Pilot Scale Demo of <u>HIGH SPEED / LOW EFFLUENT</u> 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 (>100 L and commercial scale (4,500+). 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.

<u>BPSC-15: Productivity and Cell Viability</u>

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:

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

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

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 $k_{\varepsilon\nu m}$ 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 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 :

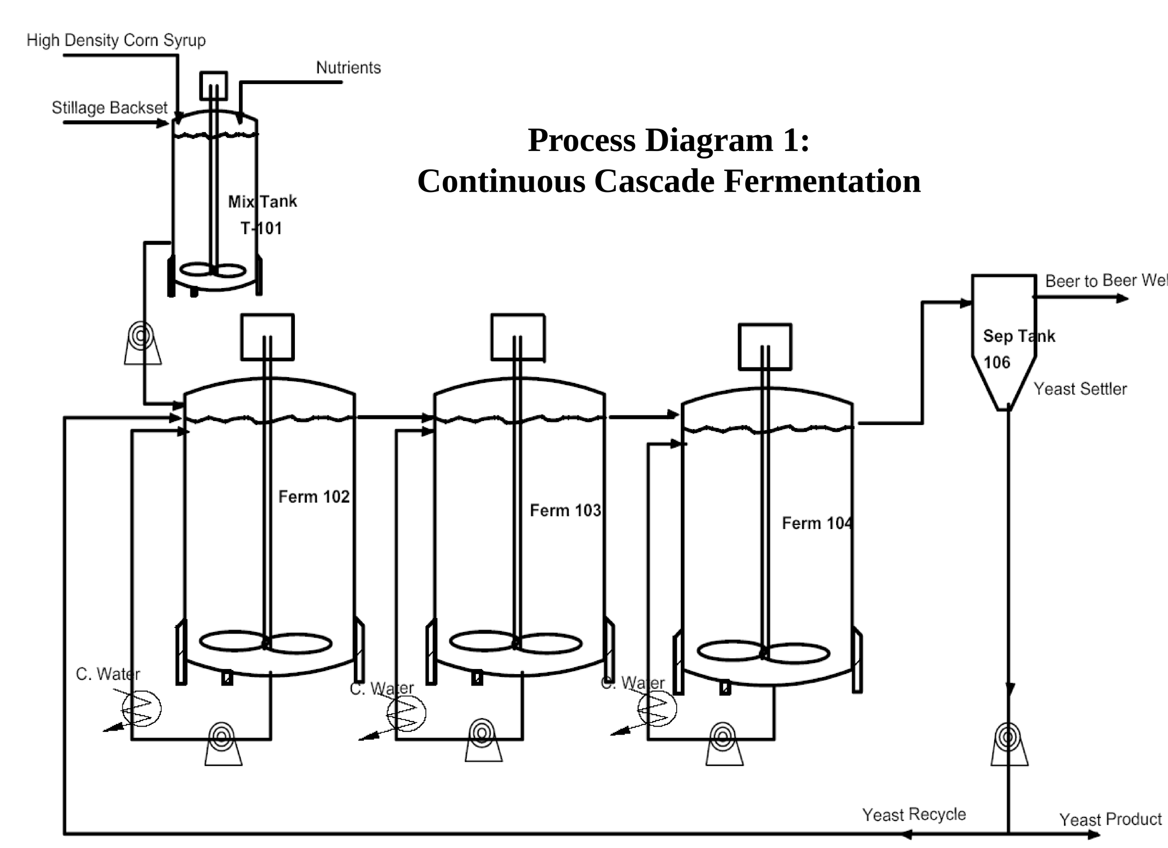
$$X_{ssl} = [\mu/ (\mu + K_d)]$$

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.

<u>HS/LE Wet Mill Ethanol Application</u>

Lab Scale Consecutive Batch- Corn Wet Mill Syrup
BPI has run a 20L 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 4 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

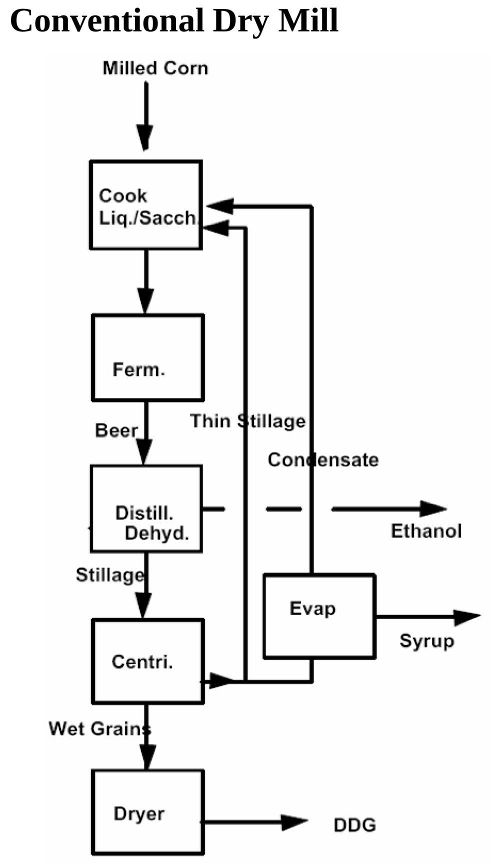
Table 1: Wet Milling Syrup Fermentation Results

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

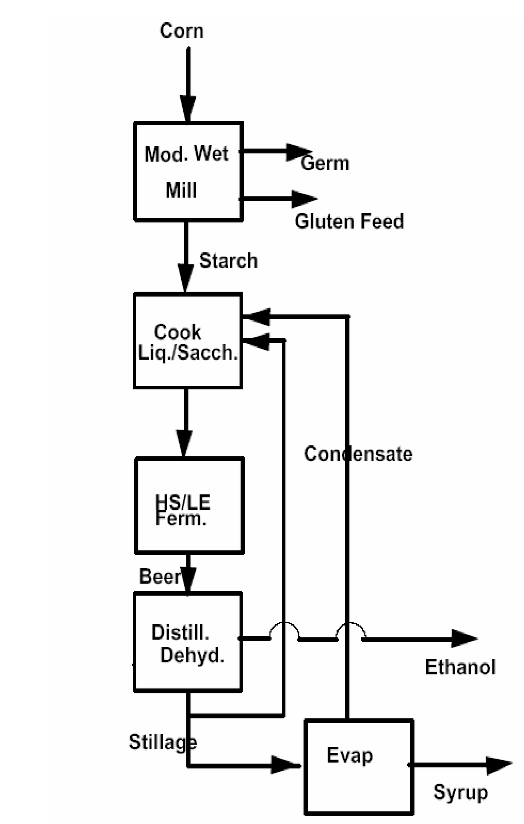
<u>HS/LE Dry Mill Ethanol Application</u>

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 4: BPI High Value Corn Processing

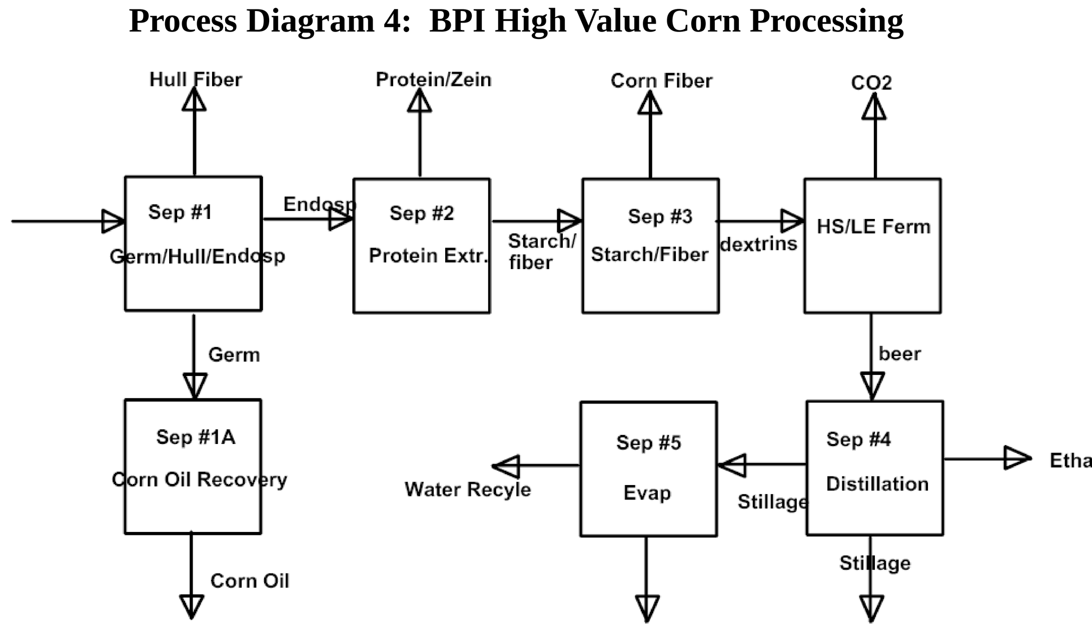


Table 2: Revenue Dry Mill Processing per Bushel of Corn

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**

<u>Demonstration of HS/LE</u>

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

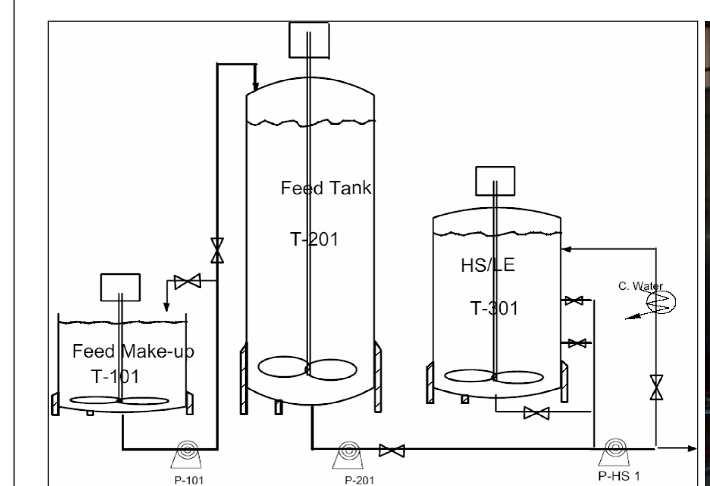


Figure 2: Actual Pilot Plant

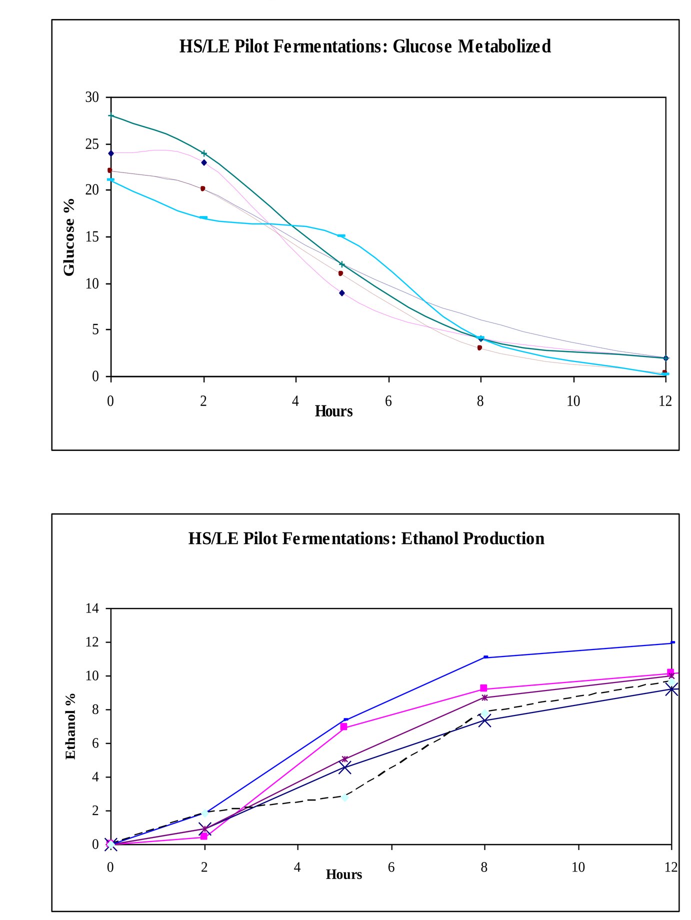
Results of Pilot Scale Demo
Twenty consecutive trials of the HS/LE fermentation process were conducted in the Xethanol Biofuels plant, April 7 – 20, 2006. The results of the fermentations and the time to completion are shown as figure 3. The pilot scale test proved to be quite successful and the following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

PROJECT SPONSORS
1) DOE- Inventions and Innov
2) Xethanol: Industrial Partner
3) Bio-Process Innovation
4) TEMA Centrifuges