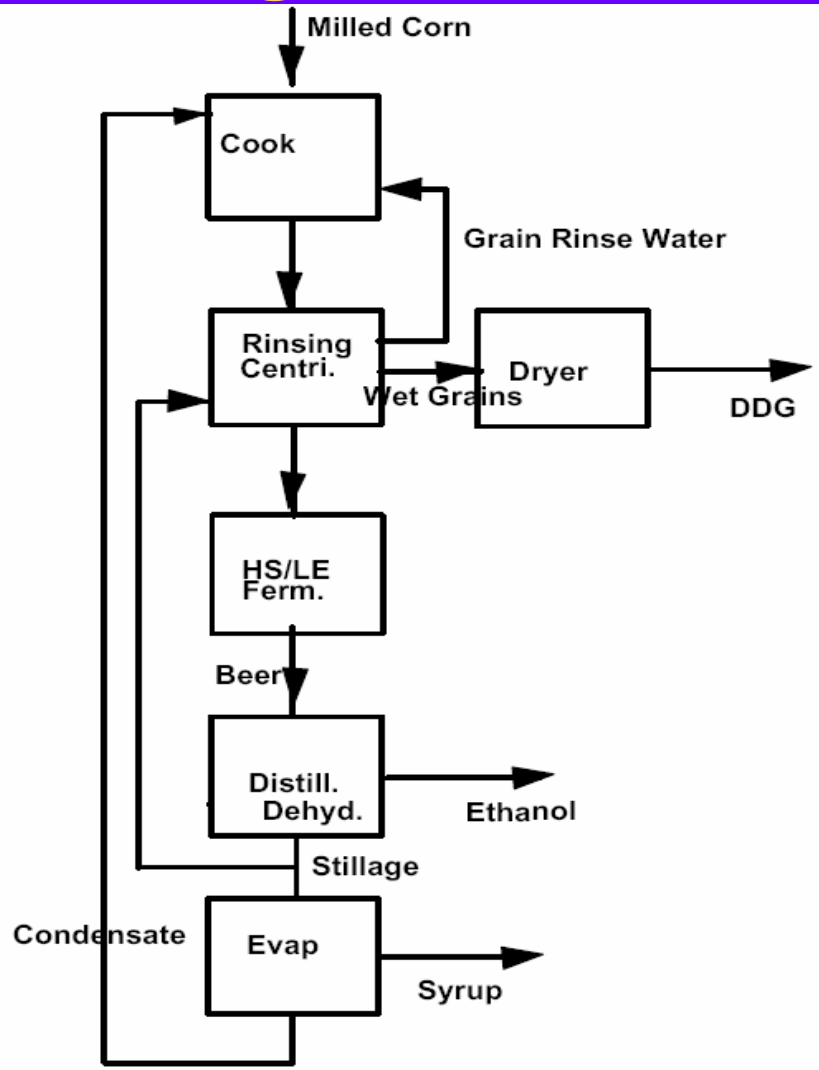
# Application of HS/LE Technology to Dry Mill Ethanol

- Remove Corn Fiber before cook
  - U of Illinois ‘Quick Fiber’ process
  - BPI Dry Mill process
  - Xethanol’s DDS process?
- Remove Corn Fiber after whole mash cook
  - Rinsing centrifuge?
  - Pneuma-Press?

# Conv.Dry Mill vs Rinsing Centrif.

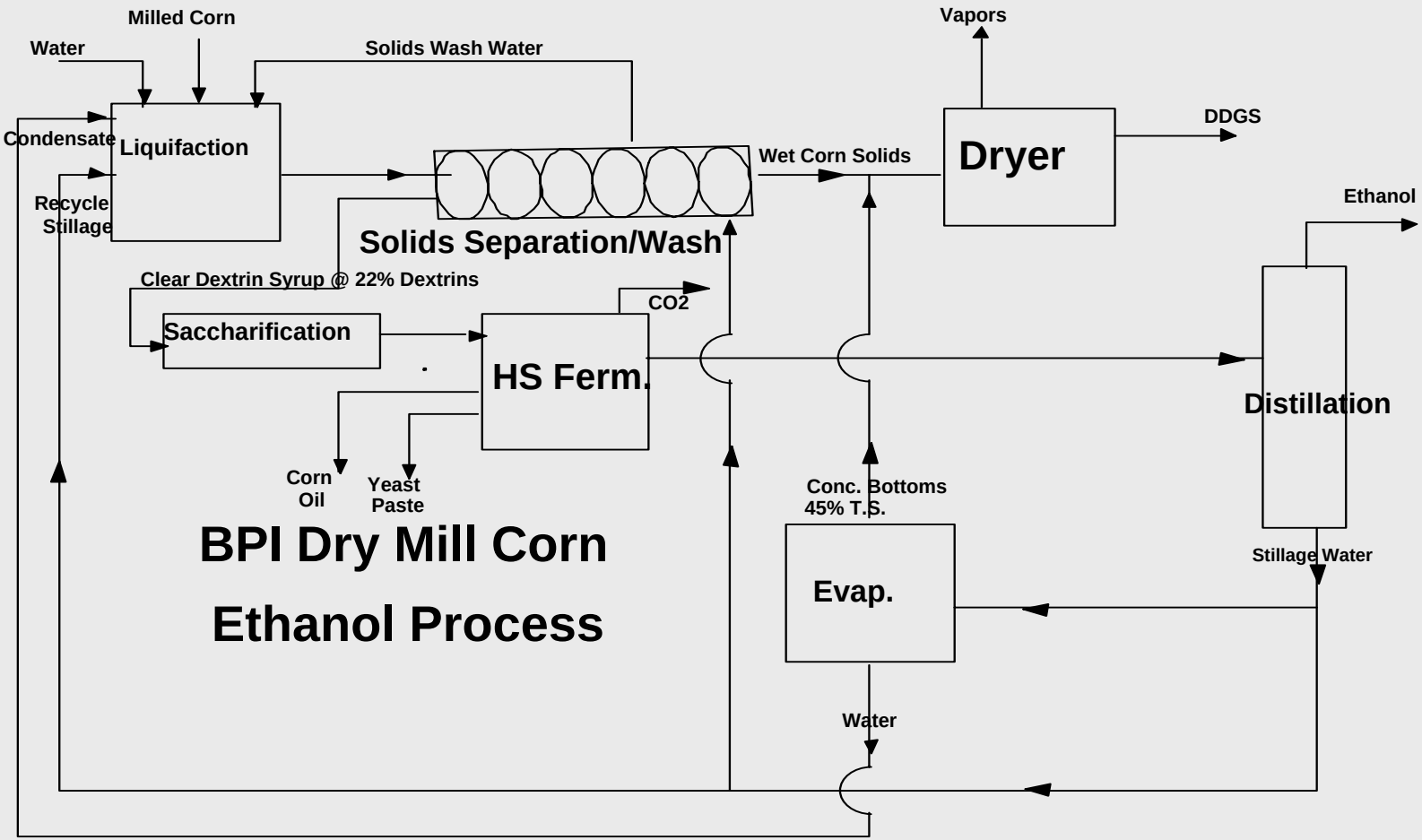



Conventional Dry Mill




Option #1. Rinsing Centrifuge (TEMA)

# Rinsing Centrifuge HS/LE to Dry Mill



# Questions for Rinsing Centrifuge HS/LE Process

- Ability to Provide Clear Syrup
- Rinse Efficiency/ Loss of dextrins in Distillers Grain
- Different quality of Distillers Grain

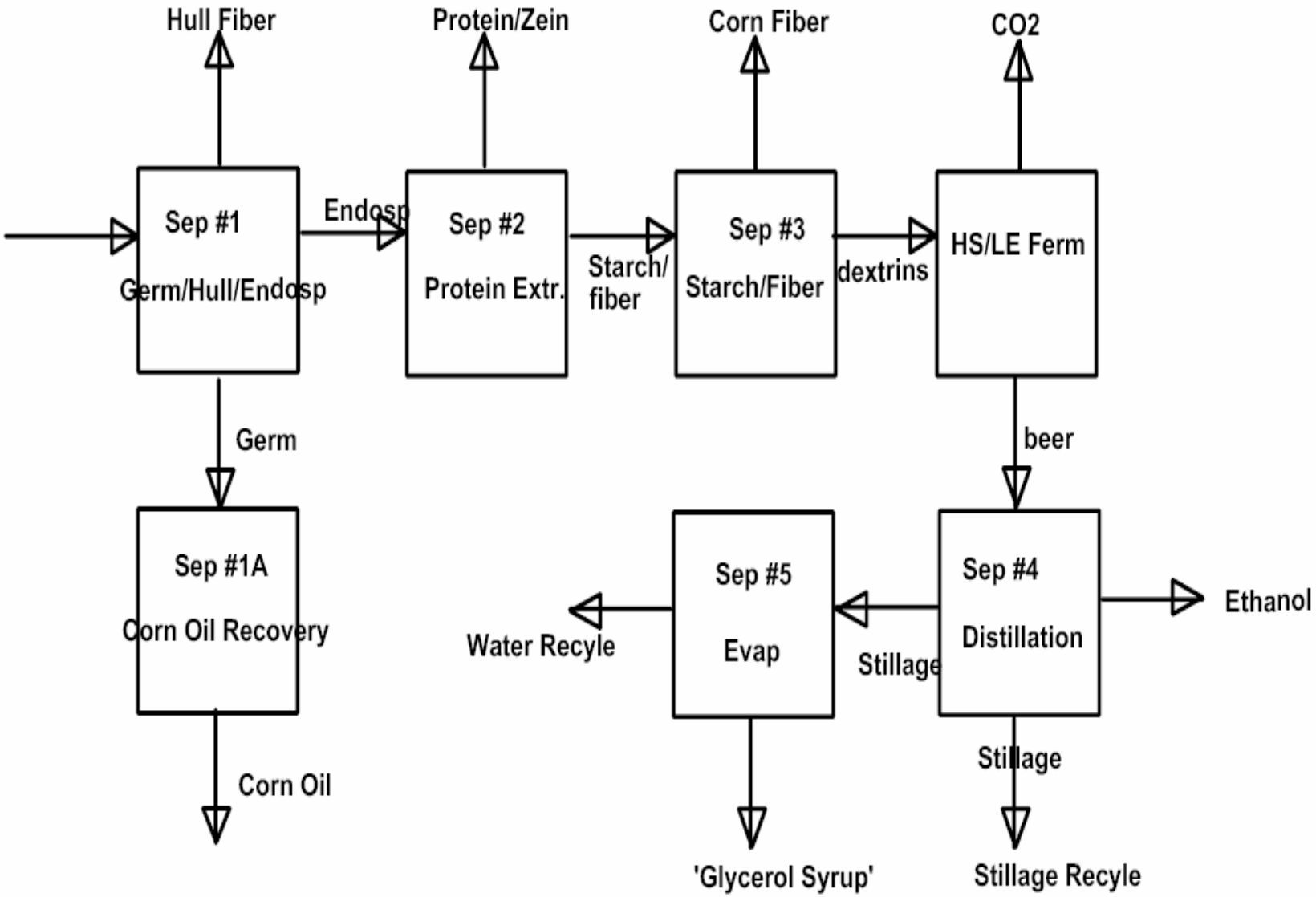
# Benefits of Rinsing Centrifuge HS/LE

- Non-fouling clean, clear beer to HX's and Distillation column
- HS/LE Process allows 60 to 80 % stillage backset versus 30 to 50% currently practiced in Dry Mill Corn Ethanol facilities
- Efficient conversion of sugars to ethanol
- Production of live yeast paste product stream

# Advantage of High Backset Rate

- Evaporation Load reduced by 30 to 50%
- Associated Energy and Capital Savings in Evaporation System

# BPI 'High Value' Corn Process



## Income from Corn Ethanol Operations

| | | | |
|---|---|---|---|
| 1) Conventional Dry Mill | | | |
| | a) ethanol | $2.99 | (2.6 gal/bu- $1.15/gal) |
| | b) DDG | $1.06 | (17#/bu- $125./ton) |
| | **TOTAL** | **$4.05** | bushel corn input |
| 2) Option 1- Rinsing Centrifuge- HS/LE | | | |
| | a) ethanol | $3.10 | (2.7 gal/bu- $1.15) |
| | b) DDG | $1.06 | (17#/bu- $125./ton) |
| | c) yeast | $0.05 | (75 g yeast bushel @ $0.31/#) |
| | **TOTAL** | **$4.21** | bushel corn input |
| 3) Option 2- Mod. Wet Mill- HS/LE | | | |
| | a) germ/oil | $0.33 | (1.6#/bushel- $0.21/#) |
| | b) gluten meal/feed | $1.01 | (15#/bushel-$135./ton |
| | c) ethanol | $3.10 | (2.7 gal/bu- $1.15) |
| | d) yeast | $0.05 | (75 g yeast bushel @ $0.31/#) |
| | **TOTAL** | **$4.49** | bushel corn input |
| 4) Option 3- BPI HV- HS/LE | | | |
| | a) corn bran | $0.39 | (3.3 #/bushel- $0.12 #) |
| | b) germ/oil | $0.33 | (1.6#/bushel- $0.21/#) |
| | c) zein protein | $5.60 | (0.8#/bushel- $7.00/#) |
| | d) hydrophilic protein | $1.52 | (1.9#/bushel- $0.80/#) |
| | e) corn fiber | $0.11 | (2.2#/bushel-$0.05/#) |
| | f) ethanol | $3.10 | (2.7 gal/bu- $1.15) |
| | f) yeast | $0.05 | (75 g yeast bushel @ $0.31/#) |
| | **TOTAL** | **$11.10** | bushel corn input |

# Opportunity- HS/LE Fermentation in New Constr/ Upgrades

- Required Fermenter Volume is reduced by a factor of 4.5 X
- Evaporation needs reduced
- Lower Cost/ Smaller facilities
- Non-fouling clear beer to HX's and Distillation
- Up to 4.5X More Ethanol Product from Same Fermenters for Plant Upgrades

# Conclusions: HS/LE Fermentation Technology

- Reduces Capital Costs
- Reduces Labor Costs
- Application to New Constr
- Application to Expansion/Retrofit

- BOOSTS NET PROFIT

# BPI: Technology Provider

- Low Energy Distillation
- Milk Lactose Fermentation
- Molasses/ Cane Syrup Fermentation
- Biomass Delignification
- Biomass Ethanol
- Low Energy/ High Density Aerobic Yeast Production

# PROCESS IMPROVMENTS FOR CORN ETHANOL
# 1) HIGH SPEED/LOW EFFLUENT FERM
# 2) HIGH VALUE PROCESS

**M. CLARK DALE & M. MOELHMAN**

**Bio-Process Innovation, 226 N 500 W., West Lafayette, IN 47906**

**Poster 6-52 27th Symposium on Biotechnology for Fuels and Chemicals May 1-4, 2005**

**Introduction**

**BPI has developed and recently received for patent protection on a *High Speed/ Low Effluent (HS/LE)* process for production of ethanol from dextrins/glucose. The process is based on a strain of yeast and operating procedures developed by BPI over the past few years. The yeast was developed to have an extreme 'floc durability' through 1) a strain selection process in which a number of highly flocculent yeast were compared, and 2) then beginning with one 'best' selected strain, improving the strain by a long process of 'natural selection'- selecting and re-selecting extremely durable osmotolerant 2 to 5 mm diam. floc yeast mutant pellets from reactors which were run for periods of months.**

**Advantages of HS/LE Process**

- Increase productivity of fermenters by a factor of 5 to 8 times
- Decrease effluent stillage by using a high degree of backset
- Decrease nutrient needs/costs
- Produce a clean, nearly sparkling clear, non-fouling 'beer' to take to the distillation column
- Produce a clean high density yeast paste by-product with no need for centrifuges
- Reduce waste water/ cleaning chemicals by eliminating need for CIP of fermenter(s) between batches.
- Reduce operator/equipment needs as process is easily automated.

**1) Inhibition of yeast growth and productivity by product** (ethanol), substrate (glucose) and other inhibitors (salts, glycerol, etc.)
Dale et al, (1994) developed an osmolality decribing both substrate and poduct inhibition of the ethanolic fermentation as:

$$v=v_m [1 - \varepsilon/k_{\varepsilon vm}] \quad \text{Eq. 1}$$

$$\mu=\mu_m [1-\varepsilon/k_{\varepsilon \mu m}] \quad \text{Eq. 2}$$

Growth is more strongly inhibited by osmolality than is productivity with $k_{\varepsilon \mu m}$ values of ranging from around 2 to 2.5 os/kg, while $k_{\varepsilon vm}$ runs 3.5 to 5.0 depending upon yeast species, osmo-tolerance, and ethanol tolerance. We have determined a value of 4.5 to 5.0 for kεvm for our flocculent yeast BPSC-15. Osmolality of the solution can be determined as a simple additive function of the osmolality of the various components of the solution broth.

$$\varepsilon=\varepsilon_s + \varepsilon_{eth} + \varepsilon_{inhib} \quad \text{Eq. 3}$$

This model allows an easy determination of the effects of stillage recycle based on the osmolality of the inerts being brought back around to the feed make-up. BPI has completed some work with recycle of molasses stillage which indicated a 27% decrease in average productivity rates for a molasses feed made up with 30% stillage. Our lab results closely followed this modeling, with Consecutive Batch Mode operation indicating an average fermentation completion in 8 hours versus 6 hours (33% decrease in average productivity).

We have completed modeling on the effects of stillage recycle for the corn syrup fermentation with the HS/LE process. These results indicate that at 70% recycle of stillage, glycerol and other non fermentables would be concentrated by a factor of 3.5X for an outlet glycerol concentration of 30 to 35 g/L (versus 9-10 g/L for no stillage recycle).

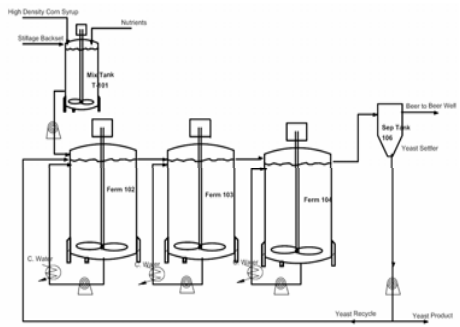
**2) Long term viability of pelletized cells**. Dale et al (1984) showed that for an immobilized cell population exposed to constant conditions of ethanol and sugar, that the steady state live cell fraction can be estimated based on a number of simplifying assumptions as:

$X_{ssl} = [\mu/ (\mu + K_d)]$

Based on this analysis, we can see that if a cell population (i.e. one particular yeast pellet) is exposed to continuous conditions of zero growth, the steady state live cell density will be zero. Thus it is important for a pellet to occasionally see conditions allowing cell growth. Thus, initial conditions in Consecutive Batch or Sstage 1 conditions in Cascade Mode should be maintained such that there is cell growth, with the overflow of younger cells refreshing the population of stages 2 and 3 where there is little cell growth due to the higher osmolality (largely due to ethanol).

**Consecutive Batch- Corn Wet Mill Syrup**
BPI has run a 2L Multi-Gen stirred fermenter using dextrins converted to glucose at 200 to 240 g/L feed concentration. We ran the system in the Consecutive Batch Mode at 3 cycles per day (8 hours per batch) over a period of 3 months (206 cycles) and determined excellent results with fermentations going to near completion in as little as 5 hours. Over this period, we determined a 'minimal nutrient' make-up of for the glucose feed stock.

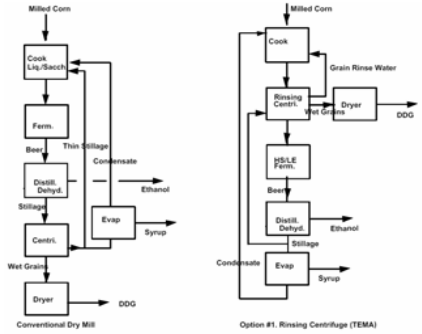
**Continuous Cascade Mode- Corn Wet Mill Syrup**
We have also run BPI's High Speed/ Low Effluent system in the continuous mode. A 1 liter Multigen reactor was used. Batches of 5 gallons feed were made-up to run the 3 experiments described in Table 1. We caught the reactor effluent in a closed pot which we held at 65C. The effluent was then transferred to the feed tanks to simulate stage 2, and once again to simulate stage 3. The volume of dead cells in the bottom of the effluent pot were measured after each stage, and the dry wt. estimated. We used a proprietary nutrient formulation consisting of inorganic N, P and K supplemented with micro-nutrients/vitamins and CSL.

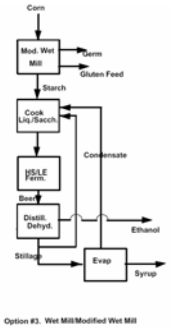



| | time (RTD) | Ethanol | Sugar | Productivity | g cells/L eff |
|---|---|---|---|---|---|
| | Hr | g/L | g/L | g eth/L hr | |
| Feed | 3 hour/stg | | 220 | | |
| Stg 1 | 3 | 73 | 57 | 24.3 | n.m. |
| Stg 2 | 6 | 105 | 4 | 10.7 | n.m. |
| Feed | 2 hour/stg | | 230 | | |
| Stg 1 | 2 | 63.6 | 98 | 31.8 | 1.1 |
| Stg 2 | 4 | 95 | 30 | 15.7 | 0.4 |
| Stg 3 | 6 | 110 | 0.2 | 7.5 | 0.4 |
| Feed | 1.3 hour/stg | | 210 | | |
| Stg 1 | 1.33 | 40 | 128 | 30.1 | 1 |
| Stg 2 | 2.66 | 68 | 77 | 21.1 | 0.7 |
| Stg 3 | 4 | 97 | 13 | 21.8 | 0.4 |

## Application of HS/LE to Dry Mill Ethanol.

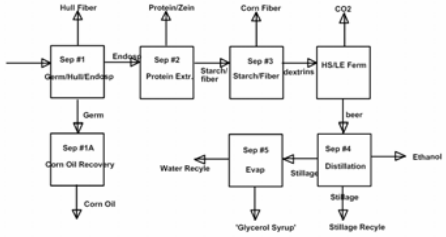
The HS/LE Fermentation Requires a 'Clear' Syrup Feed. There are a variety of Process Configurations which could be implemented.




**Benefits of Rinsing Centrifuge HS/LE**
•Non-fouling clean, clear beer to HX's and Distillation column
•HS/LE Process allows 60 to 80 % stillage backset versus 30 to 50% currently practiced in Dry Mill Corn Ethanol facilities
•Efficient conversion of sugars to ethanol
•Production of live yeast paste product stream

**Advantage of High Backset Rate**
•Evaporation Load reduced by 30 to 50%
•Associated Energy and Capital Savings in Evaporation System




Option 4. BPI Concept for High Value Corn Ethanol Processing

**Income from Corn Ethanol Operations**

| | | |
|---|---|---|
| 1) Conventional Dry Mill | | |
| a) ethanol | $2.99 | (2.6 gal/bu- $1.15/gal) |
| b) DDG | $1.06 | (17#/bu- $125./ton) |
| TOTAL | $4.05 | bushel corn input |
| 2) Option 1- Rinsing Centrifuge- HS/LE | | |
| a) ethanol | $3.10 | (2.7 gal/bu- $1.15) |
| b) DDG | $1.06 | (17#/bu- $125./ton) |
| c) yeast | $0.05 | (75 g yeast bushel @ $0.31/#) |
| TOTAL | $4.21 | bushel corn input |
| 3) Option 2- Mod. Wet Mill- HS/LE | | |
| a) germ/oil | $0.33 | (1.6#/bushel- $0.21/#) |
| b) gluten meal/feed | $1.01 | (15#/bushel-$135./ton |
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| 4) Option 3- BPI HV- HS/LE | | |
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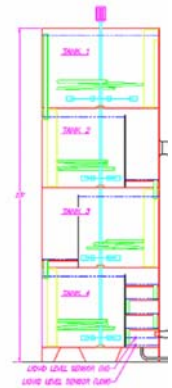
| d) hydrophilic protein | $1.52 | (1.9#/bushel- $0.80/#) |
| e) corn fiber | $0.11 | (2.2#/bushel-$0.05/#) |
| f) ethanol | $3.10 | (2.7 gal/bu- $1.15) |
| f) yeast | $0.05 | (75 g yeast bushel @ $0.31/#) |
| TOTAL | $11.10 | bushel corn input |

## Pilot Scale Demo of HS/LE

A tower cascade reactor is being modifed for semi-industrial scale pilot demonstration of the HS/LE at a small Xethanol facility in Iowa. Fabrication of piping, reactor vessel, and controls are complete&&&&&&. Trials should begin in May. The bottom chamber of the reactor (4,000L) will be run in the Consecutive Batch Mode. Later we intend to demonstrate the rinsing centrifuge (Option 1) for application to dry mill syups.



## Summary

**The HS/LE process allows complete fermentation of 18 to 28% glucose to ethanol in 4 to 8 hours, in either a continuous cascade or consecutive batch mode over extended periods of several to many months. In the Consecutive Batch (CB) mode of operation, the fermenter is available for immediate re-set after completion of fermentation and a settling period during which completed beer is decanted. This allows 3 or even 4 batches of 10 to 14% ethanol to be produced per reactor per day. In the Cascade mode, residence times of 6 hours over 3 reactors gave over 99% sugar utilization of a 220 g/L glucose feed.**

**The HS/LE process allows a high degree of backset, can be applied directly to wet mill corn syrups, and several processes are under development at BPI to apply the process to Dry Mill Ethanol production**

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# Pilot Scale Demo of HIGH SPEED / LOW EFFLUENT Process: Ethanol Production from Wet / Dry Mill Syrups & Higher Value Dry Mill Processes.

**M. CLARK DALE & RHYS T. DALE**

**Bio-Process Innovation, 226 N 500 W., West Lafayette, IN 47906**

**Corn Utilization and Technology Conference, Dallas, Texas June 5 - 7, 2006**

## Introduction to the HS/LE Process

BPI has developed and patented the *High Speed/ Low Effluent (HS/LE)* process for production of ethanol from dextrins/glucose. The HS/LE process integrates specialized operating procedures and osmotolerant mutant yeast strains that form extremely large (2 – 5 mm diameter) and durable pellets. The development of the strains economically advantageous characteristics involved both a selection processes, in which the 'best' yeast strains were selected, and improvement processes, utilizing methods of 'artificial selection'.

**Figure1: Pilot Reactor**

The HS/LE process has been proven highly effective on the lab scale (<100 L) and recently on the pilot scale (4,500+ L). Several dry mill plants are currently being designed to integrate this process for process efficiency and economic gain. The HS/LE process produces ethanol faster, cheaper, and more efficiently than current production methods as well as adding high value co-products

**Advantages of the HS/LE Process**

1) Decreased fermentation time / Increased reactor output (5 – 10 X)
2) Decreased effluent stillage by using a high degree of backset
3) Decreased fermentation nutrient input/costs
4) Production of a clean, nearly clear, non-fouling 'beer'
5) Production a clean, high density yeast paste, without centrifuging
6) Reduction in waste water and chemicals by eliminating need for CIP
7) Reduce operator/equipment needs as process is easily automated.

**BPSC-15: Productivity and Cell Viability**

**1) Inhibition of yeast growth and productivity by product** (ethanol), **substrate** (glucose) **and other inhibitors** (salts, glycerol, etc.)
Dale et al, (1994) developed an osmolality decribing both substrate and poduct inhibition of the ethanolic fermentation as:

$$\nu=\nu_m [1 - \varepsilon/k_{\varepsilon vm}] \quad \text{Eq. 1}$$

$$\mu=\mu_m [1-\varepsilon/k_{\varepsilon um}] \quad \text{Eq. 2}$$

Growth is more strongly inhibited by osmolality than is productivity with $k_{\varepsilon um}$ values of ranging from around 2 to 2.5 os/kg, while $k_{\varepsilon vm}$ runs 3.5 to 5.0 depending upon yeast species, osmo-tolerance, and ethanol tolerance. We have determined a value of 4.5 to 5.0 for kεvm for our flocculent yeast BPSC-15. Osmolality of the solution can be determined as a simple additive function of the osmolality of the various components of the solution broth.

$$\varepsilon=\varepsilon_s + \varepsilon_{eth} + \varepsilon_{inhib} \quad \text{Eq. 3}$$

This model allows an easy determination of the effects of stillage recycle based on the osmolality of the inerts being brought back around to the feed make-up. BPI has completed some work with recycle of molasses stillage which indicated a 27% decrease in average productivity rates for a molasses feed made up with 30% stillage. Our lab results closely followed this modeling, with Consecutive Batch Mode operation indicating an average fermentation completion in 8 hours versus 6 hours (33% decrease in average productivity).

We have completed modeling on the effects of stillage recycle for the corn syrup fermentation with the HS/LE process. These results indicate that at 70% recycle of stillage, glycerol and other non fermentables would be concentrated by a factor of 3.5X for an outlet glycerol concentration of 30 to 35 g/L (versus 9-10 g/L for no stillage recycle).

**2) Long term viability of pelletized cells**. Dale et al (1984) showed that for an immobilized cell population exposed to constant conditions of ethanol and sugar, that the steady state live cell fraction can be estimated based on a number of simplifying assumptions as a simple function of specific growth rate, μ, and death rate constant, $K_d$ :

$$X_{ssl} = [\mu/ (\mu + K_d)]$$

Based on this analysis, we can see that if a cell population (i.e. one particular yeast pellet) is exposed to continuous conditions of zero growth, the steady state live cell density will be zero. Thus it is important for a pellet to occasionally see conditions allowing cell growth. Thus, initial conditions in Consecutive Batch or Stage 1 conditions in Cascade Mode should be maintained such that there is cell growth.

## HS/LE Wet Mill Ethanol Application

**Lab Scale Consecutive Batch- Corn Wet Mill Syrup**
BPI has run a 2L Multi-Gen stirred fermenter using dextrins converted to glucose at 200 to 240 g/L feed concentration. We ran the system in the Consecutive Batch Mode at 3 cycles per day (8 hours per batch) over a period of 3 months (206 cycles) and determined excellent results with fermentations going to near completion in as little as 5 hours. Over this period, we determined a 'minimal nutrient' make-up of for the glucose feed stock.

**Continuous Cascade Mode- Corn Wet Mill Syrup**
We have also demonstrated BPI's High Speed/ Low Effluent system in the continuous mode using a 1 liter Multigen reactor. Batches of 5 gallons feed were made-up to run the 3 experiments described in Table 1. Reactor effluent was captured in a closed vessel which was held at 65C. The effluent was then transferred to the feed tanks to simulate stage 2, and once again to simulate stage 3, as shown below in Process Diagram 1. The volume of dead cells in the bottom of the effluent pot were measured after each stage, and the dry wt. estimated. We used a proprietary nutrient formulation consisting of inorganic N, P and K supplemented with micro-nutrients/vitamins and CSL.

**Process Diagram 1: Continuous Cascade Fermentation**

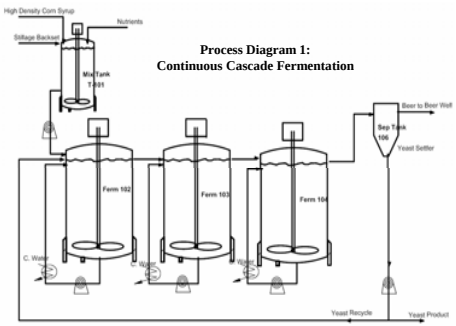


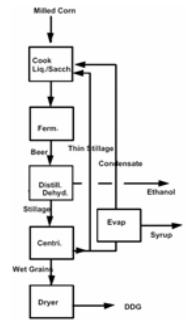

**Table 1: Wet Milling Syrup Fermentation Results**

| STAGE | Time (hour) | Sugars (g / L) | Ethanol (g / L) | Ethanol (g / L / hour) | Cell Mass (g / L effluent) |
|---|---|---|---|---|---|
| THREE (3) HOUR STAGES | | | | | |
| Feed | 0 | 220 | - | - | - |
| Stage 1 | 3 | 57 | 73 | 24.3 | - |
| Stage 2 | 6 | 4 | 105 | 10.7 | - |
| TWO (2) HOUR STAGES | | | | | |
| Feed | 0 | 230 | - | - | - |
| Stage 1 | 2 | 98 | 63.6 | 31.8 | 1.1 |
| Stage 2 | 4 | 30 | 95 | 15.7 | 0.4 |
| Stage 3 | 6 | 0.2 | 110 | 7.5 | 0.4 |
| ONE (1.3) HOUR STAGES | | | | | |
| Feed | 0 | 230 | - | - | - |
| Stage 1 | 2 | 98 | 63.6 | 31.8 | 1.1 |
| Stage 2 | 4 | 30 | 95 | 15.7 | 0.4 |
| Stage 3 | 6 | 0.2 | 110 | 7.5 | 0.4 |

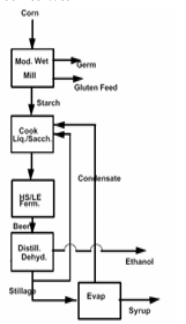
## HS/LE Dry Mill Ethanol Application

The HS/LE Fermentation Requires a 'Clear' syrup feed. The dry mill process (Process dia. 2) does not produce a clear syrup but there are a variety of process configurations, such as modified wet milling (Process dia. 3) and dry fractionation (Process dia. 4) that would allow utilization of HS/LE fermentation while producing higher value co-products.

**Process Diagram 2: Conventional Dry Mill**




**Process Diagram 3: Modified Wet Mill**




**Process Diagram 2: BPI High Value Corn Processing**

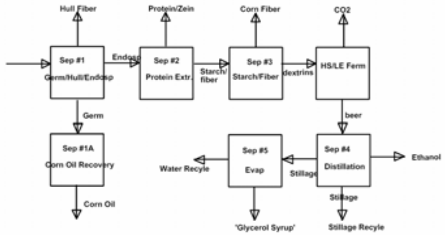



**Table 2: Revenue Dry Mill Processing per Bushel of Corn**

| Processing Method | Co-Products | Production | Price | Revenue |
|---|---|---|---|---|
| 1) Conventional Dry Mill | Ethanol | 2.7 gal | $2.20 gal | $5.94 |
| | DDGS | 17 lbs | $80 ton | $0.68 |
| Total Revenue 'Dry Mill' Per Bushel of Corn | | | | **$6.62** |
| 2) Modified Wet Mill HS/LE | Ethanol | 2.75 gal | $2.20 gal | $6.05 |
| | Gluten Meal / Feed | 15 lbs | $140 ton | $1.05 |
| | Germ / Oil | 1.6 lbs | $.33 lb | $0.53 |
| | Yeast | .17 lbs | $.35 lb | $0.06 |
| Total Revenue 'Modified Wet Mill' Per Bushel of Corn | | | | **$7.69** |
| 3) BPI High Value Corn Processing HS/LE | Ethanol | 2.75 gal | $2.20 gal | $6.05 |
| | Corn Bran | 3.3 lbs | $.12 lb | $0.90 |
| | Corn Fiber | 2.2 lbs | $0.05 | $0.11 |
| | Zein Protein | .8 lbs | $5.00 lb | $4.00 |
| | Hydrophilic Protein | 1.9 lbs | $.80 lb | $1.52 |
| | Germ / Oil | 1.6 lbs | $.33 lb | $0.53 |
| | Yeast | .17 lbs | $.35 lb | $0.06 |
| Total Revenue 'BPI HV Corn Processing' Per Bushel of Corn | | | | **$13.17** |

## Pilot Scale Demonstration of HS/LE

A set of stainless steel vessels were fabricated for pilot (semi-industrial scale) demonstration of the HS/LE process at the Xethanol Bio-Fuels dry mill facility in Iowa. Fabrication of piping, reactor vessel, and controls were completed and 20 trials completed in April 2006. The trials showed:

**1) The pilot HS/LE process closely matched lab scale results when scaled up by 1,000 - 2,000 X.**
**2) In industrial, non-sterile, environments the HS/LE process performed well.**
**3) HS/LE sets were complete in 10 – 16 hours (design rate of 12 hrs).**
**4) The HS/LE reactor can be cooled on an industrial scale via external HX's.**
**5) The HS/LE process in 'Consecutive Batch' mode is easily scaled up on clear syrups.**

**Process Diagram 5: Pilot Scale Design**

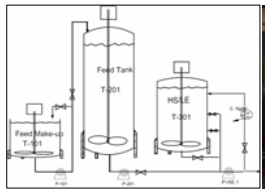



**Figure 2: Actual Pilot Plant**

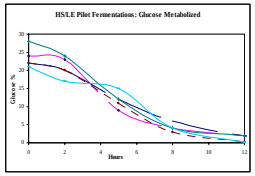
**Results of Pilot Scale Demo**
Twenty consecutive trials of the HS/LE fermentation process were conducted in the Xethanol Biofuels plant, April 7 – 20, 2006. The results of the fermentations and the time to completion are shown as figure 3. The pilot scale test proved to be quite successful and the following conclusions were made:

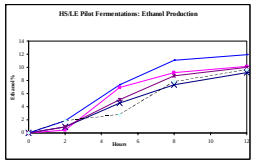
1) The HS/LE process can be scaled up 2,000 – 4,000 X from laboratory scale to industrial scale ( 2 L to 4,500 L) successfully.

2) The HS/LE process can be utilized in non-sterile, industrial environments with out requiring a CIP between batches.

3) The HS/LE fermentations demonstrated a nearly complete utilization of glucose in 10 to 16 hours yielding beer ethanol concentrations of 8 – 11% over 20 consecutive trials.

**Figure 3: Results**




## Summary

**The HS/LE process allows complete fermentation of 18 to 24% glucose to ethanol in 4 to 8 hours, in either a continuous cascade or consecutive batch mode over extended periods of several to many months. In the Consecutive Batch (CB) mode of operation, the fermenter is available for immediate re-set after completion of fermentation and a settling period during which completed beer is decanted. This allows 3 or even 4 batches of 10 to 12% ethanol to be produced per reactor per day. In the Cascade mode, residence times of 6 hours over 3 reactors gave over 99% sugar utilization of a 220 g/L glucose feed.**

**The HS/LE process allows a high degree of backset, can be applied directly to wet mill corn syrups, and several processes are under development at BPI to apply the process to Dry Mill Ethanol production. These Pilot scale demonstrations of the HS/LE process found no barriers to immediate industrial application.**

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