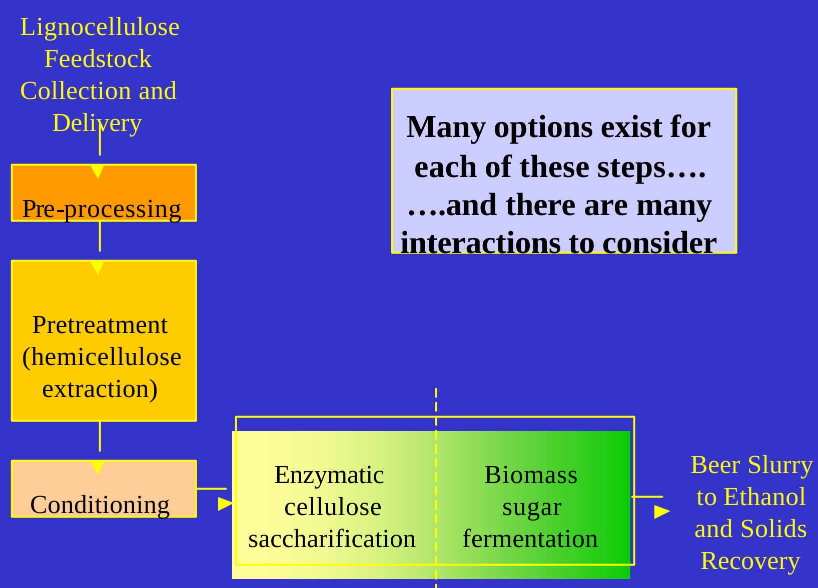
NREL National Renewable Energy Laboratory

Competitive Technology/ Detailed Technical Assessment: **Process Evaluation/Development**

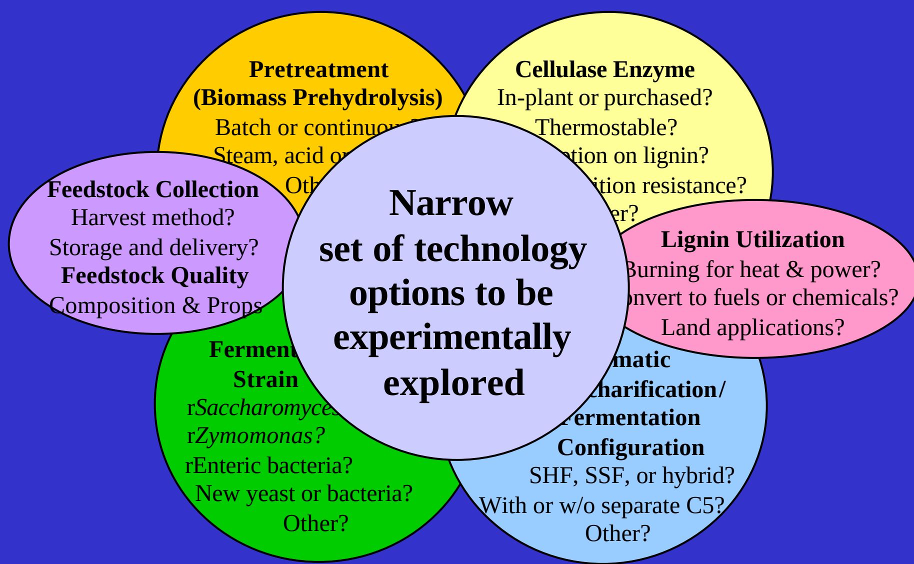
Jim McMillan

Major Steps in an Enzymatic Process



Many options exist for each of these steps….
….and there are many interactions to consider

Process Development Options



Critical Success Factor for Pioneer Plants

✍Accurately estimate cost and performance!*

– Plant cost growth strongly correlated with:
  - Process understanding (integration issues)
  - Project definition (estimate inclusiveness)

– Plant performance strongly correlated with:
  - Number of new steps
  - % of heat and mass balance equations based on data
  - Waste handling difficulties
  - Plant processes primarily solid feedstock

* "Understanding Cost Growth and Performance Shortfalls in Pioneer Process Plants", a study by the Rand Corp. for DOE (1981)

Technology Selection

- ✍Focus on pretreatment and fermentation strains….. Final enzyme characteristics remain unknown
- ✍Apply 2-step screening methodology
  - 1º screen: Reported efficacy
    - Obtained from the literature or personal communications
  - 2º screen: Quantitative performance and readiness
    - Mass balanced performance data?
    - Process models show favorable economic potential?
    - Showstopper regulatory/permitting issues?
    - Ready for pilot testing?
    - Available for third party commercialization?

Part II: Presentations

- ✍Feedstock – John Sheehan
- ✍Pretreatment – Dan Schell
- ✍Enzymes – Jim McMillan
  - ✍Genencor – Bill Dean and Mike Knauf
  - ✍Novozymes – Joel Cherry
- ✍Fermentation strains – Kiran Kadam

Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis
- ✍ **Feedstock**
- Pretreatment
- Enzyme
- Fermentation Microorganism
- Business plan
- High-level Stage 3 plan

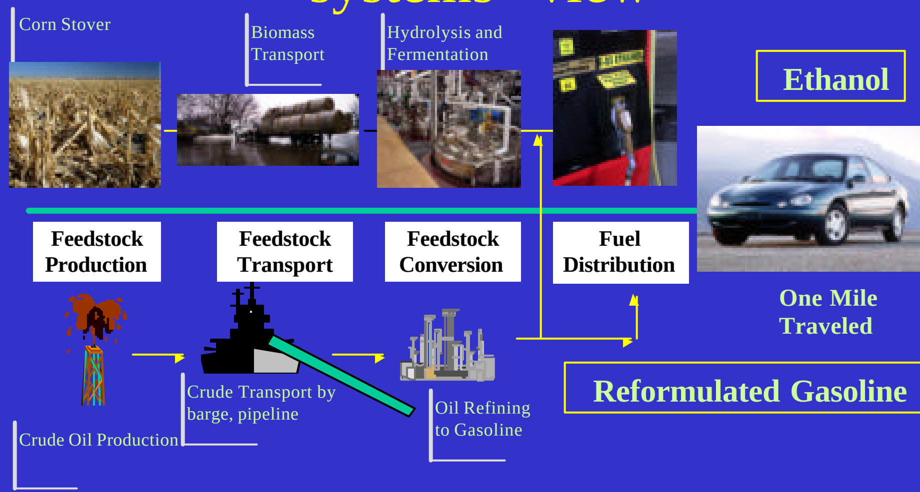
Feedstock

Agricultural residues like corn stover are the cornerstone of DOE's first generation of sugar platform biorefineries.

Critical success factors

- Adequate supply of stover
- Ability to sustainably collect stover
  - Soil health
  - Environmental issues
  - Economic impacts of stover collection and use
- Ability to collect stover cost-effectively
  - Stover costs contribute significantly to ethanol cost

We have used LCA to keep a "systems" view

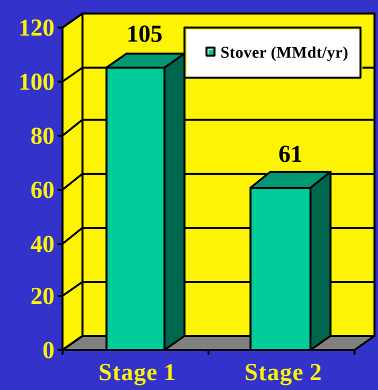


Stage 2 feedstock work involved experts from many organizations within DOE and USDA

- ORNL
  - Collection logistics, resource assessment and macroeconomic impacts
- USDA ARS
  - Soil science research and modeling
- USDA NRCS
  - Soil conservation issues
- USDA Office of Energy
  - Agronomics of bioenergy supply

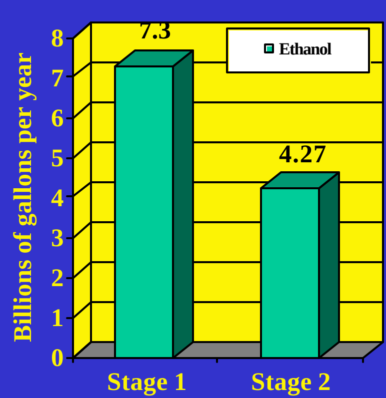
Stage 2 findings
Available supply of stover

- Stage 1 analysis estimated 105 MMdt per year of stover in top 10 corn states
- Stage 2 analysis has further refined that estimate at 61 MMdt
- These are conservative estimates based on no changes in current farm practices



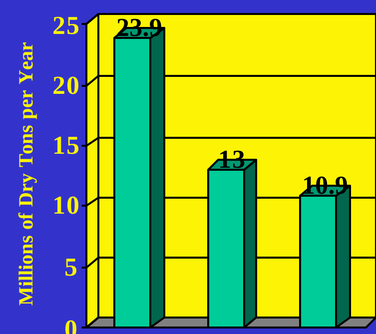
Stage 2 findings
Available supply of stover

- Potential ethanol supply and demand are well matched
  - The available market for stover derived ethanol is at least 3 billion gallons beyond the future supply from corn grain
  - Stage 2 estimates of supply confirm the ability to support this market



Stage 2 findings
More rigor in assessment of sustainable stover supply

- The stage 1 preliminary analysis for Iowa showed 23.9 MM dry tons per year available
- In stage 2, we continued to improve our understanding of sustainable collection
  - Stage 1
  - Stage 2
    - Erosion control limits
    - Soil carbon limits



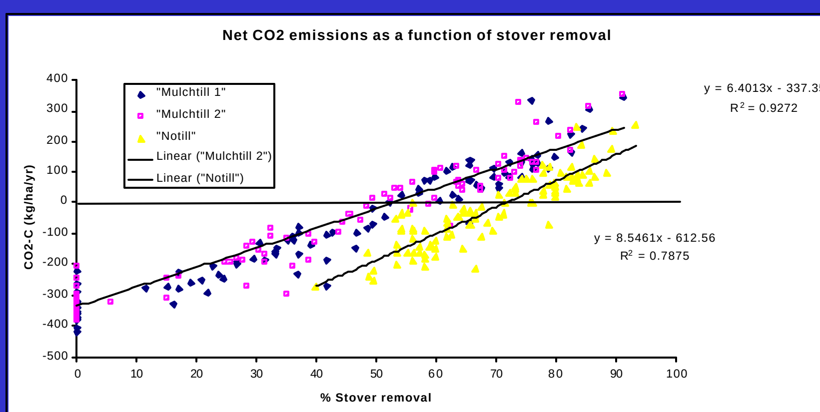
Stage 2 findings
Economic impacts of stover to ethanol in Iowa

- At a selling price of $1.25 per gallon ethanol
  - 15 economically feasible plants, producing almost 1 bgy of Ethanol
  - Create 14,253 annual jobs in industrial (57%), transportation (24%) and ag sectors (19%)
  - Create $2.4 billion in annual Total Product Output in industrial (71%), transportation (12%) and ag sectors (17%)
  - Create $950 million annual Value Added in industrial (62%), transportation (15%) and agricultural sectors (23%)

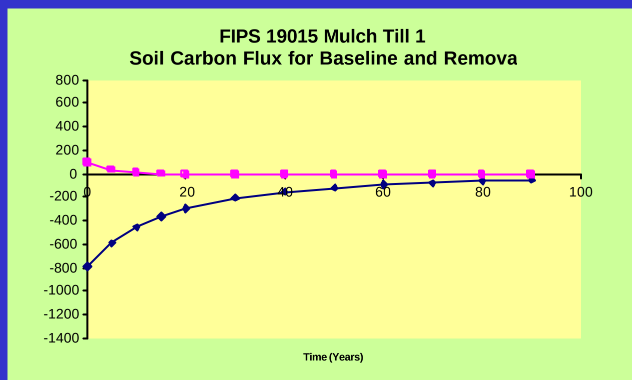
Stage 2 findings
Sustainability

- Four big issues have surfaced in our evaluation of corn stover as a feedstock
  - Land use
  - Soil health
  - Greenhouse gas emissions
  - Water quality
- Soil health, soil carbon levels and greenhouse gases are inextricably intertwined
- Water quality remains an unknown

Stage 2 findings
Sustainability and soil carbon



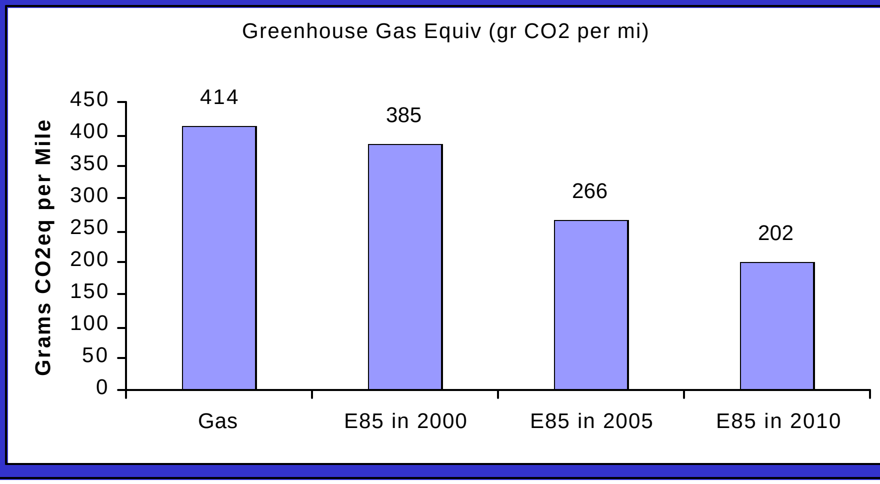
Stage 2 Findings
Sustainability and soil carbon



Stage 2 Findings
Sustainability and soil carbon

- DOE/ORNL sponsored research at USDA:
  – Lignin as a soil amendment
  – Field studies on the effects of residue removal

Stage 2 findings
Sustainability and Greenhouse Gas Effects



Stage 2 findings
Technology risks—today's approach to stover harvest

- Collect stover *after* grain harvest
- Our base case collection steps include:
  - shredding and raking in one operation
  - round baling [580 dry kg (1270 dry lb)]
  - transporting from the field to an intermediate storage facility 8 km (5 miles) away using a bale wagon pulled by a tractor
  - stacking the bales 5 high under a shed using a telescopic handler

Stage 2 findings
Technology risk—today's approach to stover harvest

	Low	Base	High
Yield, ton/ac	1.1	1.5	2.5
$/ton	$31.10	$26.90	$22.20
Density, lb/ft^3	7	9	10
$/ton	$30.90	S26.90	$25.50
Operating hours	50%	100%	150%
$/ton	$29.80	$26.90	$25.80
Combinations*, $/ton	$41.00	$26.90	$21.00

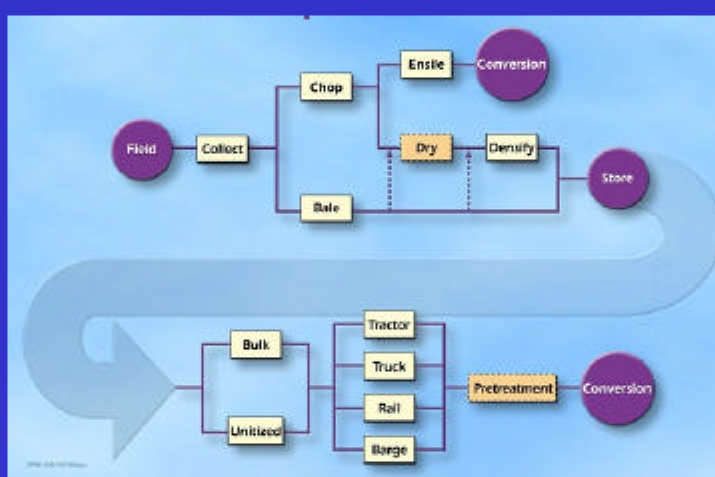
Stage 2—Technology Risks Feedstock Composition

- Corn stover is a complex material
- Economics of ethanol are highly dependent on carbohydrate and lignin content
- Rapid analysis techniques developed at NREL are leading to a more robust understanding of composition and how it will vary
- Groundbreaking analysis techniques not only help to quantify the technical issues in the development stages for a stover-based refinery, but offer vital real-time tools for use in the ethanol plant and in the field

Stage 2 findings
Technology risks—today's approach to stover harvest

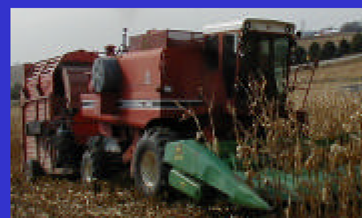
- Timing is tricky—constrained by grain harvest, stover moisture content and weather
- Inefficient
- Leads to poor quality feedstock
- In Stage 1 we had identified these problems
- In Stage 2, we focused on benchmarking the existing collection strategy and identifying improvements

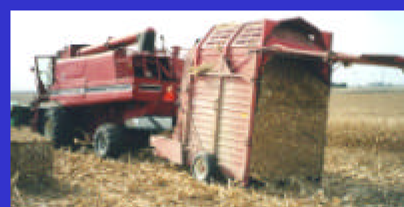
Stage 2 findings
Improving collection technology



Stage 2 Findings
Improving collection technology

- International Harvester 1460 Axial Flow Combine with Row Bean Head
  - Collects the Whole Plant
  - Harvests and Separates Grain From Stover
- Stover Conveyed to Hesston Stakhand 10
  - Density of Stover Increased
  - Dirt Free Collection





Feedstocks—what have we learned in stage 2?

- As stage 2 comes to an end, we can conclude that:
  - Environmentally sustainable collection of stover is possible
  - There is a sufficient supply of stover to meet our projected market opportunities
  - Soil sustainability is not a showstopper
  - BUT, soil sustainability and its impact on stover cost and climate change are still poorly understood
  - There are opportunities to reduce the delivered cost of stover collection

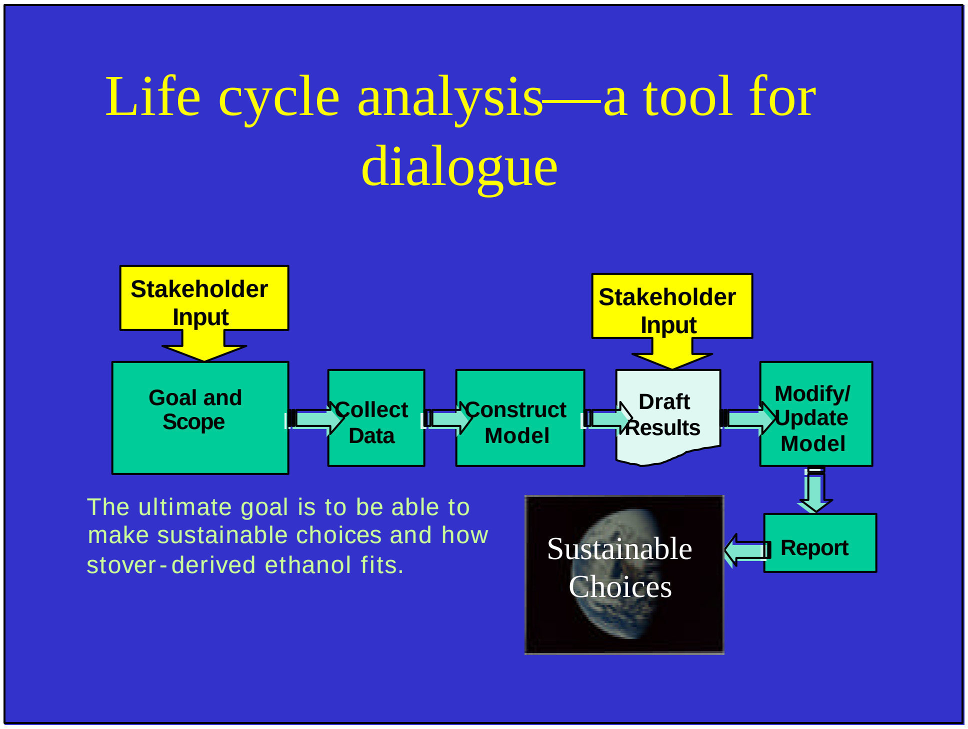
Feedstocks—what do we need to do in stage 3?

- Improve our understanding of soil sustainability
- Reassess the size of stover as a resource in light of improved information on soil sustainability
- Develop more efficient collection strategies that systemically reduce cost and risk
- Use life cycle analysis as a tool for dialogue about the benefits and risks of a stover-based biorefinery as a sustainable option for society

Talking about sustainability

- "Systems oriented"
- "Expanding Resources"
- "Quality of Life"
- "Earth"
- "Ethic"

- Life Cycle
- Renewable Resources
- Economics
- Environment
- Dialogue


Life cycle analysis—a tool for dialogue
Stakeholder Input
Goal and Scope
Collect Data
Construct Model
Stakeholder Input
Draft Results
Modify/ Update Model
Report
Sustainable Choices
The ultimate goal is to be able to make sustainable choices and how stover-derived ethanol fits.

National Renewable Energy Laboratory

Competitive Technology/ Detailed Technical Assessment: **Pretreatment**

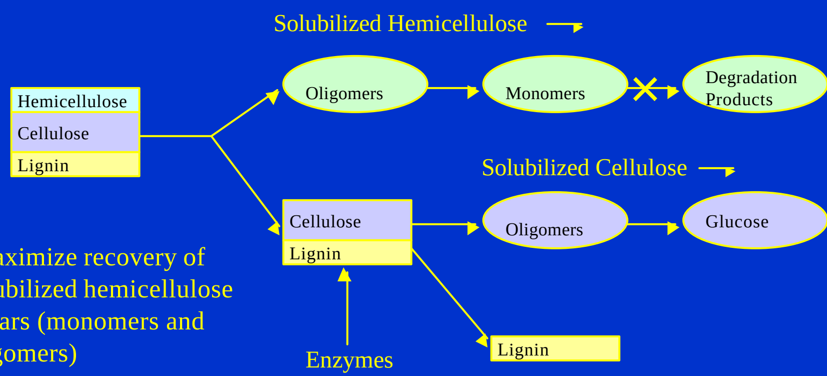
Dan Schell

Presentation Outline

- Background
- Technology selection
  - Selection process
  - Information gathering process
  - Results
  - Recommendations
- Pretreatment technology status
  - Investigating technical feasibility
  - NREL capabilities and recent results
- Findings

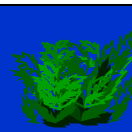
Goals of Biomass Pretreatment

Example for an Acid-Catalyzed Process



- Maximize recovery of solubilized hemicellulose sugars (monomers and oligomers)
- Produce solid fraction that can be efficiently converted to glucose by cellulase enzymes

Pretreatment Challenges



Challenging Reaction Chemistry and Heat and Mass Transfer Conditions

- Many options with poorly understood chemistry
- Heterogeneous solid feedstock that is difficult to chemically analyze
- Broad particulate distribution
- Achieving multiple objectives
  - Maximizing hemicellulose sugar yield
  - Maximizing cellulose conversion

Pretreatment Challenges

Challenging Process Conditions

- High temperature and highly corrosive environment (some options)
- High solids loading
- Understanding post-processing requirements to recover oligomeric sugars
- Understanding downstream consequences (i.e., effects on integrated performance, solid precipitates, etc.)
- Understanding environmental and safety issues

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Pretreatment Selection

Aggressive Project Schedule

Technology must already be undergoing performance testing/validation at the pilot scale

Goal for Selection Effort

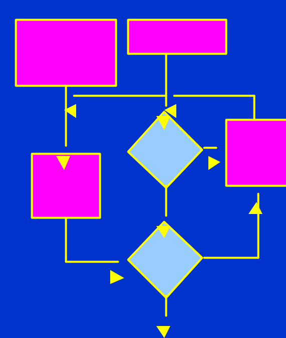
- Objective: To select from the many varieties the best technologies for process integration efforts in Stage 3.
  - Select a pretreatment now to begin stage 3 work
  - Review all technologies and make final selection by late FY02

Variety of Biomass Pretreatments

Pretreatment Category	Pretreatment Types
Base-Catalyzed	AFEX/FIBEX
	Alkaline-Peracetic
	Alkaline-Peroxide
	Alkaline-Solvent
	Ammonia
	Lime
	Sodium Hydroxide
Non-Catalyzed	Autohydrolysis
	Comminution
	Hot Water
	Hot Water-pH Neutral
Acid-Catalyzed	Hydrochloric
	Nitric
	Peracetic
	Phosphoric
	Sulfur Dioxide
	Sulfuric
Solvent-Based	Organosolv
	Solvents
Chemical-Based	Peroxide
	Wet Oxidation
	Supercritical Carbon Dioxide
Others	Biological
	Radiation

Selection Process

- Applied two-tiered screening process to reduce number of pretreatment options
  - First screen (technical performance)
  - Second screen: data quality and technology readiness/availability
- Biofuel Program's Advanced Pretreatment Task is tracking all pretreatments



Criteria for First Screen

Is the pretreatment effective? Does it meet minimum performance criteria?

1. hemicellulose sugar yield (total sugars) ? 75%

   AND

   enzymatic cellulose conversion ? 80%

   OR

2. total sugar yields equivalent to 1.

Criteria for Second Screen

Is the pretreatment sufficiently developed to consider it for Stage 3?

- Data quality criteria:
  - Performance data is required that is supported by carbon & mass balances
- Readiness criteria:
  - Pilot scale work reported and facilities available by June, 2002
  - Small amounts of pretreated material available for bench-scale testing by Dec. 2001
  - Sufficient (drum-scale) pretreated material available to meet process development needs by June 2002

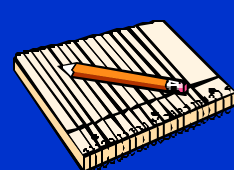
Approach to Information Gathering

- Comprehensive literature survey of pretreatment technologies
  - Applied tiered screening to identify top candidates
  - Information is available in a Microsoft Access™ database
- Obtained information from pretreatment researchers and technology developers (e.g., Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI))
  - Followed suggestions of gate 2 reviewers to engage pretreatment community
  - Presented at two CAFI meetings, sent questionnaire to the pretreatment community in September 2001, followed up with phone and emails contacts

Questionnaire

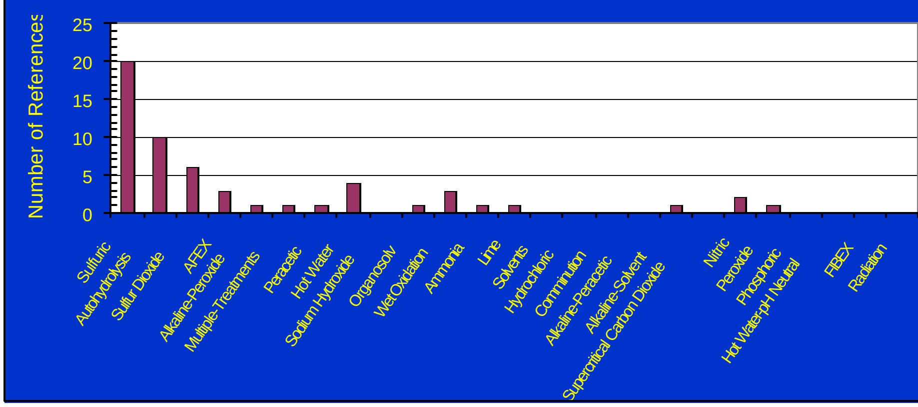
Information Requested from Technology Developers

- Technical criteria-experimental data
  - Hemicellulose (Xylose) yield data?
  - Cellulose digestibility?
  - Ethanol yield?
  - Type of feedstock?
- Quality criteria
  - Carbon/mass balance data available?
- Readiness criteria
  - Economic analysis available?
  - Supply small quantities by Dec. 2001?
  - Supply large quantities by June 2002?
  - Where could pretreatment be performed at the pilot scale?



Highlights from Literature Survey

- ~ 600 unique citations found from literature search and discussions with technology developers
  - New reference continue to be added to database
- 54 papers provided hemicellulosic sugar yield and cellulose conversion data



Passing to the Second Screen

- Pretreatments from literature meeting first screen criteria
- All pretreatments being actively developed
  - Provide feedback to technology developers

Pretreatments Undergoing Second Screen

Pretreatment Category	Pretreatments Undergoing Second Screen	Technology Developers and Providers
Base-Catalyzed	AFEX/FIBEX	Bruce Dale/Michigan State, MBI
	Ammonia*	Y.Y.Lee/Auburn
	Lime	Mark Holtzapple/Texas A&M
Non-Catalyzed	Hot Water (batch)	Charlie Wyman/Dartmouth, Mike Antal/Hawaii Natural Energy Institute
	Hot Water (percolation)	Mike Antal, Charlie Wyman
	Hot Water-pH Neutral	Michael Ladisch/Purdue
Acid-Catalyzed	Nitric Acid	Lee MacLean/HFTA
	Sulfur Dioxide	Jack Saddler/UBC, Esteban Chornet/ University of Sherbrooke
	Sulfuric Acid	BC International, Iogen, NREL, TVA, Charlie Wyman
	Sulfuric Acid (hot wash process)	NREL
Solvent-Based	Organosolv (Clean Fractionation)	NREL
Chemical-Based	Peroxide	
	Wet Oxidation	Ed Lehrburger/Pure Vision

* Pretreatments passing first screen criteria based on literature

Results of Selection Process

- Sulfuric acid was the only pretreatment to meet all of the first and second screen criteria
- Other pretreatments did not meet all the criteria
  - Data supported by mass/carbon balances
  - Pilot scale testing

Recommendations

- Sulfuric acid selected for initial stage 3 process development work
- Review all technologies in late FY02, in collaboration with an industrial partner
  - Generic Aspen-based process models for acid, alkaline, and non-catalyzed processes are being developed in collaboration with technology developers
  - Technology developers to supply data

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- Findings

Pretreatment Status

Investigating Technical Feasibility

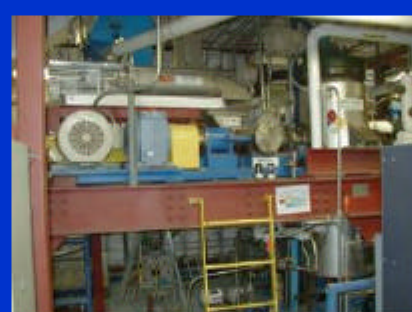
- Assess state of dilute sulfuric acid technology
  - Exploratory pretreatment of corn stover at pilot scale
    - Supply enzyme developers with pretreated feedstock
    - Bench mark current capabilities
    - Identify showstoppers to process development

⇨ Essential to have pretreated materials to move forward with fermentation strain screening and early integration work.

Sunds Reactor Capabilities

Parameter	Range
Reactor Solids Concentration	19-28% (most at 20%)
Residence Times	3-12 min*
Acid Concentration	0%-5% (w/w)
Temperature	140°-195°C

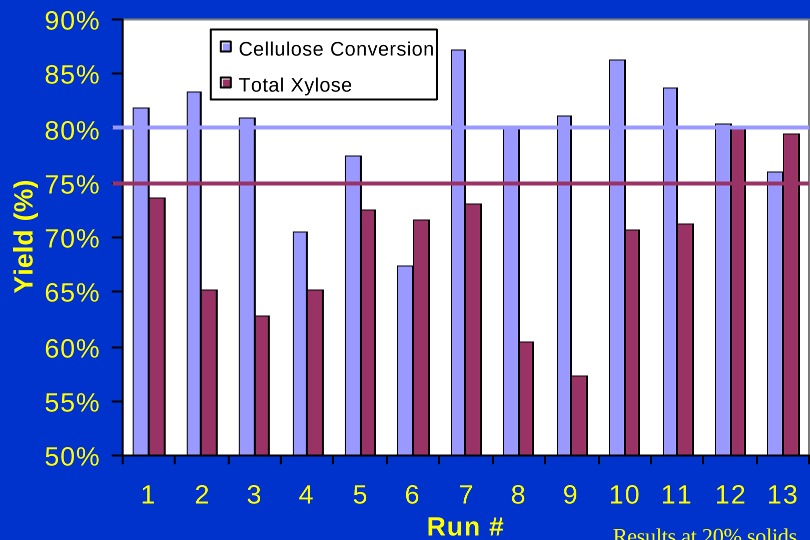
* Flow-through operation permits a residence time of ~ 0.5-2.0 min (estimated)



⇨ Good capability for generating pretreatment and other process residues for Biofuels Program and external client needs

Recent Performance Results

Dilute Sulfuric Acid Pretreatment



Results at 20% solids

Improved Capability

Sunds Reactor

Parameter	Can Achieve Now	With Further Optimization
Solids Concentration (%)	25-28	? 30
Total Xylose Yield (%)	80	? 85
Cellulose Conversion (%)	80	? 90

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Findings
Technology Assessment

- Dilute sulfuric acid ready for Stage 3
  - Insufficient data and/or readiness for other approaches
- Other technologies will be re-assessed if data becomes available by 8/02
  - A single pretreatment will be recommended to carry forward into FY03 work

Findings

Capabilities Assessment

- Demonstrated 80% total xylose yield and >80% cellulose conversion at 20% solids concentration
  - Demonstrates technical feasibility
  - 28% solids concentrations has been achieved

? Anticipate ability to achieve critical success factors for pretreatment

? Stage 3 work needed to verify this

INTERMISSION

10 minute break

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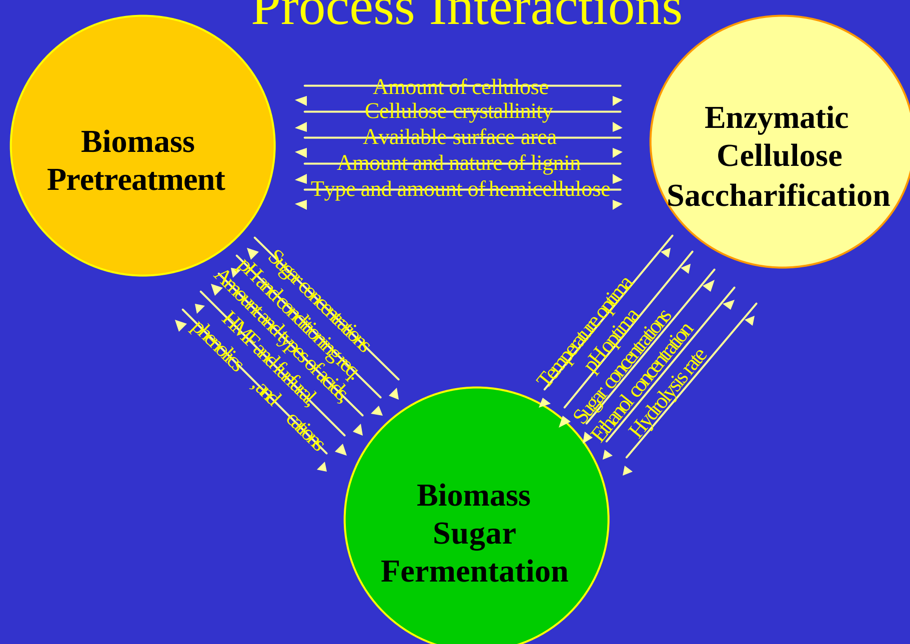
Competitive Technology/ Detailed Technical Assessment: **Enzymes**

Jim McMillan

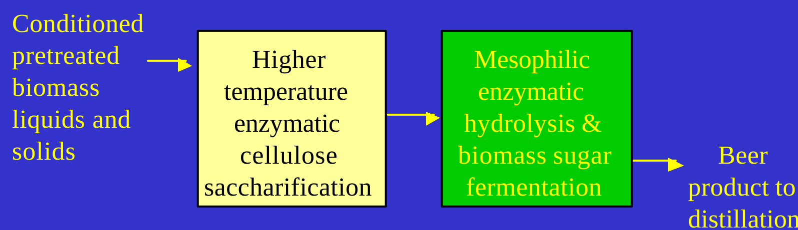
Operated for the U.S. Department of Energy by Midwest Research Institute • Battelle • Bechtel

Process Interactions



Outlook Favors Hybrid Configuration

- Anticipate using a hybrid hydrolysis and fermentation (HHF) process configuration that begins like SHF and ends like SSF.



Hybrid Hydrolysis and Fermentation (HHF)

? *Process economics will determine the most economic route. Difficult to assess before technology selection completed.*

Enzymes — Current Status

- Next generation enzymes under development; lower cost cellulases anticipated in 2003-2004

- We will hear brief reports on the status and outlook from the enzyme developers
  - Genencor – Bill Dean and Mike Knauf
  - Novozymes – Joel Cherry

Genencor Report

Bill Dean and Mike Knauf

Novozymes Report

Joel Cherry

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- Life Cycle Analysis
- Feedstock
- Pretreatment
- Enzyme
- ✍ **Fermentation Microorganism**
- Business plan
- High-level Stage 3 plan

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Competitive Technology/ Detailed Technical Assessment: **Fermentation Strain Selection**

Kiran Kadam

January 30, 2002

Operated for the U.S. Department of Energy by Midwest Research Institute • Battelle • Bechtel

Presentation Outline

- Background
  - Importance of effective ethanologen
  - Fermentation challenges/issues
- Strain selection
  - Methodology/Screening criteria
  - Screening results
- Recommendations for Stage 3
- Process implications
- Technical showstoppers
- Outlook for Stage 3

Background

- Economical bioconversion of corn stover requires the following:
  - a well-pretreated substrate
  - an efficient cellulase system
  - an effective ethanologen (ethanol producing microorganism)
- Potential ethanologen
  - Important process technology component
  - Aggressive project schedule
    - Project is not developing new strains
    - Existing strains whose potential has been demonstrated

Fermentation Challenges/Issues

- Feedstock cost: key economic factor
  - Corn stover: 37–40% six-carbon sugars, 23–25% five-carbon sugars ? need cofermenting strains
- Desired strain characteristics
  - Efficient fermentation of both C6 and C5 sugars *(cost)*
  - Hydrolyzate tolerance *(industrially relevant conditions)*
  - Nutrient requirements *(cost)*
  - Thermotolerance *(compatibility w/2nd generation thermostable enzymes)*
  - Stability under extended operation *(industrially relevant conditions)*
- Environmental permitting
  - More complicated for cofermenting recombinant strains
  - Corn-to-ethanol technology uses wild type yeasts

Fermentation Strain Selection Objective and Approach

- Objective:
  Review available ethanologenic strains and recommend 2-4 strains for Stage 3 studies with corn stover hydrolyzate
- Approach:
  - Survey the literature on fermentative strains
  - Evaluate and compare performance based on literature data

Survey of Reported Strains

- About 150 references scrutinized
- Culling criterion:
  Strains must have ability to ferment glucose and at least one pentose sugar
- 34 fermentative strain options identified
  - A few options based on using two microorganisms

Fermentative Strain Options

	Bacteria		
1	*Escherichia coli* KO11	20	*E. chrysanthemi* and *E. carotovora*
2	*E. coli* SL28, SL40	21	*E. chrysanthemi* B374 pZM15
3	*E. coli* KO11, SL40	22	*Clostridium thermosaccharolyticum*
4	*E. coli* FBR3 (plasmid pLO1297)		**Yeasts**
5	*E. coli* ST09, ST32	23	*Saccharomyces cerevisiae* 1400 (pLNH32)
6	*Klebsiella oxytoca* P2	24	*S. cerevisiae* 1400 (pLNH33)
7	*K. oxytoca* M5A1 (pLOI555)	25	*S. cerevisiae* 1400 424A(LNH-ST)
8	*K. oxytoca* SZ2(pCPP2006) and SZ6(pCPP2006)	26	*S. cerevisiae* YHM4 and YHM7
9	*K. planticola* ATCC 33531 pZM15	27	rDNA *S. cerevisiae*
10	*Zymomonas mobilis* 39676 (pZB206)	28	*S. cerevisiae* 424 and T1 w/ pLNH32
11	*Z. mobilis* ZM4 (pZB5)	29	*S. cerevisiae* 424A(LNH-ST)
12	*Z. mobilis* 39676 (pZB4L)	30	rDNA *S. cerevisiae*
13	*Z. mobilis* C25		
14	*Z. mobilis AX101*		**Fungi**
15	*Bacillus stearothermophilus* pNW-PET	31	*Paecilomyces sp.* NF1
16	*B. stearothermophilus* LLD-15, LLD-16, T13		**Combinations**
17	*Lactobacillus casei* 686 (pRSG02)	32	*S. cerevisiae* (ATCC 60868) and *P. stipitis* Y-7124
18	*Bacteroides polypragmatus* Type strain GP4	33	*Z. mobilis* and *C. saccharolyticum*
19	*Erwinia chrysanthemi* EC 16 pLOI 555	34	*S. cerevisiae* and *C. acetobutylicum*

Methodology

- Methodology similar to that used in pretreatment selection
- Primary screen with a broad set of criteria
  - Basic efficacy screen
- Secondary screen with quantitative criteria
  - Impact on MESP
- Recommend strains for Stage 3
  - Select top ranking strains
  - Availability for licensing by a third party

Primary Screen

- Minimum performance criteria
  - ? 80% ethanol yield on sugars
  - ? 4% (w/v) ethanol concentration
- Identify other compelling traits
  - Thermotolerance
  - Secretion of endoglucanases/hydrolytic enzymes
  - Ability to metabolize cellobiose
  - Demonstration at pilot scale

Strains Passing Primary Screen

Strain	Ownership
E. coli KO11	U. of Florida/BCI
E. coli SL28, SL40	U. of Florida/BCI
E. coli FBR3 (plasmid pLO1297)	USDA/ U. of Florida/BCI
K. oxytoca P2	U. of Florida/BCI
K. oxytoca SZ2/6(pCPP2006)	U. of Florida/BCI
E. chrysanthemi EC 16 pLOI 555	U. of Florida/BCI
Z. mobilis 39676 (pZB4L)	NREL
Z. mobilis AX101	NREL
Paecilomyces sp. NF1	NREL
S. cerevisiae 1400 (pLNH32)	Purdue U./Iogen
S. cerevisiae 1400 424A(LNH-ST)	Purdue U./Iogen
S. cerevisiae 424A(LNH-ST)	Purdue U.
S. cerevisiae	U. of Stellenbosch, S. Africa
S. cerevisiae (ATCC 60868) and *P. stipitis* Y-7124	n/a
B. stearothermophilus LLD-16	Imperial College/ Agrol Technologies Ltd., UK
C. thermosaccharolyticum	MIT
Z. mobilis and *C. saccharolyticum*	n/a

Substrate Utilization Range of Strains Passing Primary Screening

	Glucose	Xylose	Other Hexoses	Arabinose	Cellobiose
rDNA *E. coli*	?	?	?	?	
rDNA *K. oxytoca*	?	?	?	?	?
rDNA *E. chrysanthemi*	?	?	?		?
rDNA *Z. mobilis*	?	?		?	
wt *Paecilomyces* sp.	?	?	?	?	
rDNA *S. cerevisiae*	?	?	?		
wt *S. cerevisiae* and wt *P. stipitis*	?	?	?		
wt *B. stearothermophilus*	?	?			?
wt *C. thermosaccharolyticum*	?	?			
wt *Z. mobilis* and wt *C. saccharolyticum*	?	?			

? indicates ability to ferment to ethanol; wt indicates wildtype species

Secondary Screen

- Net change in MESP as screening criterion
- Cost impacts of key strain traits
  - Ethanol production efficiency
  - Thermotolerance
- Traits for which cost impacts not quantified:
  - Nutrient requirements
    - Estimate costs from literature but don't use results to reject strain
  - Ability to secrete endoglucanases
    - Lower enzyme loading?
  - Ability to directly metabolize cellobiose
    - Lower ?-glucosidase requirement?

Impacts on MESP

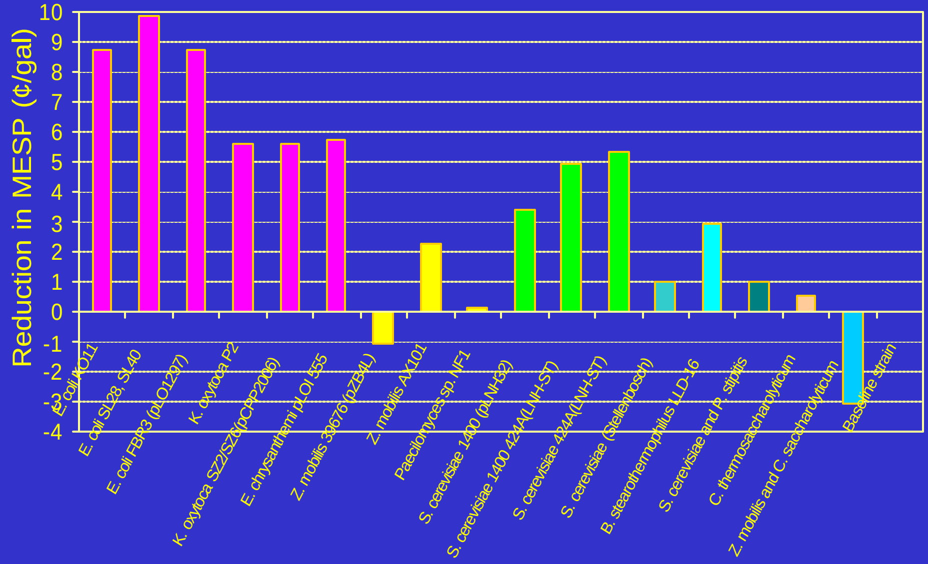
- Ethanol production efficiency

	Baseline conversion, %	¢ per additional 10% converted/unconverted to ethanol
Xylose	85	±4.0
Arabinose	85	±0.6
Galactose/mannose	0	+0.7

- Thermotolerance
  – Benefits of high-temperature SSF not quantified

Contamination loss	Cost impact, ¢/gal ethanol
5% (Baseline)	0.0
1% (Assume for thermophilic strains)	6.0

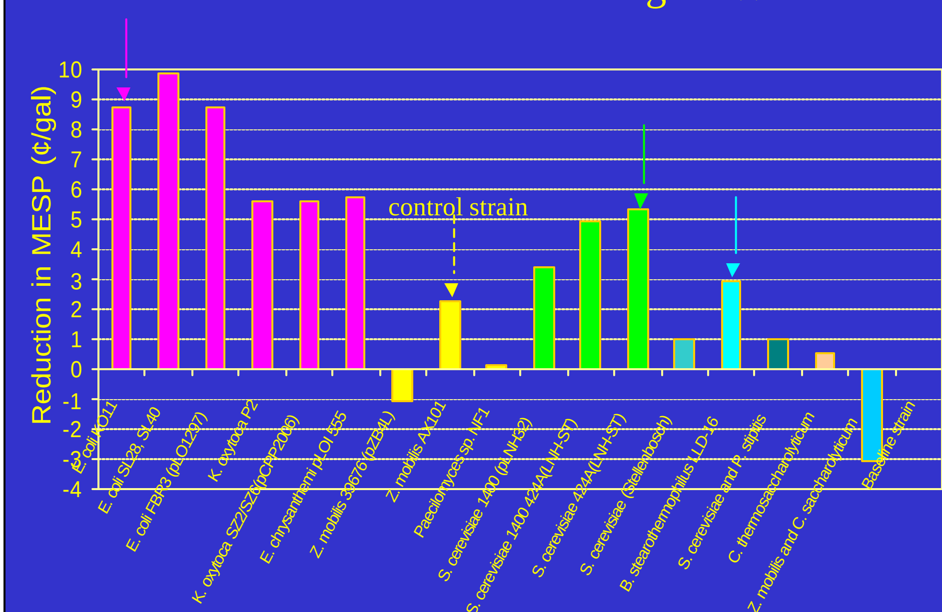
Results of Secondary Screen



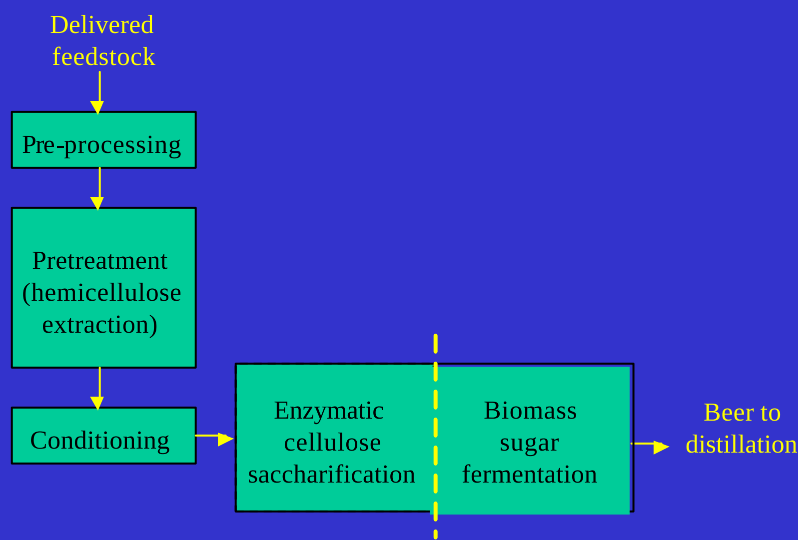
Top Strains Passing Secondary Screen

Strain	Reduction in MESP, ¢/gal ethanol	Other attributes/comments
E. coli KO11, SL40	9.3	Best of Ingram strains.
E. coli FBR3 (pLO1297)	8.8	Based on plasmid developed by U. of Florida.
E. chrysanthemi pLOI 555	5.7	Secretes endoglucanases and metabolizes cellobiose.
K. oxytoca P2	5.6	Secretes endoglucanases and metabolizes cellobiose.
S. cerevisiae 424A(LNH-ST)	5.4	Owned by Purdue U. Easy to license.
S. cerevisiae 1400 424A(LNH-ST)	4.9	Demonstrated at pilot scale. Owned by Iogen.
B. stearothermophilus LLD-16	2.9	Thermotolerant and metabolizes cellobiose. Nonrecombinant strain. Demonstrated at pilot scale.
Z. mobilis AX101	2.3	Can serve as baseline strain.

Strains Recommended for Stage 3 Work



Major Steps in an Enzymatic Process



Process Implications

- pH and thermostability of cellulases being developed not yet known
- Acid or neutral cellulases best depending on strain
- Strain and enzyme characteristics affect process configuration

	pH range	Temperature range, °C	Process Implications
E. coli **KO11**	**6.0–6.8**	**32–37**	**Neutral cellulases; HHF mode**
S. cerevisiae **424A(LNH-ST)**	**5.2**	**38**	**Acid cellulases; HHF mode**
B. stearothermophilus **LLD-16**	**6.5**	**65–70**	**Neutral cellulases; HHF or SSF mode**

Technical Showstoppers

- Fermentation technology appears feasible
  - Meet aggressive conversion/rate goals set in process engineering model
    - Using realistic levels of cellulase enzyme(s)
  - "Robustness" under industrially relevant conditions
    - Demonstrate integrated process performance at a large enough scale
  - Hydrolyzate conditioning costs
    - High nutrient levels (used in reported studies) mitigate hydrolyzate toxicity
  - Nutrient costs: need to be ?3¢/gal ethanol

Legal/Regulatory Compliance

- Patent/IP positions
  - All strain developers/owners open to third party licensing
  - Terms and agreements for such licensing need to be negotiated
- Waste steams, emissions, safety, permitting issues
  - More complicated for GMOs (genetically modified organisms)
  - But issues not insurmountable
  - Related extra costs, if any, need to be identified

Outlook

- Early in Stage 3
  - Assess strain performance using corn stover hydrolyzate
  - Knowledge gap about sugar utilization patterns/rates re: corn stover w/minimal nutrients
  - For promising strains
    - Determine sequence of sugar consumption and rates
    - Characterize requirements for hydrolyzate conditioning
  - For the most promising strain(s)
    - Develop/demonstrate low-cost media
- Later in Stage 3
  - Optimization of HHF
  - Process integration
  - Identify terms for licensing by a third party (i.e., the technology commercializer)

Questions?

Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis
- Feedstock
- Pretreatment
- Enzyme
- Fermentation Microorganism
- **Business plan**
- High-level Stage 3 plan

NREL National Renewable Energy Laboratory

Enzyme Sugar-Ethanol Platform
Business Plan

James D. McMillan

Gate 3 Project Review, Golden, Colorado

January 30, 2002

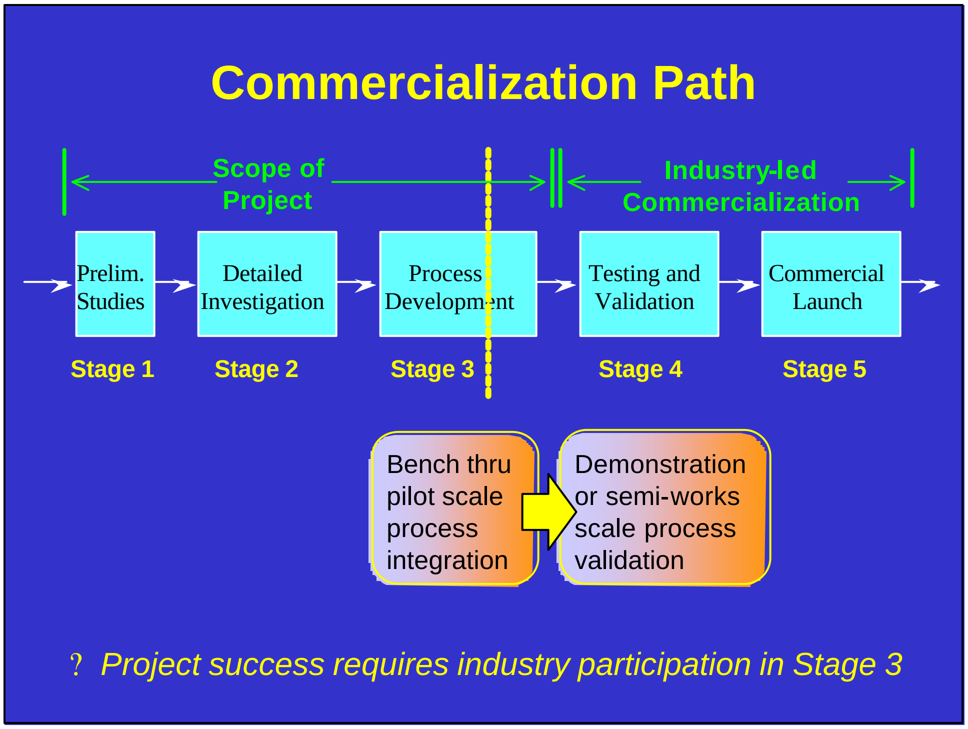
Operated for the U.S. Department of Energy by Midwest Research Institute • Battelle • Bechtel

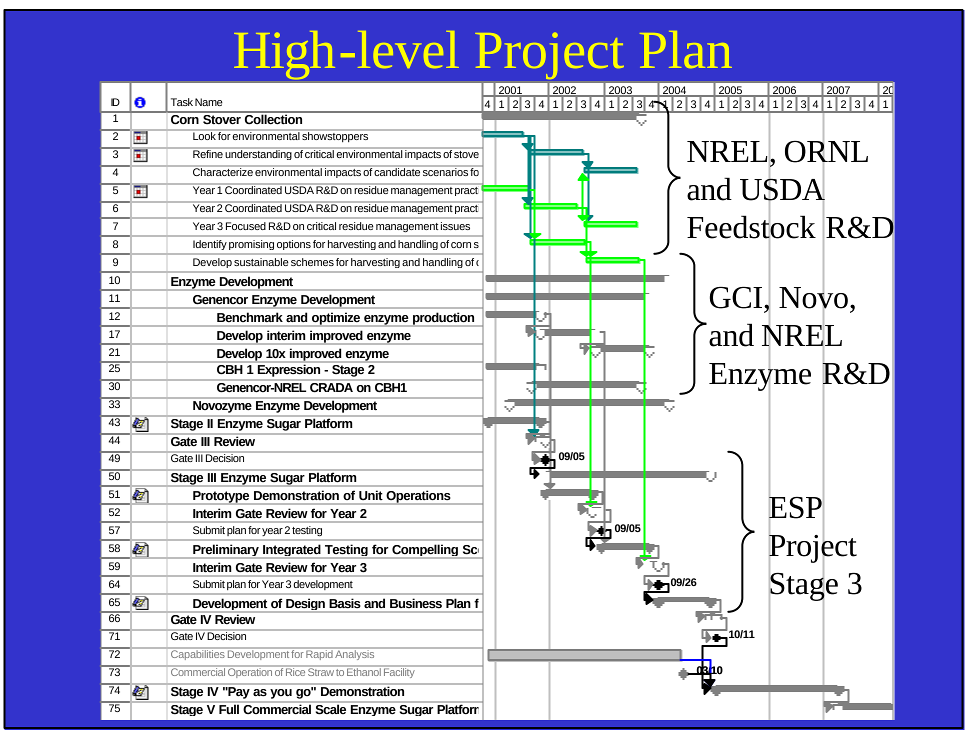
Part III: Presentations

- Business Plan
  - Overall Plan – Jim McMillan
  - Colloquy Results – Jim Hettenhaus (cea)
  - LOI for Stage 4 Demo. Plant – John Ashworth
- Stage 3 Overview – Jim McMillan

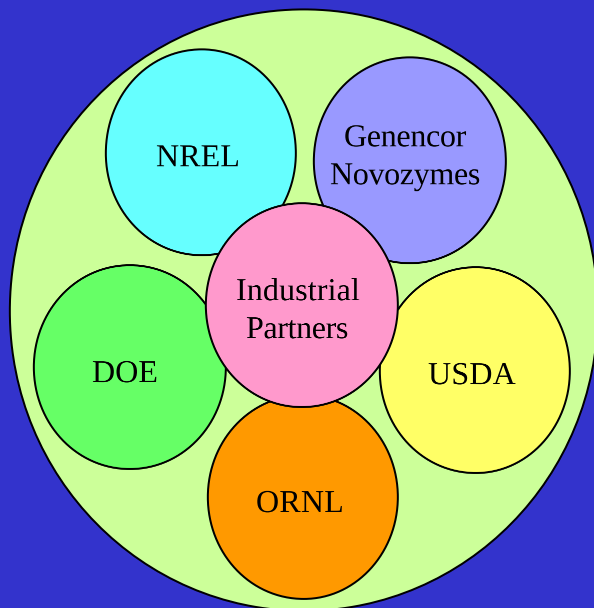
Overall Business Plan Outline

- Commercialization path
- High-level project plan
- Project coordination
- Anticipated timeline


Commercialization Path
Scope of Project
Industry-led Commercialization
Prelim. Studies
Detailed Investigation
Process Development
Testing and Validation
Commercial Launch
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
Bench thru pilot scale process integration
Demonstration or semi-works scale process validation
? Project success requires industry participation in Stage 3


High-level Project Plan
ID
Task Name
2001
2002
2003
2004
2005
2006
2007
1 Corn Stover Collection
2 Look for environmental showstoppers
3 Refine understanding of critical environmental impacts of stove
4 Characterize environmental impacts of candidate scenarios fo
5 Year 1 Coordinated USDA R&D on residue management pract
6 Year 2 Coordinated USDA R&D on residue management pract
7 Year 3 Focused R&D on critical residue management issues
8 Identify promising options for harvesting and handling of corn s
9 Develop sustainable schemes for harvesting and handling of c
10 Enzyme Development
11 Genencor Enzyme Development
12 Benchmark and optimize enzyme production
17 Develop interim improved enzyme
21 Develop 10x improved enzyme
25 CBH 1 Expression - Stage 2
30 Genencor-NREL CRADA on CBH1
33 Novozyme Enzyme Development
43 Stage II Enzyme Sugar Platform
44 Gate III Review
49 Gate III Decision
50 Stage III Enzyme Sugar Platform
51 Prototype Demonstration of Unit Operations
52 Interim Gate Review for Year 2
57 Submit plan for year 2 testing
58 Preliminary Integrated Testing for Compelling Sc
59 Interim Gate Review for Year 3
64 Submit plan for Year 3 development
65 Development of Design Basis and Business Plan f
66 Gate IV Review
71 Gate IV Decision
72 Capabilities Development for Rapid Analysis
73 Commercial Operation of Rice Straw to Ethanol Facility
74 Stage IV "Pay as you go" Demonstration
75 Stage V Full Commercial Scale Enzyme Sugar Platforr
09/05
09/05
09/26
10/11
03/10
NREL, ORNL and USDA Feedstock R&D
GCI, Novo, and NREL Enzyme R&D
ESP Project Stage 3

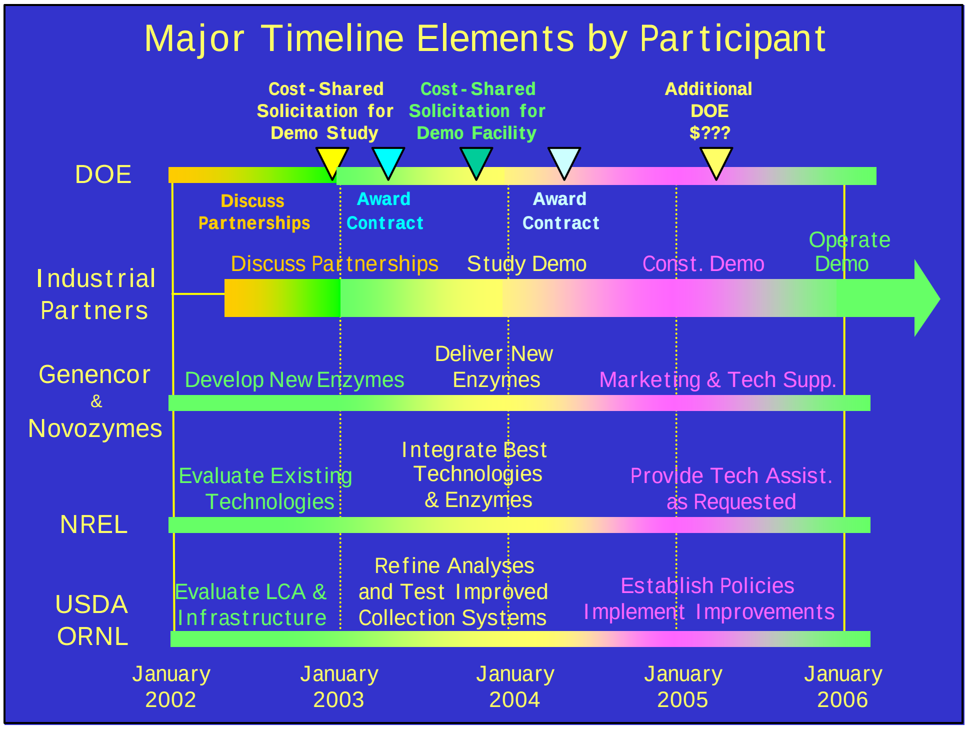
Large Project Team



Project Coordination

- Parallel efforts must succeed on schedule
  - Sustainability studies (LCA, etc.) must show that corn stover is a renewable and abundant feedstock
  - Collection infrastructure and policies must exist and be able to supply corn stover at low cost
  - Enzymes must be available for later Stage 3 process integration and demonstration work
- Stage 3 is a large effort
  - a variety of potential systems must be evaluated

✍ *Coordination is essential for project success!*



Key External Tasks

– Increase rigor of sustainability analysis

- Continue farmer and environmental community outreach
- Create a public forum to discuss collection issues
- Extend efforts to reduce feedstock cost
- Begin working with USDA resource conservation districts and with non-profit groups focused on rural development

– Build support and expertise for policy development

- Increase recognition of project's alignment with Congress's bioenergy & bioproducts R&DD goals
- Facilitate USDA ownership of infrastructure development
- Bring EPA into the picture to address regulatory issues
- Demonstrate GHG reduction role for corn stover to bioenergy & bioproducts

Colloquy Results

LOI Issuance

Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis
- Feedstock
- Pretreatment
- Enzyme
- Fermentation Microorganism
- Business plan
- ✍**High-level Stage 3 plan**