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ENG 300

Introduction to Biofuels

Part 1: Overview and Ligno-Cellulosics

Blake Simmons

Part 2: Biofuels in Energy-Water Context

Ron Pate

November 13, 2007

Sandia is a multiprogram laboratory operated by Sandia Corporation, a LockheedMartin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

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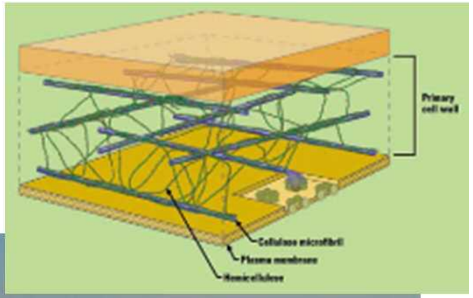
- The nation pays a high price for oil
  - Critical national security issues
  - Unprecedented environmental harm
  - Dwindling supply and price fluctuations

Energy from biomass holds sustainable energy promise, however:



- Currently cellulosic ethanol production is expensive and energy intensive
- Revolutionary breakthroughs are needed to create energy-efficient, cost-effective cellulosic biofuel
- Other starch, sugar, and oil crop based feedstocks and biofuels have sustainable scale-up issues and/or impacts on other food/feed/fiber markets

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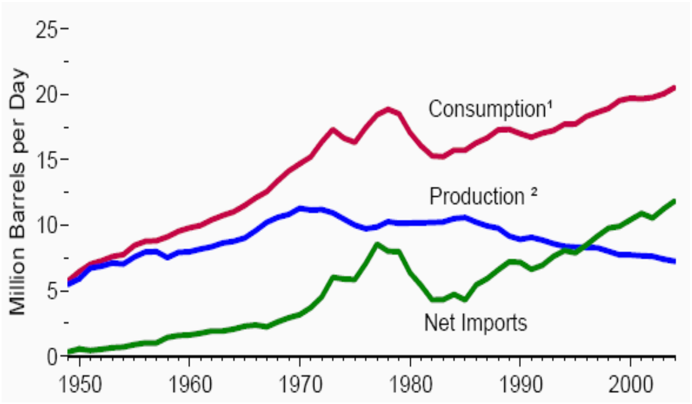
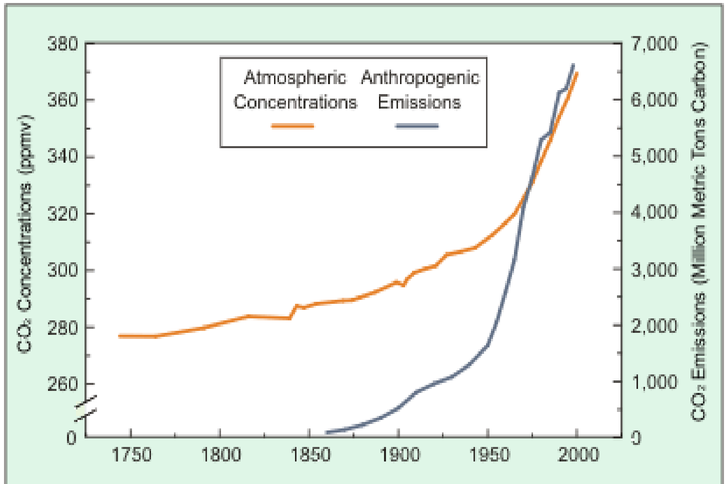
We Are Facing Unprecedented Transportation Fuels Challenges

- Largest end use of energy by sector
- 97% of transportation energy comes from petroleum
- Two-thirds of petroleum is used for transportation -- 60% for ground





Source: Oak Ridge National Laboratory, Carbon Dioxide Information Analysis Center, http://cdiac.esd.ornl.gov/.

- Gasoline and diesel both produce about 20 pounds of CO_2 per gallon
  - 7 tons of carbon/vehicle-year
- Transportation presents a unique challenge because onboard sequestration is not credible

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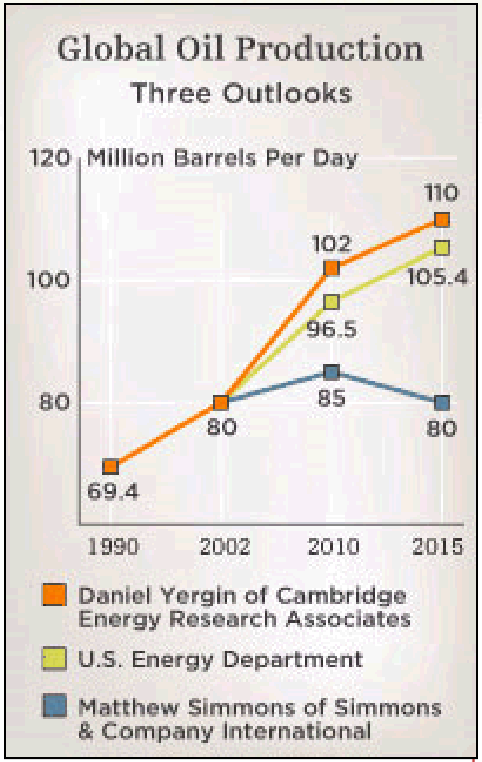
Biofuels Interest & Motivation

- **Energy Security … Heavy U.S. dependence on petroleum imports**
  - Oil imports of ~10-M bbl/day (150+ B-gal/yr)
    … two thirds for transportation fuels
  - Subject to supply disruption from volatile regions
  - Represents $300+ B/yr burden on U.S. economy
    … supports interests hostile to US
  - Increasing competition (China, India, etc.)
    & price volatility for limited global supplies
  - "Peak Oil" concerns
    … decades away?
    ... In 10-years?
    … happening now?
    → Place your bets ! →



- **Finding best paths for build-up/integration with existing production, energy/fuels, and transportation infrastructure**
  - Costs/Benefits/Impacts Tradeoffs
  - Technology, Processes, Systems R&D needs and priorities
- **Energy-Water-Environmental Nexus Concerns**
  - Mitigate adverse impacts on land use, water, GHG footprint, etc.

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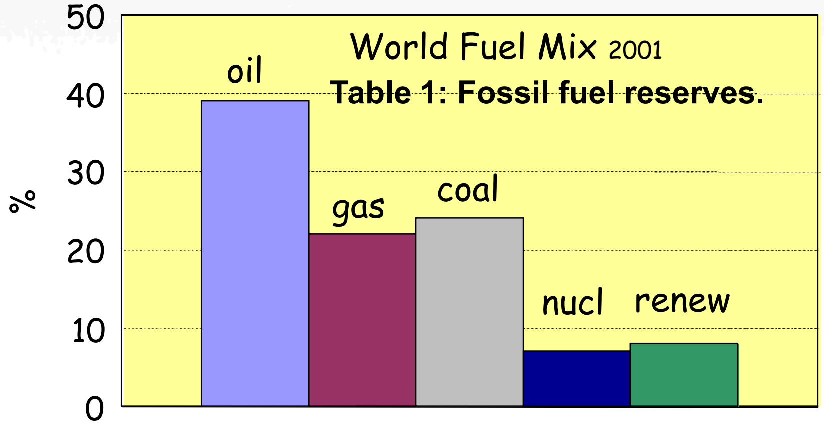
Research Drivers – Energy Security and Environmental Concerns



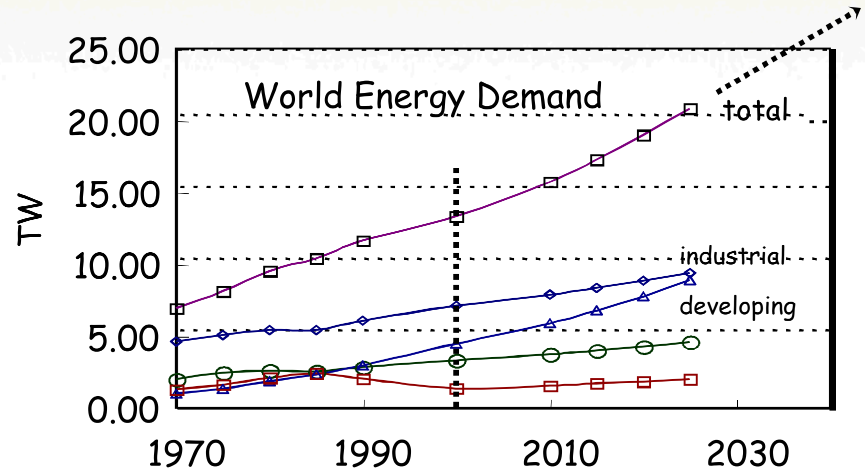
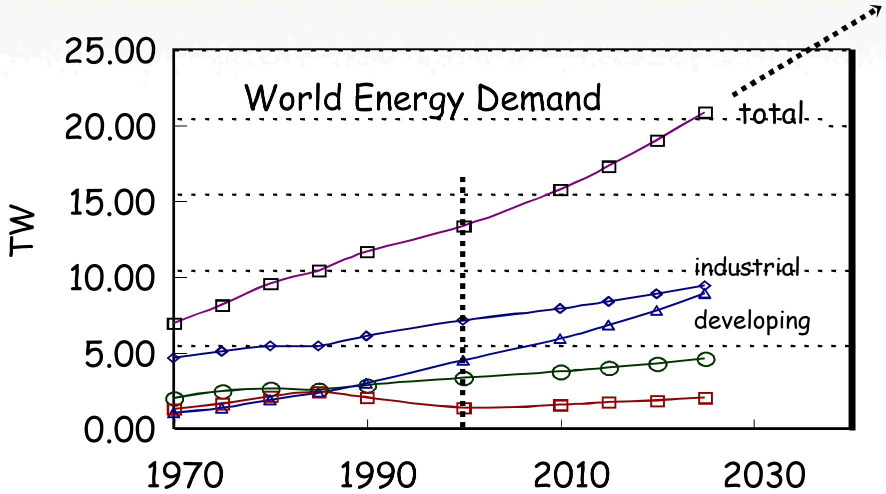


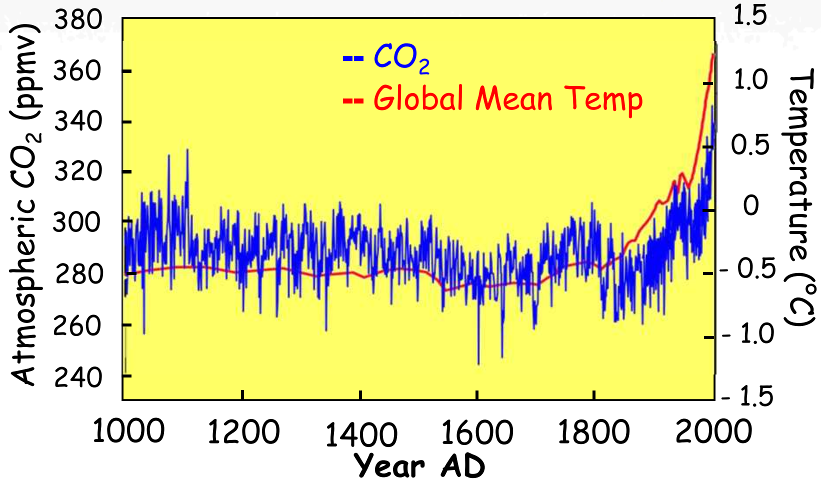
Table 1: Fossil fuel reserves.

Feedstock	Recoverable Reserves (Gigaton Carbon)[A]	Reserve Life At Current Consumption Rate (Years)[B]	Reserve Life At Projected Gdp Growth (Years)[C]
Oil	120	35	25
Natural Gas	75	60	45
Coal	925	400	100

a)Source: Energy Information Administration website (www.eia.doe.gov).
b)Estimated reserves divided by current consumption.
c)Source: Population trends for each geographic sector of the world were taken from the Population Reference Bureau website (www.prb.org) and GDP per Capita for every country were taken from a table at www.photius.com/wfb1999/rankings/gdp_per_capita_0.html. Estimates were made for how fast GDP/Capita (in constant dollars) might grow in each country, and were then multiplied by the expected population growth in each country and summed for the whole world to get a ratio of how energy demand will grow (energy demand grows historically at half the rate of GDP growth). Provided courtesy of Jeffrey Siirola.

Research Drivers – Energy Security and Environmental Concerns





- Growing demand for energy and finite availability of traditional energy feedstocks (oil and gas) motivates the consideration of alternative fossil feedstocks (tar sands, shale, coal) for the short term
- Biomass conversion offers the possibility of a sustainable source of fuel
- Generation of H_2 from H_2O and H_2/CO from H_2O/CO_2 should be considered using non-thermal sources of energy (e.g., photons and electrons)

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Research Drivers – Energy Security and Environmental Concerns

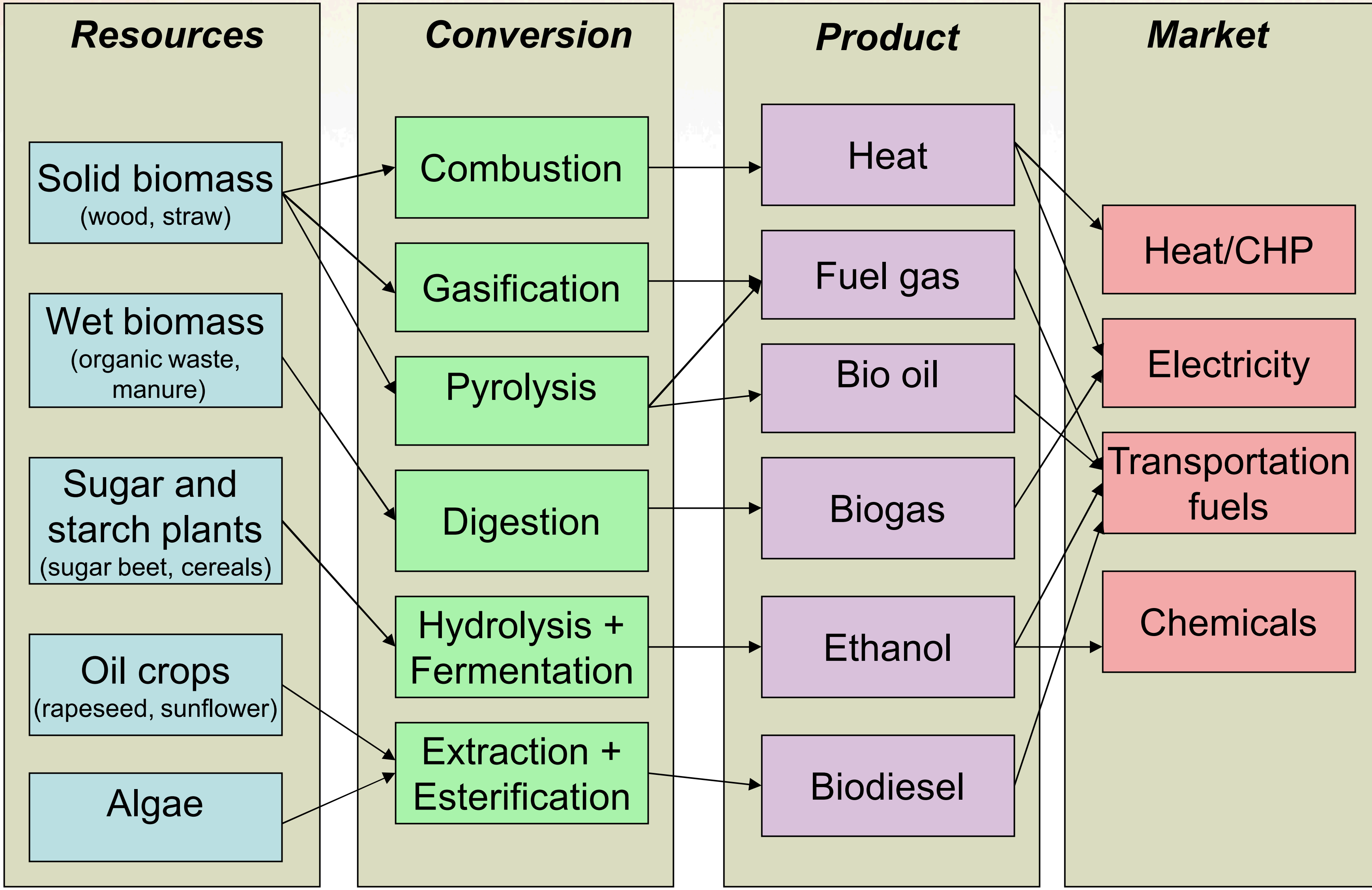
Conclusions:

- Changes in the feedstocks from which fuels are produced are likely to occur in this century
- Future fuel-supply technologies must be sustainable
- Novel catalytic technologies will be required for the production of fuels

Implications:

- Research should be directed at developing a fundamental understanding of how future feedstocks (shale oil, tar sands, biomass) can be converted to fuels efficiently w/ minimal adverse environmental impacts
- Basic research aimed at understanding catalyst structure and catalytic phenomena will contribute to the knowledge base used to guide the discovery and development of new catalysts

Biomass Interconversion Pathways



Biofuels: Status

- **Bio-derived liquid fuels address two significant national risks:**
  1) Dependence on foreign oil
     → Biofuels can be produced domestically

  2) Climate impact of CO_2 emissions from fossil fuels
     → Biofuels are potentially carbon neutral

- **Current pathways for biomass-based fuels:**
  – Ethanol (e.g., from corn seed wet/dry milling) : 4.9-5.2 billion gal produced
    • Compare to 140 billion gal/yr for petroleum gasoline

  – Lignocellulosic ethanol
    • USDA/DOE: ~1.3 billion tons per year available for conversion
    • Typical yield: 65-100 gallons/ton

  – Biohydrogen from microorganisms

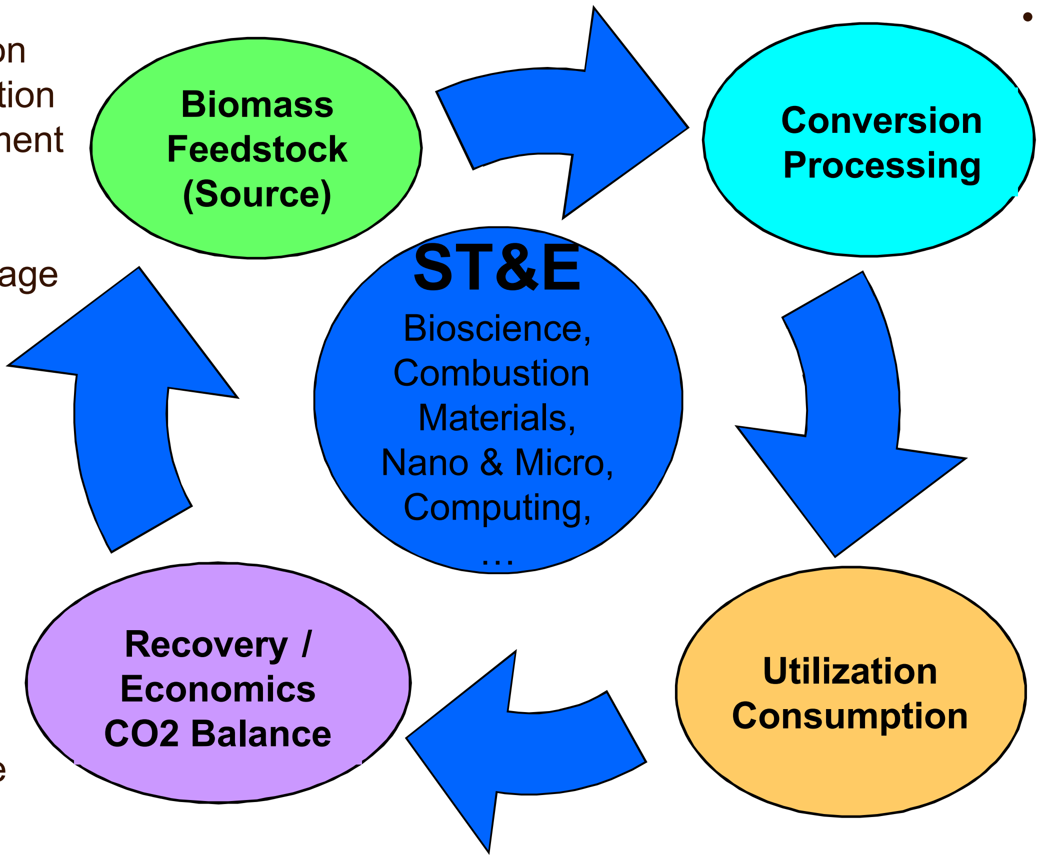
  – Fuels and/or intermediates (alkanes, alcohols, syngas) derived from processing of biomass via gasification, pyrolysis, solar heating

  – Biodiesel (e.g., from soy beans): ~250 million gal/yr
    • Compare to 62 billion gal/yr for petroleum diesel fuel
    • Potential market for up to 1-3 billion gal/yr domestic production from vegetable oils with room for further enhancements

  – Other high-value fuels from bio-oil sources in development (e.g. DARPA)

Cost Effective, Sustainable Biofuels for New Transportation Fuels Systems

Challenges
- Biomass
    - Production
    - Optimization
    - Pretreatment
    - Scale-up
- Sustainability
    - Water usage



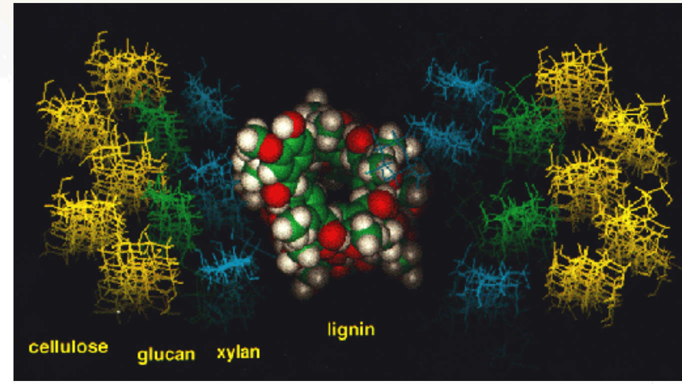
Challenges
- Biomass processing
    - Catalysis
    - Thermochemical
    - Biochemical
    - Scale-up
    - Microbial Communities

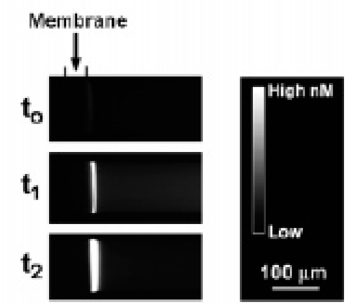
Challenges
- Engine design
- Fuel Distribution
- Fuel Storage
- Materials Compatibility
- US Infrastructure Implications (Systems)

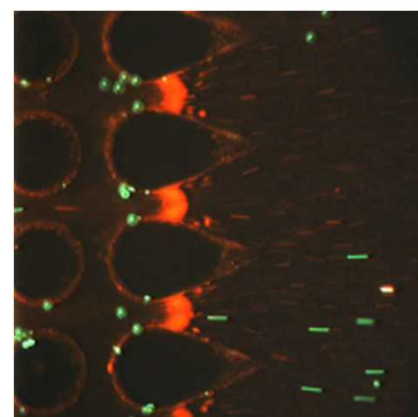
Challenges
- Carbon Implications
- Co-location w/coal-fired generators
- Viable Lifecycle Costs

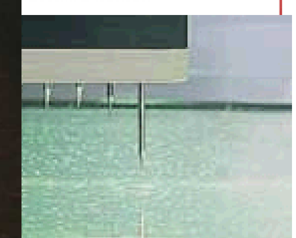
Advanced new disciplines and capabilities are key

- New platforms are required to enable discovery and realization of breakthrough S&T
- Materials science based approaches to understanding enzyme-substrate interactions
- High-throughput microsystem-based techniques for producing and characterizing proteins
- New instruments for high-resolution imaging at different scales (molecular, cellular, microbial)
- Computational modeling
- Synthetic enzymes and new enzyme architectures









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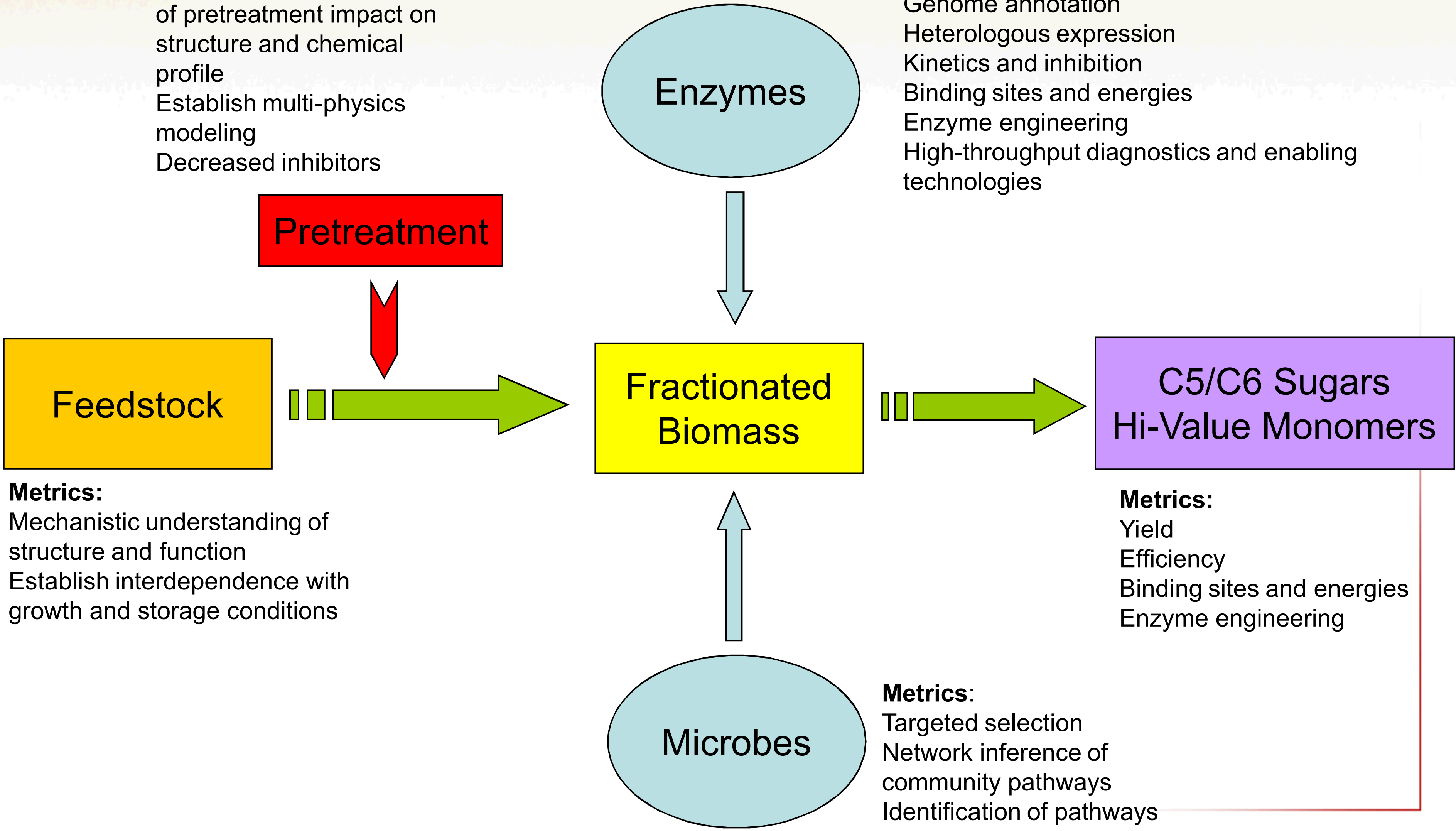
Ethanol Production Today

- Primary mode of fuel ethanol production: corn kernel (starch) wet and dry milling
  - 4.9 billion gallons produced in 2006
  - Took 13 years to reach 1 billion gallon production levels
- New mandate: double the amount of ethanol blended with gasoline by 2012
- Current tax breaks: $0.51 per gallon
- Nearly half of the gasoline sold in the US contains 10 percent ethanol
- 76 corn ethanol refineries under construction (112 in place already)
- Food vs. fuel – corn prices have spiked because of increased demand
  - $4 per bushel, highest in ten years (recent price drops)
  - Will result in higher prices across the board for associated products (meat, etc.)
- Ethanol is on track to consume 50% of corn yield (last year 20%)
- Must develop alternative sources of feedstocks and processing to meet Federal goals
- Ethanol derived from cellulosic material is the most viable alternative
  - Believed to cost 5x more today to establish a cellulosic biorefinery

Biomass Processing Flow



Metrics:
Mechanistic understanding of pretreatment impact on structure and chemical profile
Establish multi-physics modeling
Decreased inhibitors

Metrics:
Library development
Genome annotation
Heterologous expression
Kinetics and inhibition
Binding sites and energies
Enzyme engineering
High-throughput diagnostics and enabling technologies

Metrics:
Mechanistic understanding of structure and function
Establish interdependence with growth and storage conditions

Metrics:
Yield
Efficiency
Binding sites and energies
Enzyme engineering

Metrics:
Targeted selection
Network inference of community pathways
Identification of pathways
Isolation of key enzymes
Genome annotation

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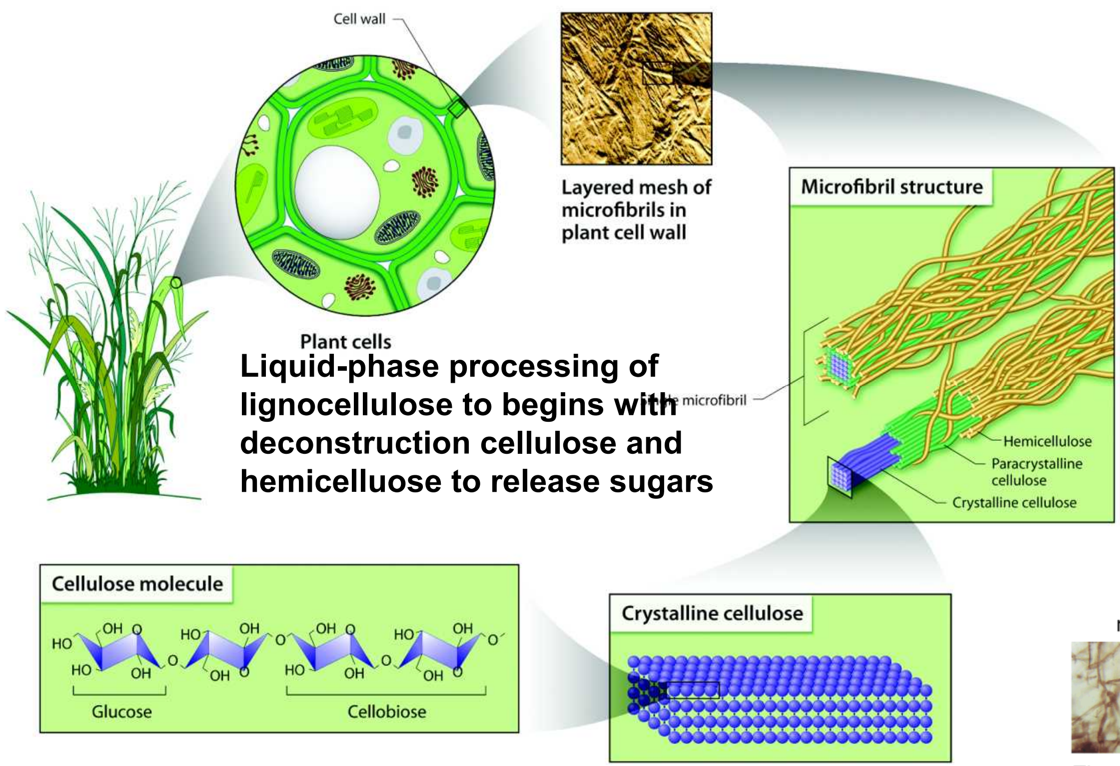
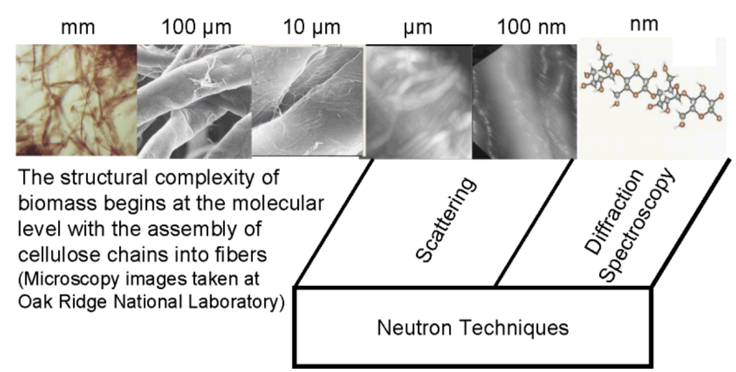
Advanced Catalysts for Conversion of Biologically-Derived Feedstocks



Liquid-phase processing of lignocellulose to begins with deconstruction cellulose and hemicelluose to release sugars

Biomass can be converted to fuels by:

- **Pyrolysis – complex liquid products requiring further processing**
- **Gasification – produces CO/H_2 that can be converted further to diesel**
- **Deconstruction – produces sugars that can be converted to fuels by enzymatic or non-enzymatic catalysts**



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Pretreatment

Pretreatment Summary (combined with enzymatic hydrolysis)

Pretreatment	Conditions	Total Process Yield	Disadvantages
Ionic Liquids	Temp - 90-140 °C	Glucose ~ 75-90% Xylose - TBD%	Expensive
Ammonia Fiber Explosion	5-15% Ammonia; Temp - 150-180 °C; pH - 9-11	Glucose - 89% Xylose - 94%	Must recycle ammonia stream; sugar degradation as a function of temperature and time
Organosolv	Hot aqueous ethanol, catalysts	Glucose - 91% Xylose - 94%	Expensive; Handling requirements
Dilute Acid	Temp - 140-200 °C; pH - 2-4; Time - 20-60 minutes	Glucose - 91% Xylose - 90%	Unwanted inhibitory byproducts; capitalization cost
Hydrothermolysis	Temp - 200-230 °C; time - 15 min.; pH - above 4-5; Pressure - 350 - 400 psig	Glucose - 88% Xylose - 100%	Not efficient at softwood degradation

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Pretreatments: Pros and Cons

- Goal: improved pretreatment approaches (to decrystallize cellulose for enhanced enzymatic hydrolysis)

- Organosolv
  - Very efficient with lignin solubilization
  - What if ethanol was free?
  - Environmental impact key

- Acid pretreatments most dominant
  - Pros: efficient, relatively cheap
  - Cons: inhibitor formation through glucose degradation

- Steam explosion widely used as well
  - Pros: simple
  - Cons: limited efficiacy against lignin and other feedstocks

- Ionic liquids (e.g., butyl-methylimidazolium chloride & other solvents)
  - Innovative approach, nascent

R&D Opportunities

- Innovative solvents with enhanced solubilization properties of all three major biomass constituents
- Establish computational modeling activity around biomass pretreatment beyond simple kinetics
- High-throughput, combinatorial approaches to discovery
- Establish fundamental, science-based understanding of biomass in different environments
  - Tie-in to multi-scale modeling coupled with diffusive/active transport mechanisms within lignocellulosic materials
  - Imaging - methods to quantify pretreatment effectiveness
    - HSI, TEM, STM, AFM, XRD, LC-MS, GC-MS
  - Spectroscopic investigations as a function of pretreatment/processing conditions
    - FTIR, Raman, SERS, NMR

Enzymatic Hydrolysis

Cellulosic Biomass: Big Potential, Complex Problems

- About half of the carbonaceous compounds in terrestrial biomass are cellulose, which is the most prominent single organic compound on earth.
- The net primary production of biomass was estimated to be 60 Gt/annum of carbon in terrestrial and 53 Gt/annum in marine ecosystems (1 Gt = 1012 kg) (Cox et al. 2000).
- Almost all of the biomass produced is mineralized again by enzymes which are provided by microorganisms.
- Cellulose is a chemically homogeneous linear polymer of up to 10 000 D-glucose molecules, which are connected by ß-1,4-bonds. As each glucose residue is tilted by 180° towards its neighbors, the structural subunit of cellulose is cellobiose
- The chemical uniformity provokes spontaneous crystallization of the cellulose molecules, the tightly packed microfibrils. Cellulose thus is a sturdy material ideally suited to insure the structural stability of land plants where it is a main component of the primary cell wall, especially in wood.
- Although crystalline cellulose is chemical homogeneous, no single enzyme is able to hydrolyze it, whereas soluble cellulose derivatives are easily degraded by a single endo-ß-1,4-glucanase.
- Enzyme mechanisms generally depend on single molecules fitting in their substrate pocket - with cellulose the substrate is much larger than the enzyme
- The crystalline material is hydrolyzed by a number of simultaneously present, interacting enzymes, or alternatively by a multienzyme complex found in anaerobic micro-organisms (cellulosome).
- Cooperation with non-catalytic specific binding modules (the carbohydrate binding proteins or modules) the enzymes are able to disrupt the crystal surface at the solid-liquid interphase, to make single cellulose fibers accessible for hydrolysis.
- The investigation of the hydrolysis mechanisms of cellulases opens up a new way of looking at enzymatic activity: the dualism between mechanical and structural "preparation" of the insoluble (crystalline) substrate followed by the hydrolytic activity on a released molecule (Sheehan and Himmel 1999).

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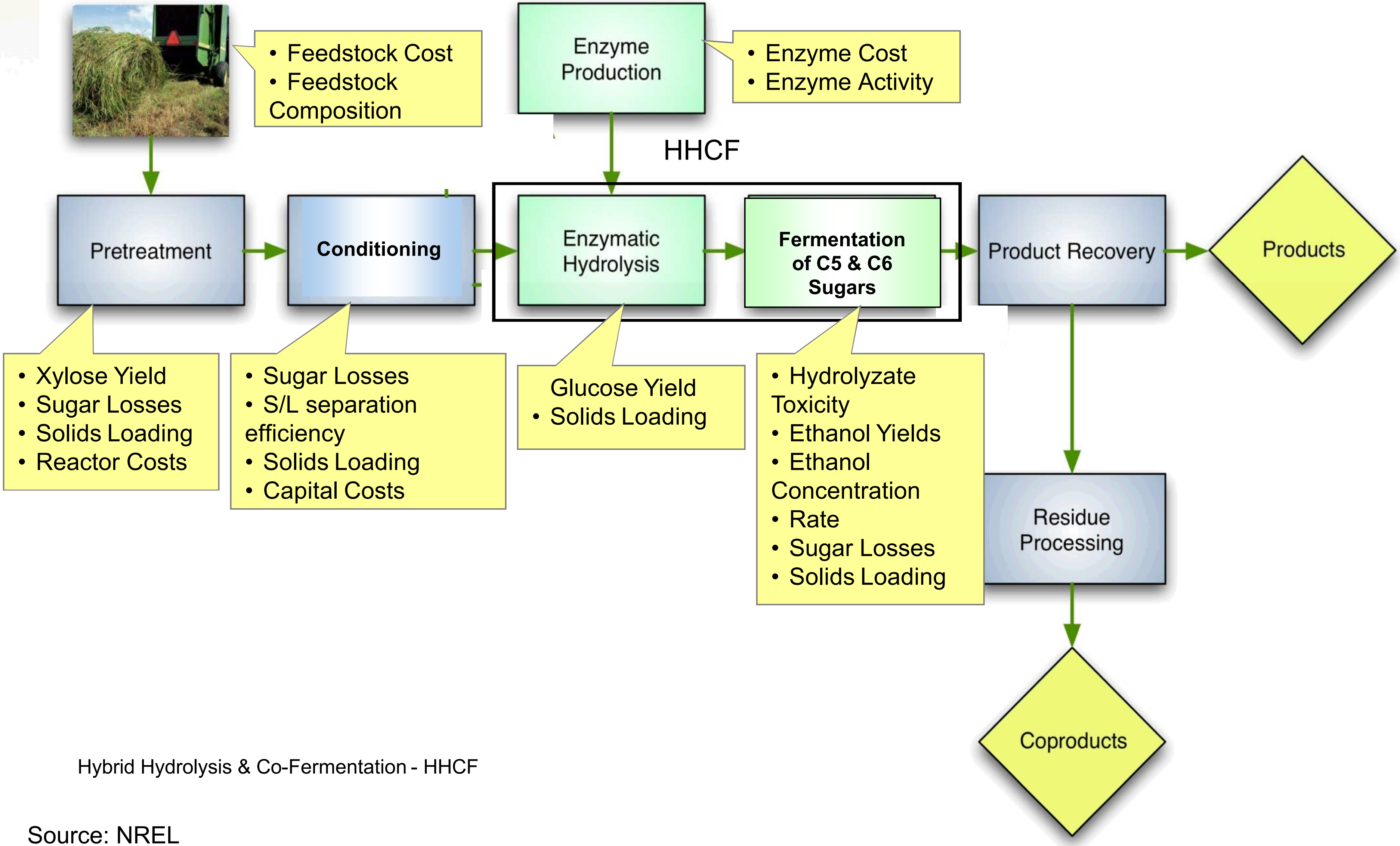
Role of Enzymes in Ethanol Production

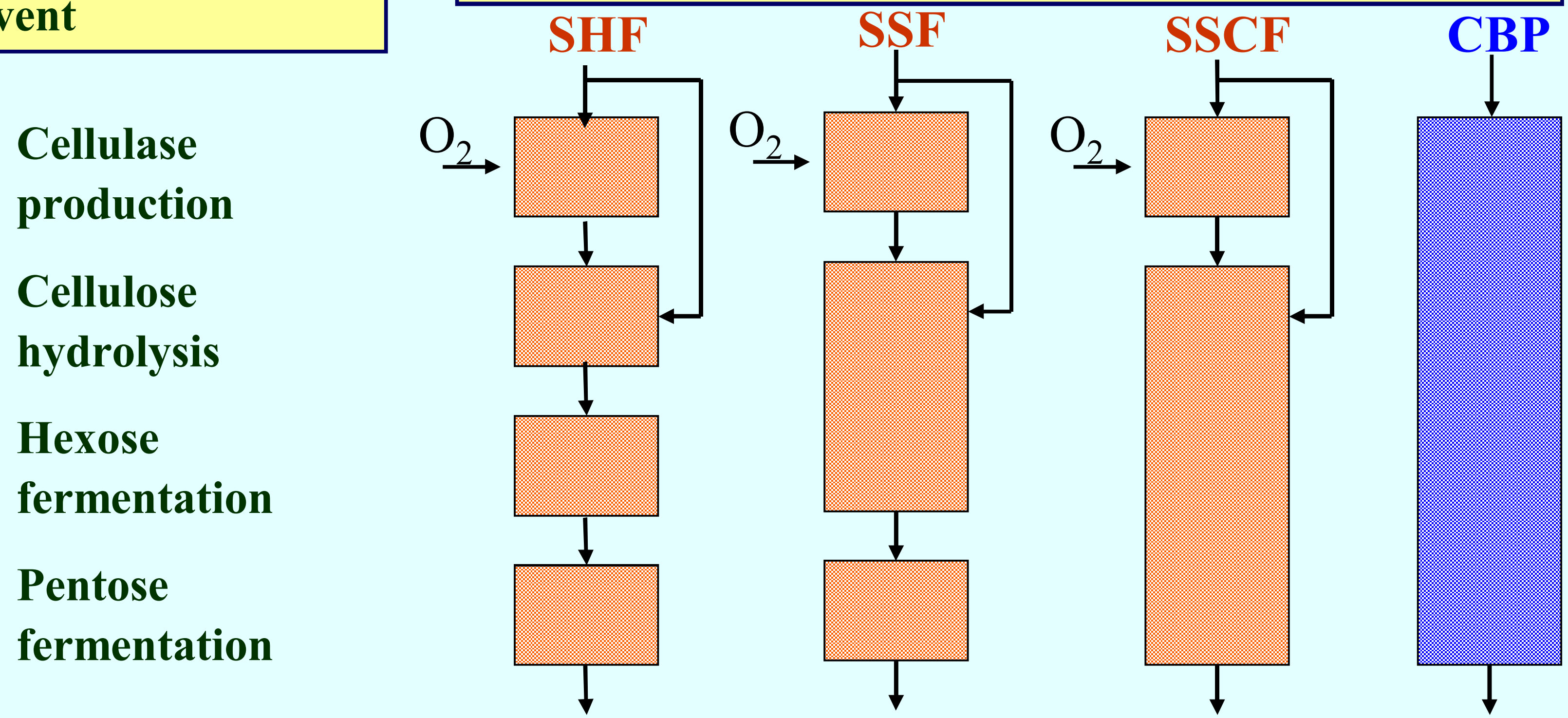


Source: NREL

Evolution of Biomass Processing Featuring Enzymatic Hydrolysis

Biologically-Mediated Event

Processing Strategy
(each box represents a bioreactor - not to scale)



SHF: Separate hydrolysis & fermentation

SSF: Simultaneous saccharification & fermentation

SSCF: Simultaneous saccharification & co-fermentation

CBP: Consolidated bioprocessing

Fundamental Mechanisms of Cellulase Hydrolysis

- Three basic cellulase enzymes: endoglucanase, exoglucanase, β-glucosidase
- Cellulases differ not only in the action mode (endo or exo), but also in the way they bind to the crystalline surface of the substrate.
- There are two sites in the enzymes which mediate binding: the active site of the catalytic domain and the separately folded and functionally independent carbohydrate binding module (CBM) which usually is attached through a PTS-box.
- The essential function of the CBM was shown for cellobiohydrolase CBHI from T. reesei, for which a detailed 3-dimensional model was constructed (Lee and Brown 1997).
- The catalytic domain without the CBM (the core enzyme) has a limited activity on cellulose.
- The deletion of CBMs has no effect for activity on soluble substrates (like CMC or barley ß-glucan) where the possible sites of activity on the substrate are not limited. (Tomme et al. 1995; Bolam et al. 1998).
- Members of each group have been investigated for their binding capacity for a number of polysaccharides: crystalline and amorphous cellulose, ß-1,3-glucan, xylan, starch, chitin and others (Tomme et al. 1998).
- Even within one family binding to different substrates is possible (Zverlov et al. 2001). Although CBMs bind to the cellulose with a high association constant and sometimes irreversibly, they show, in conjunction with a catalytic domain, surface diffusion and redistribute on the surface (Jervis et al. 1997; Carrard et al. 2000).
- Although CBMs are important for the processivity of cellulases (Irwin et al. 1998), there is no hint for a driving force, neither by the CBM nor by the catalytic unit.

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Ligninase

- **Lignases (a.k.a. ligninase)**
  - Goal: Develop more efficient conversion of lignin into hi-value products and/or alternative fuels through biochemical or chemical conversion technology. Develop model lignin system for study.
  - Currently a huge gap in understanding this system
  - Enzyme structure/function studies
    - Rational design/directed evolution
    - Mechanisms of lignin breakdown
    - Determine the mechanisms of lignin conversion, the role of enzyme binding
    - Catalytic and binding domains within lignin – new pretreatments?
    - Kinetic studies of lignin conversion
    - Alleviate product inhibition through chemical and structural modifications
  - Lignin studies
    - Fundamental science of lignin composition and structure
    - Imaging and
    - Modeling coupled with active transport

Overall Enzyme Research Goals

Fundamental R&D Opportunities:

- Develop advances in S&T that enable revolutionary progress in the efficient and cheap pretreatment and conversion of lignocellulosic materials into fermentable sugars
- Develop a fundamental understanding of enzyme-substrate and enzyme-enzyme complexes that play a role in biomass depolymerization and hydrolysis
- Development of new microsystem-based high-throughput screening technology for enhanced rational design of enzymes
- Utilization of BES funded world-class imaging and tools to generate new insight into mechanism of lignocellulose deconstruction and enzymatic hydrolysis
- Utilization of BES funded world-class biophysical characterization tools to generate new insight into enzyme kinetics and local environments of lignocellulose degradation
- Apply massive parallel computational modeling resource to understand enzymatic complexes and their role in biomass hydrolysis
- Synthetic -> biological -> synthetic

Road Blocks

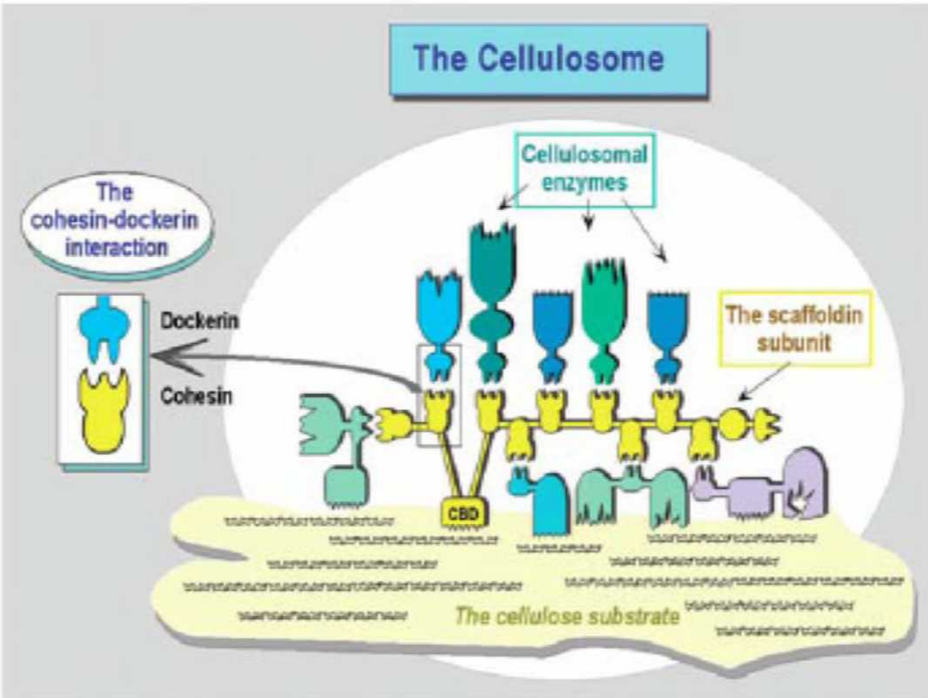
- Robust information on enzyme characteristics/crystal structures outside of enzymes derived from the dominant model system: *T. reesei*
- Efficient processing and annotation of vast genomics information directly applicable for the rational design of biomass-related enzymes
- Process compatibility – consolidated bioprocessing as a model system
- Accurate and robust kinetic assays (new molecules, new diagnostics) amenable to high-throughput screening techniques
- Lack of fundamental knowledge of lignocellulose as a composite material with unique and distinct binding sites and cross-linked structures as a function of feedstock
- Efficient pretreatment with minimal production of adverse co-products
- Lignin

Cellulosome

Cellulosomes: Bacterial Assemblages of Cellulolytic Enzymes

- Cellulosomes are cell protuberances which tightly bind to crystalline cellulose (Lamed et al. 1987; Mayer et al. 1987). T
- They mediate a close neighborhood between cell and substrate and thus minimize diffusion losses of hydrolytic products, which is thought to be a major advantage for attached cells.
- A cellulosome preparation contains a number of different proteins, most of them having enzymatic activity. However, attempts for mild denaturation, purification of single components and reconstitution were only partially successful (Beattie et al. 1994; Bhat et al. 1994; Choi and Ljungdahl 1996).
- in all cellulosomes investigated so far the components of the multienzyme complex are strongly bound to each other by a duplicated, non-catalytic segment of 22 amino acid residues found to be conserved in all enzymes which are located in the cellulosome (Tokatlidis et al. 1991).
- This dockerin module binds specifically to the cohesin modules, located in a non-catalytic cellulosome component, for which the term "scaffoldin" was coined (cellulosome structure).
- The catalytic components themselves are complex proteins consisting of catalytic and non-catalytic modules. Binding of the cellulosome to the crystalline substrate is mainly mediated by a very strongly binding CBM IIIa module of the scaffoldin.
- The production of the multienzyme-complex "cellulosome" may have a number of advantages for the effective hydrolysis of cellulose:
  - synergism is optimized by the correct ratio between the components, which is determined by the composition of the complex;
  - non-productive adsorption is avoided by the optimal spacing of components working together in synergistic fashion;
  - competitiveness in binding to a limited number of binding sites is avoided by binding the whole complex to a single site through a strong binding domain with low specificity
  - stop of hydrolysis on depletion of one structural type of cellulose at the site of adsorption is avoided by the presence of other enzymes with different specificity.

Cellulosome: Structure and Function



A Typical Cellulosome: Scaffoldin Organizational Protein Contains a cellulose-binding domain (CBD); Multiple copies of subunit-binding cohesins; Catalytic subunits are integrated by cohesin-dockerin interactions (Bayer et al, 2004)

Cellulose degradation images taken from culture of known cellulosome producer, *C. thermocellum*,

R&D Opportunities: Cellulosome

- Role of location, structure, and complexation in overall efficiency
- Is there any process gain in the cellulosome vs. free bulk enzymes?
- Cellulosomes in extreme environments
  - Enhanced shielding and stability
- Make a synthetic scaffolding structure relevant to industrial processes
- Fundamental understanding of the cellulose-cellulosome interface

completing the energy sustainability puzzle

ENERGY and WATER

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PART 2: Biofuels and Energy-Water Interdependencies

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