WRRC Research on Biomass Conversion: Strategies for Developing Flexible Biorefineries

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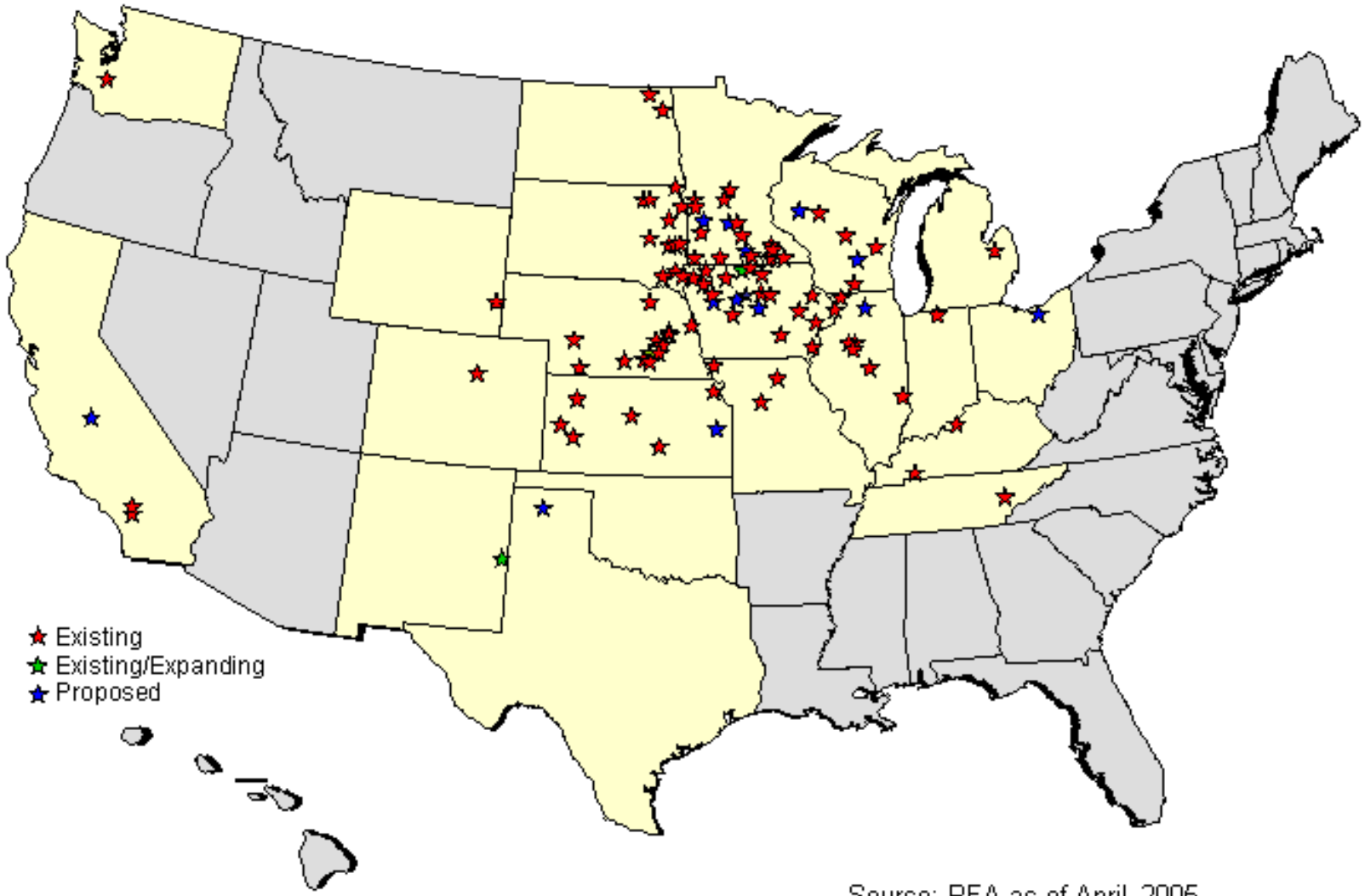
30 November 2006

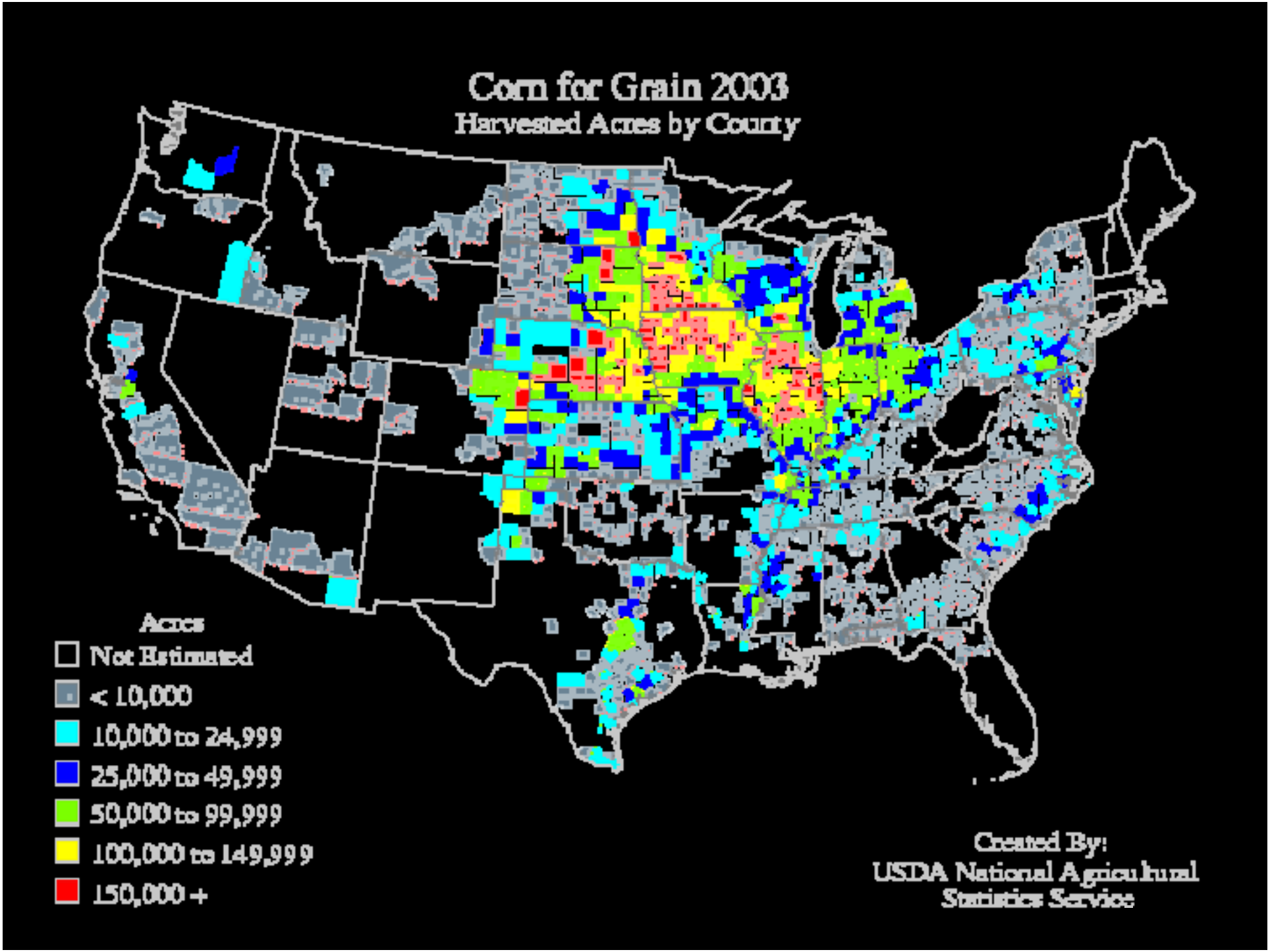
USDA
Agricultural Research Service ars

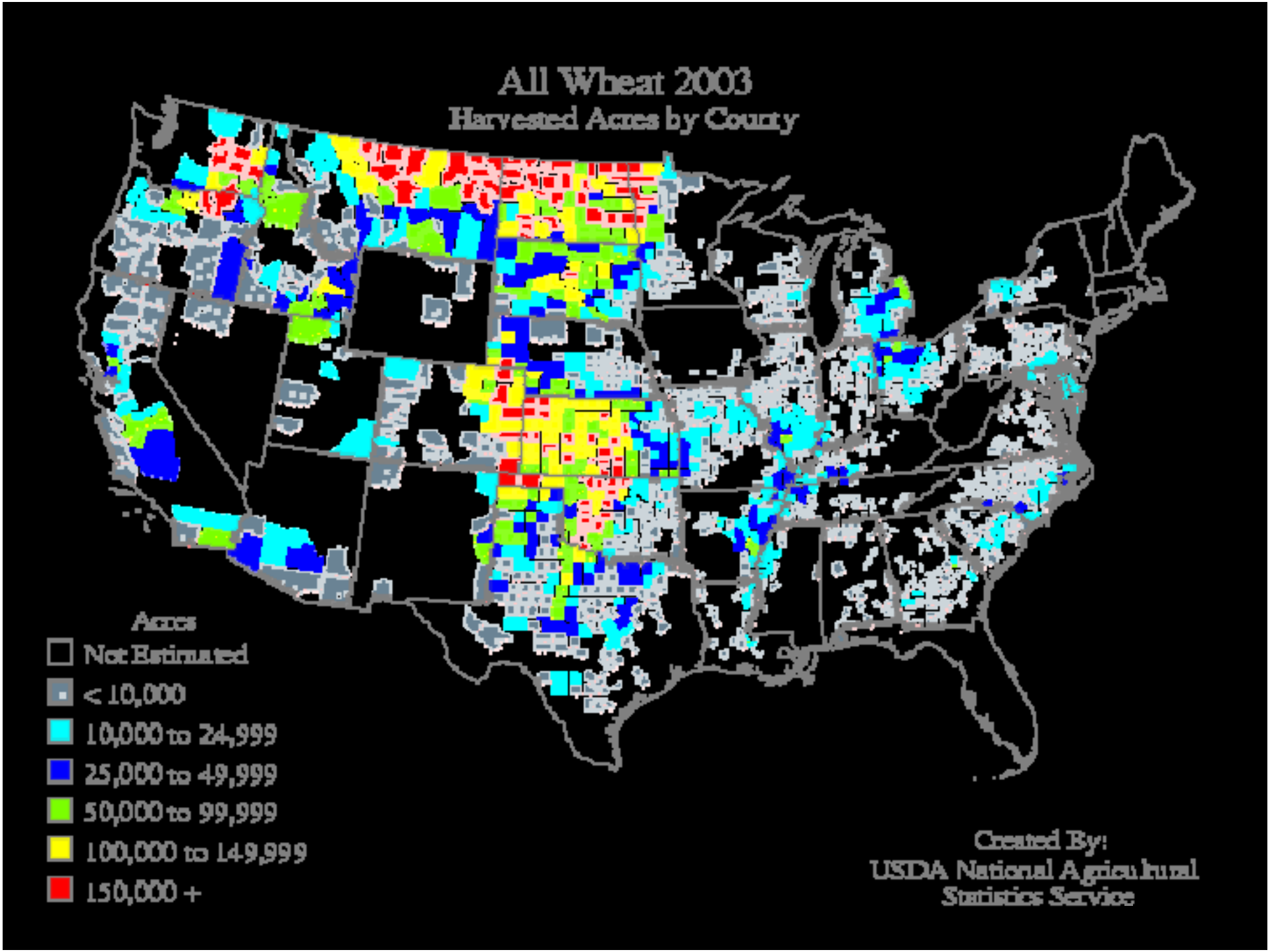
WRRC
Albany, California

U.S. ETHANOL MANUFACTURING LOCATIONS

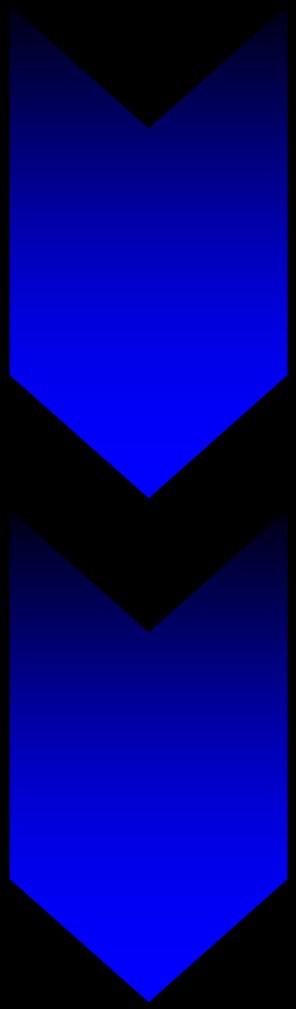


Source: RFA as of April, 2005


Corn for Grain 2003
Harvested Acres by County
Acres
Not Estimated
< 10,000
10,000 to 24,999
25,000 to 49,999
50,000 to 99,999
100,000 to 149,999
150,000 +
Created By:
USDA National Agricultural
Statistics Service


All Wheat 2003
Harvested Acres by County
Acres
Not Estimated
< 10,000
10,000 to 24,999
25,000 to 49,999
50,000 to 99,999
100,000 to 149,999
150,000 +
Created By:
USDA National Agricultural
Statistics Service

Corn-to-Ethanol: U.S trends



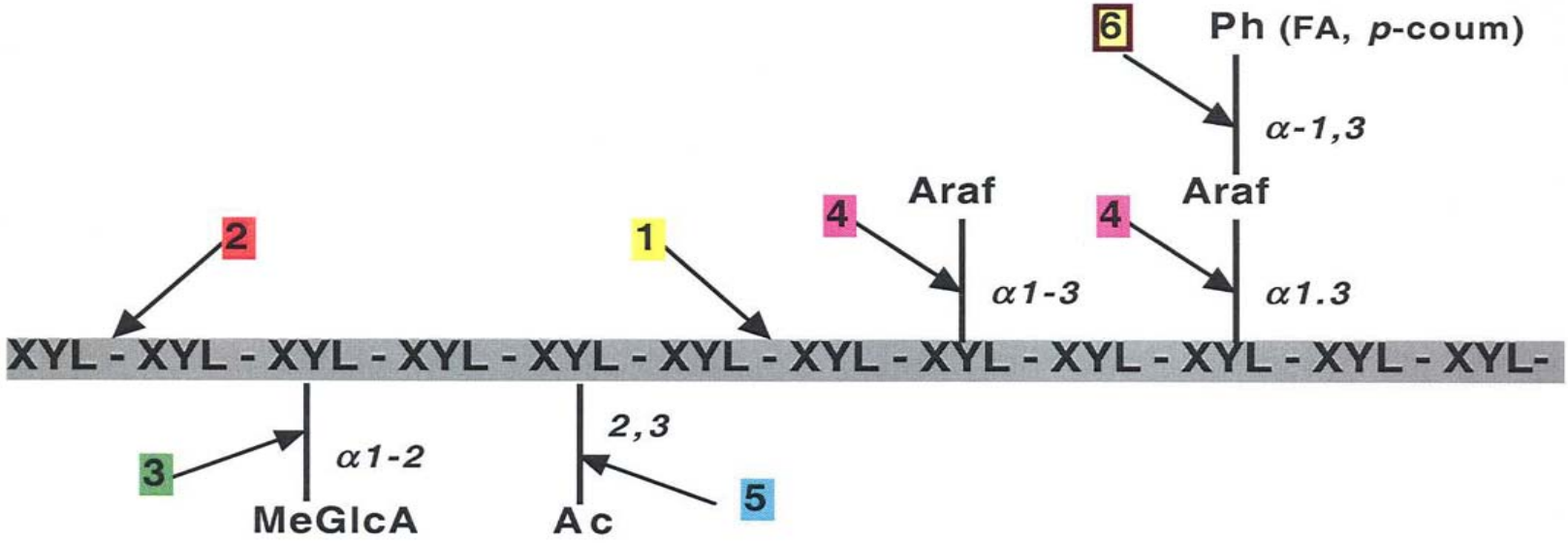
- Ethanol production is at 5-6 billion gals/yr (~25 billion litres)
- ~2% of transportation fuel
- Ethanol uses ~20% of US corn
- Most ethanol is not produced near refineries
- It is not widely produced in the most populated states.

Biomass Cellulose-to-ethanol

Evolutionary Enzyme Design For Improved Biorefining of Crops and Residues

Dominic Wong
Charles Lee
Kurt Wagschal
Michael Smith

Xylanolytic enzymes



1 endo-β-1,4-xylanase (EC 3..2.1.8)

2 β-xylosidase (EC 3.2.1.37) or exo-β-xylanase

3 α-glucuronidase (EC 3.2.1.139)

4 α-L-arabinofuranosidase (EC 3.2.1.55)

5 acetylxylan esterase (EC 3.1.1.72)

6 feruloyl esterase (EC 3.1.1.73)

- Wong, Lee & Wagschal

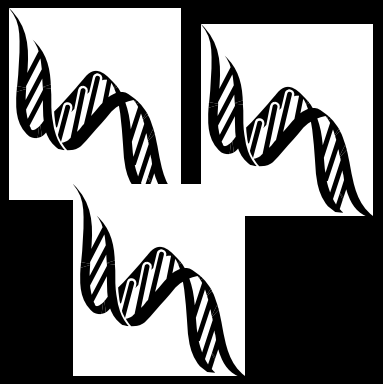
Hypothetical plant xylan and the enzymes for its complete hydrolysis (Adapted from Biely 2003)

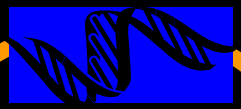
Novel Genes from Metagenomes

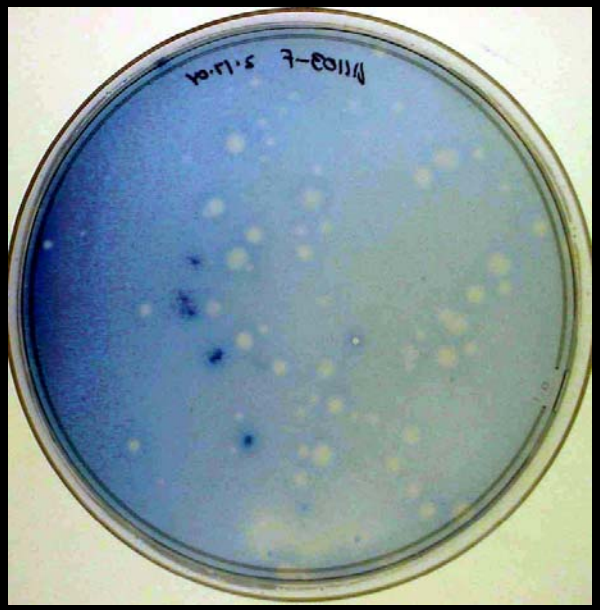
"Metagenome" =

the collective genomes of all microorganisms in a given habitat

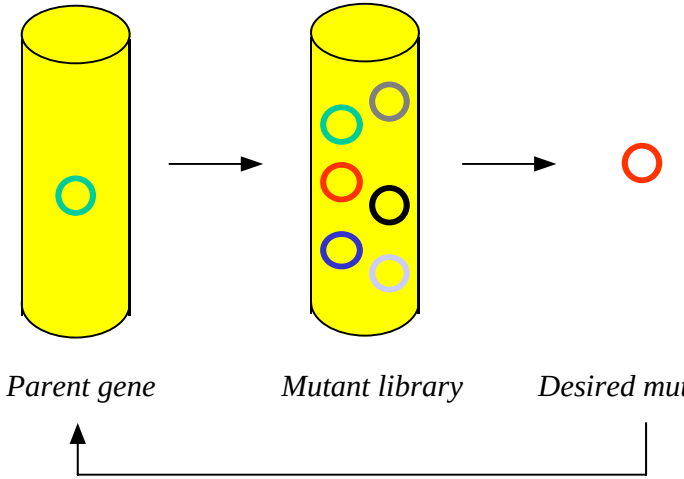
Gene Discovery

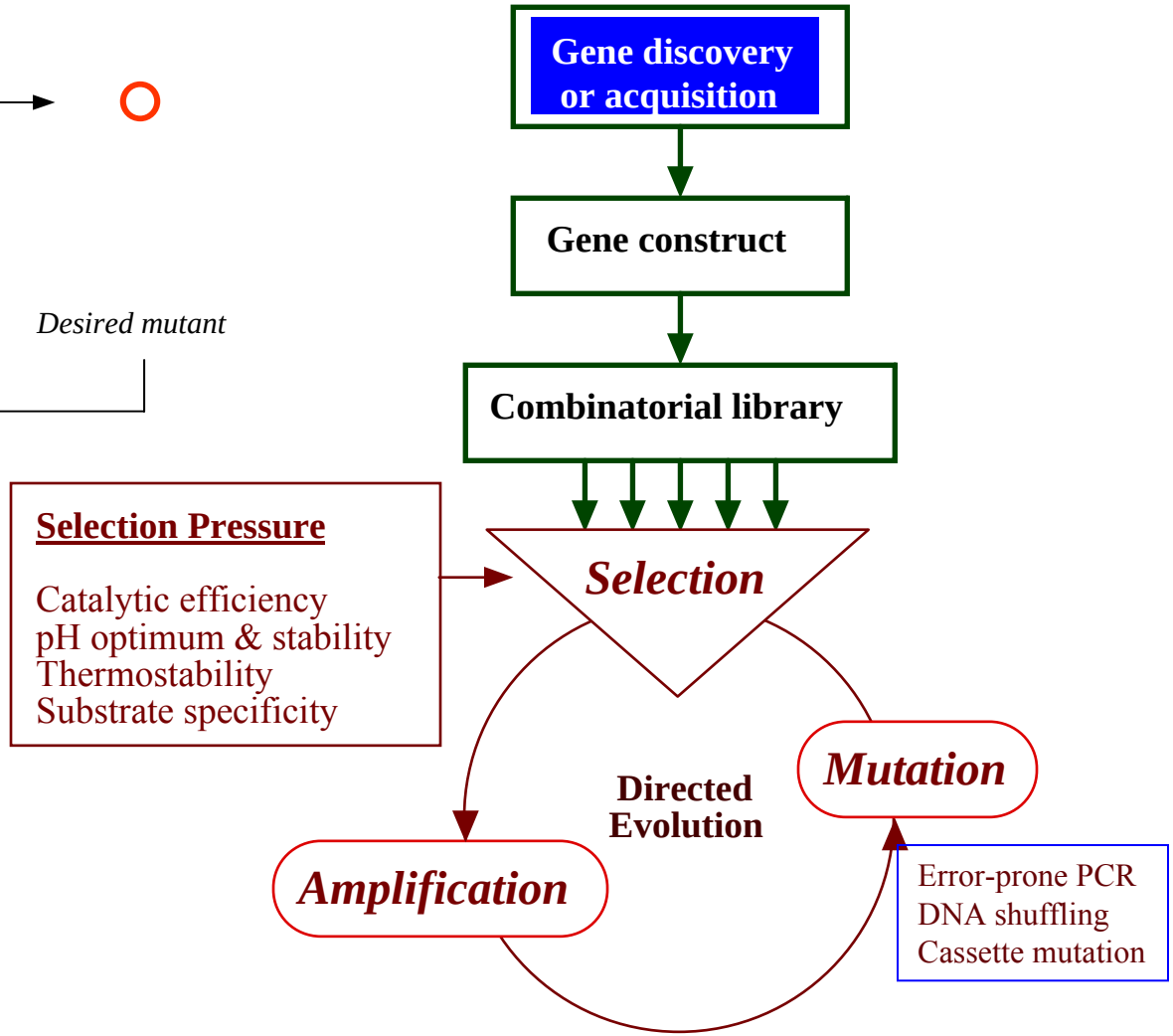




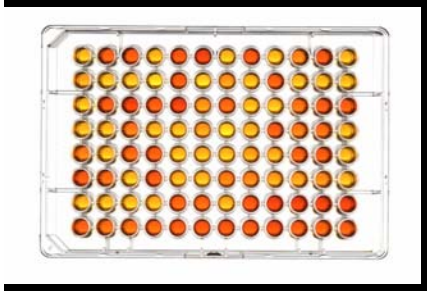


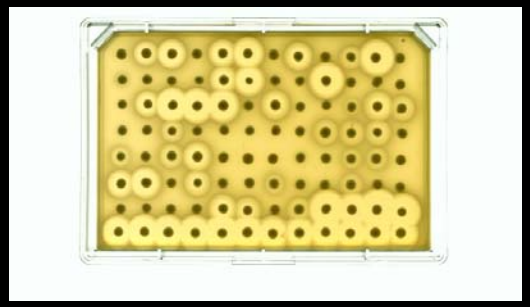
Directed Evolution

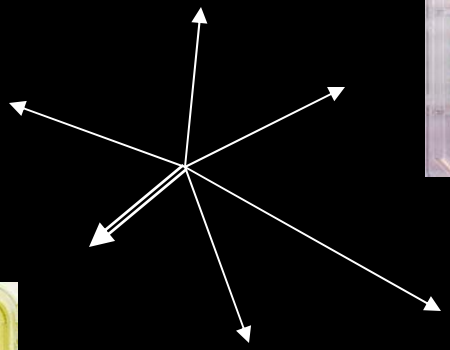


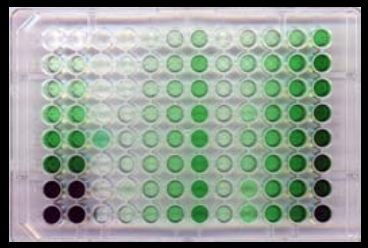


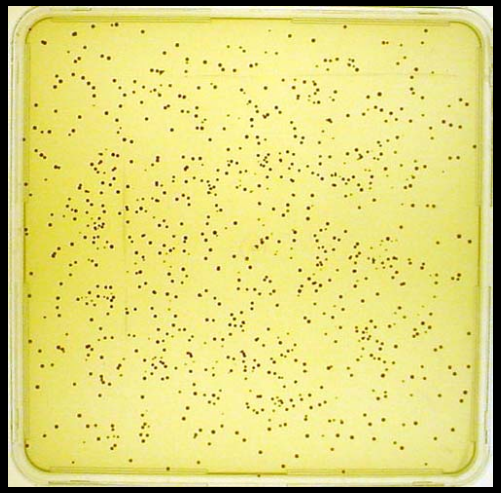
High-throughput Assay Screening

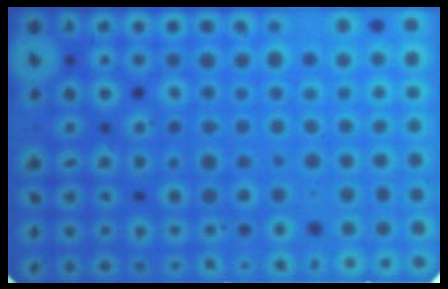






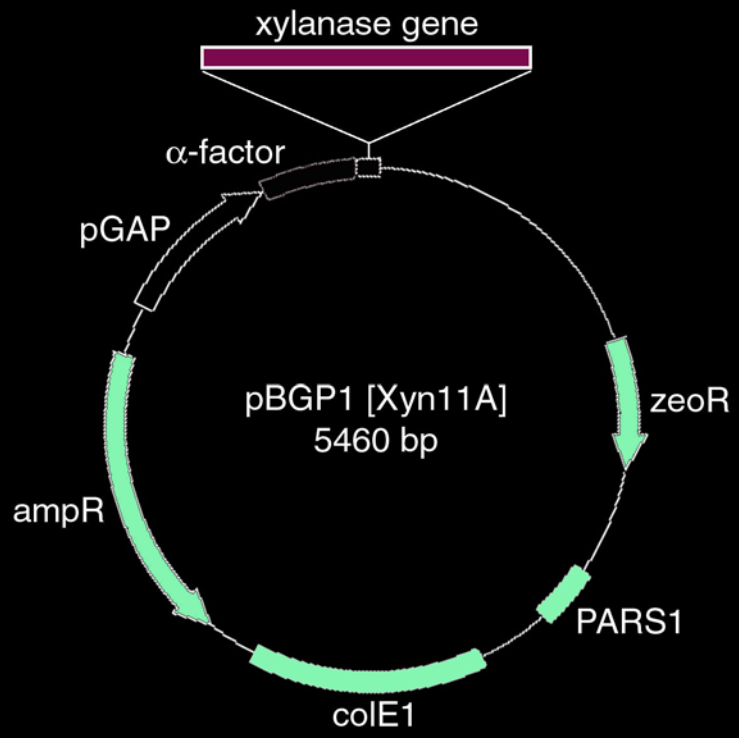






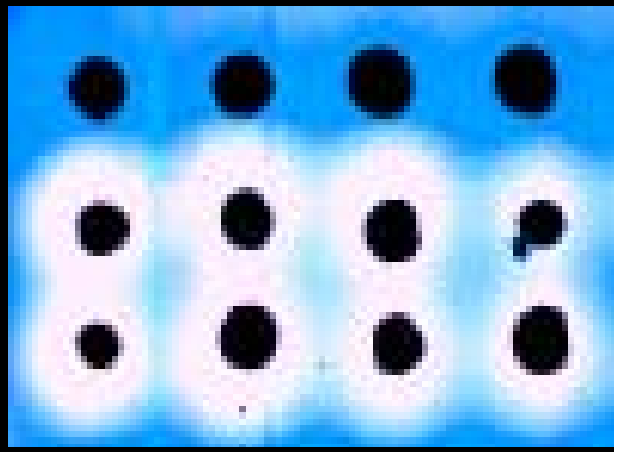
Q-tray

Hemicellulose hydrolysis



control

+ xylanase



WRRC modified vector;

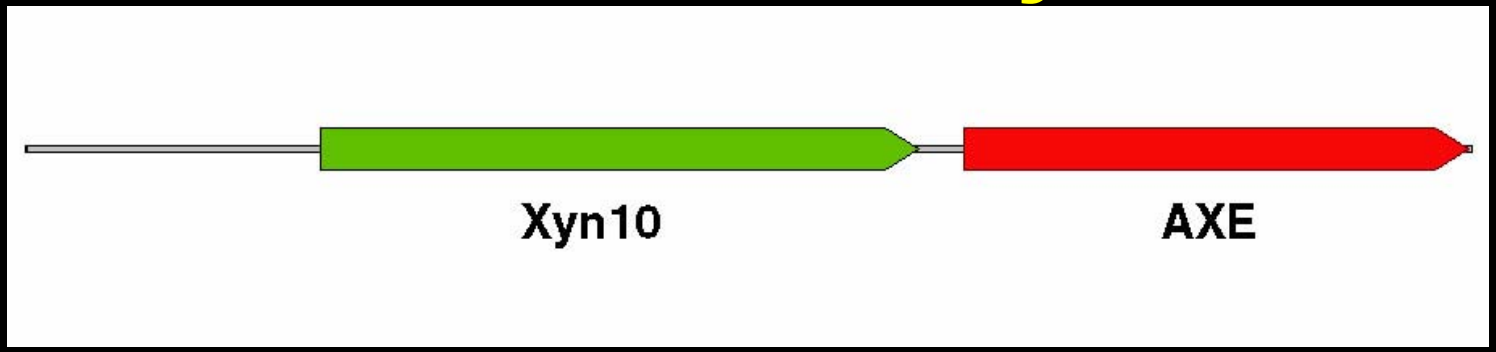
P.pastoris

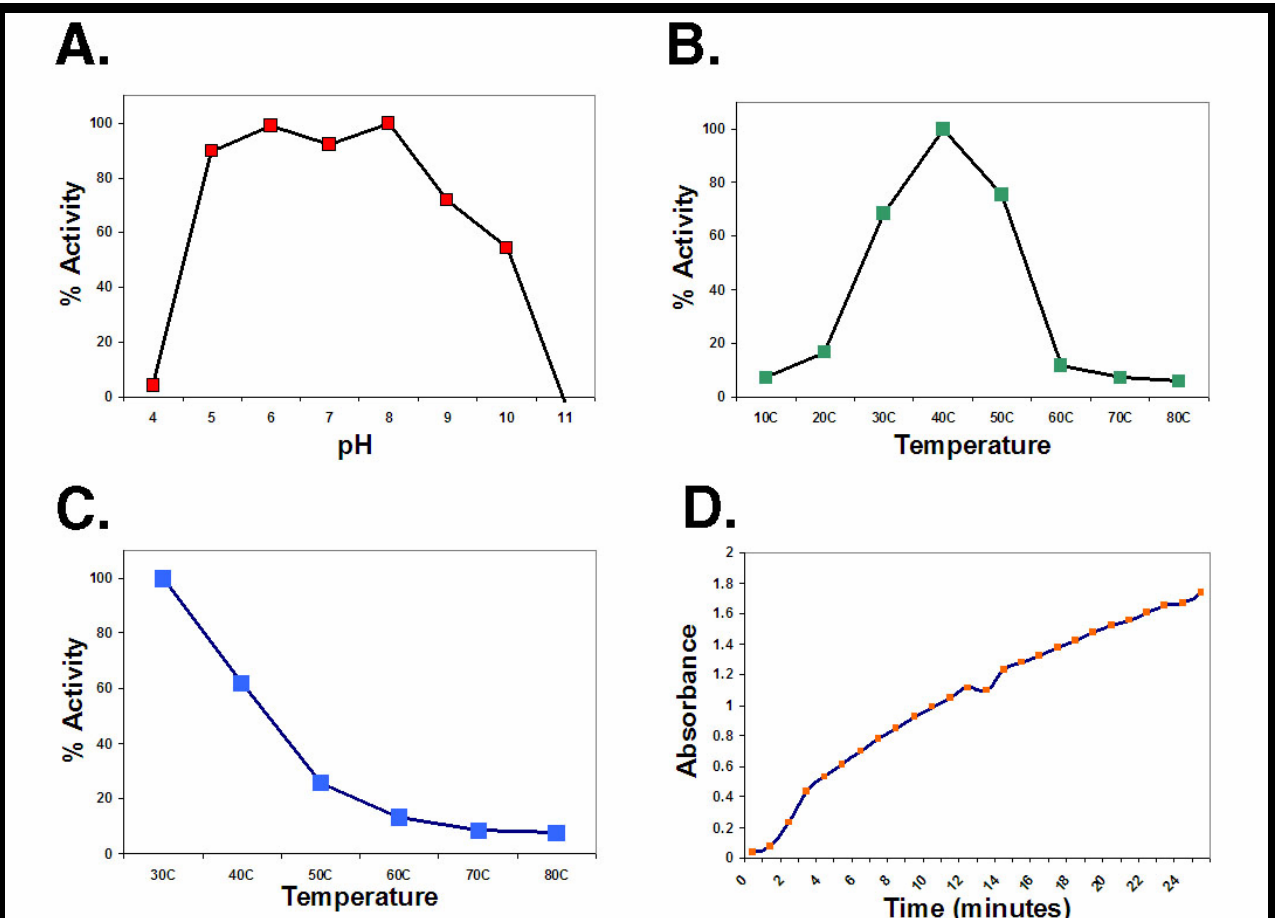
- Charles Lee

Genes & Enzymes: Isolation, expression

- α-amylases
- glucoamylases
- β-1,4-Xylanases
- β-xylosidases
- α-L-arabinofuranosidases
- α-glucuronidases
- feruloyl esterases
- xylanase-acetylxylan esterases
- endoglucanase-xylanase

Bifunctional enzyme

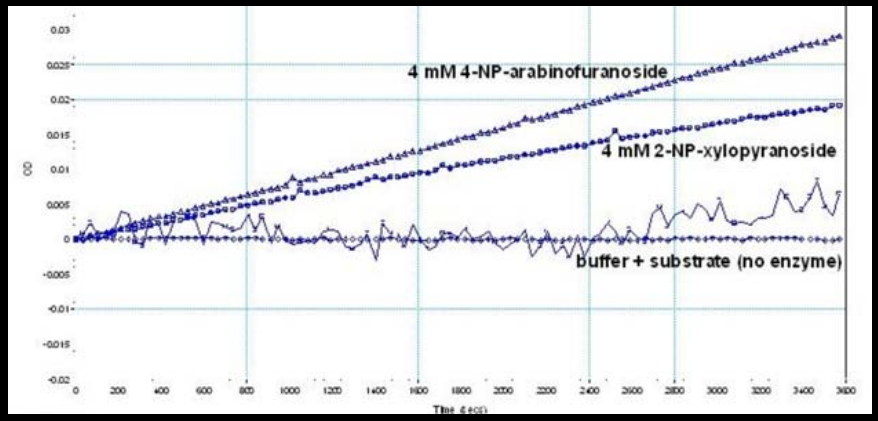


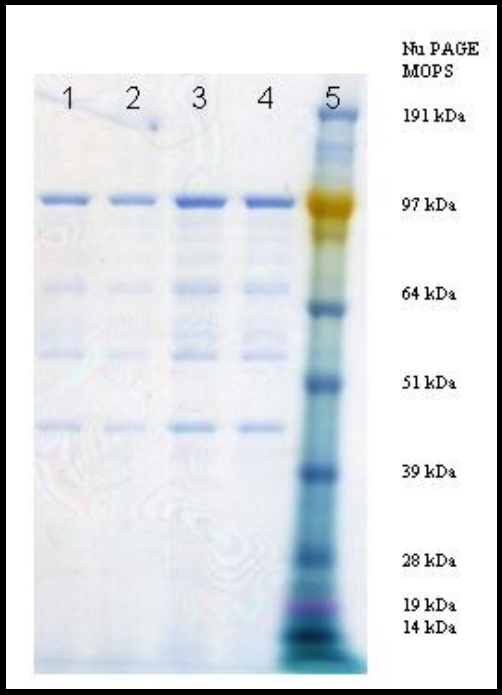


Recent Research Developments

II. Chimeric enzymes

- β-Xylosidase-Arabinofuranosidase



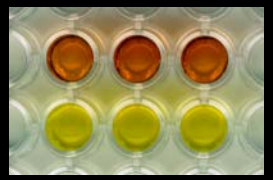


- Xylanase-Feruloyl Esterase

Fae activity

Control

Ethyl ferulate solid assay



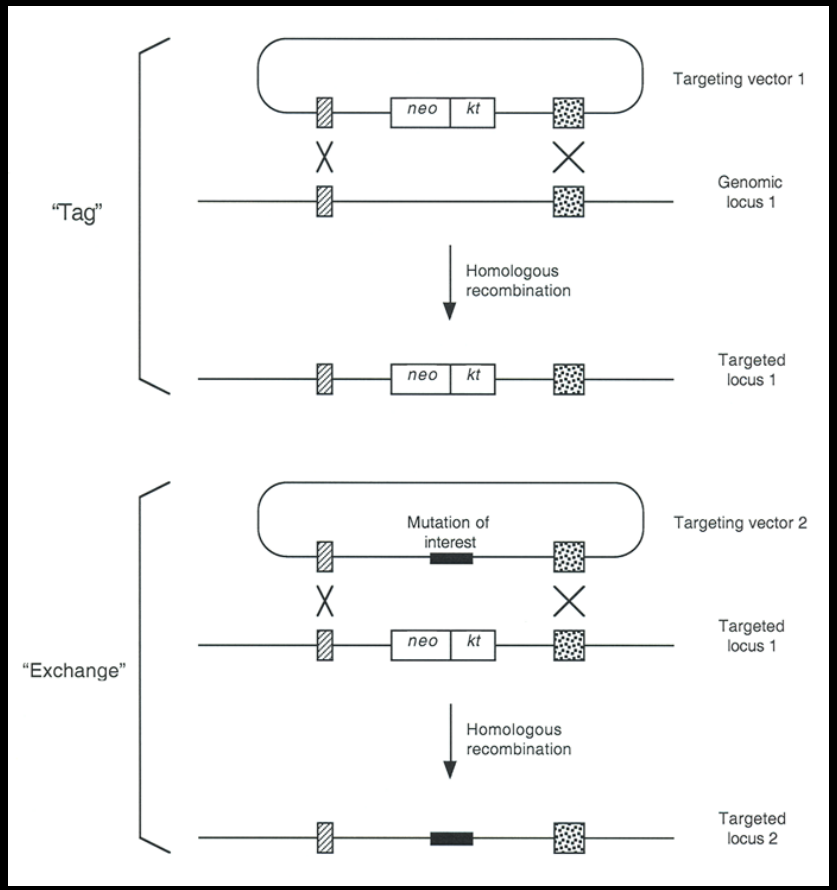
Xylanase activity

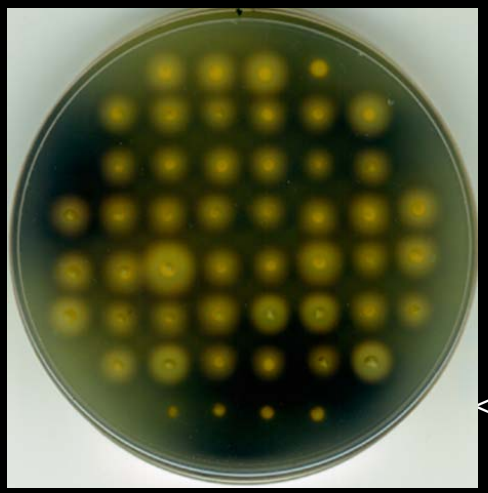
Control

Rye arabinoxylan hydrolysis

Recent Research Developments

III. Yeast Chromosomal Integration



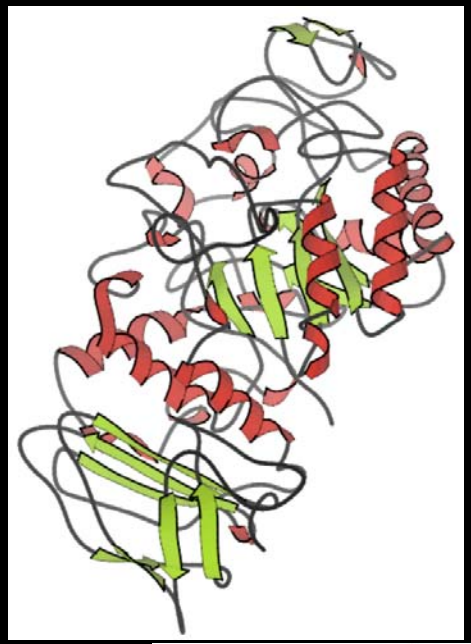


<= Control

- Integration of multiple genes
- at precise locations in the yeast chromosome

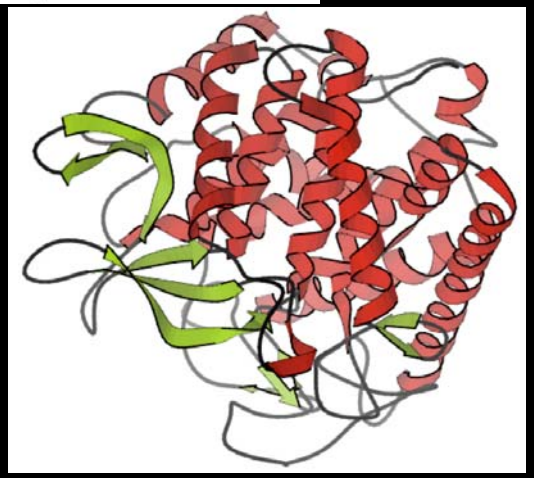
Fig. 19.3 Double-hit replacement

Signature Enzymes

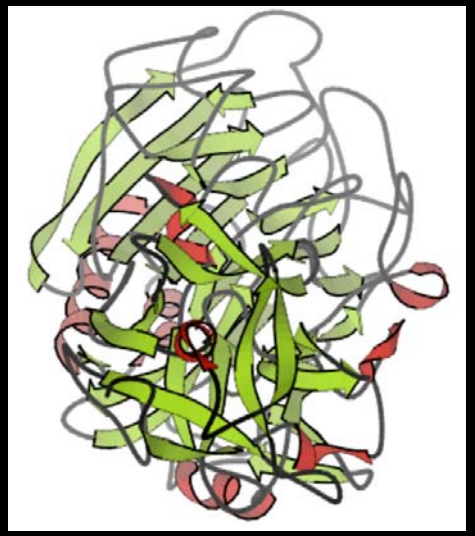


Mike Smith

Kurt Wagschal



Charles Lee



Dominic Wong

Straw for cellulose-to-ethanol

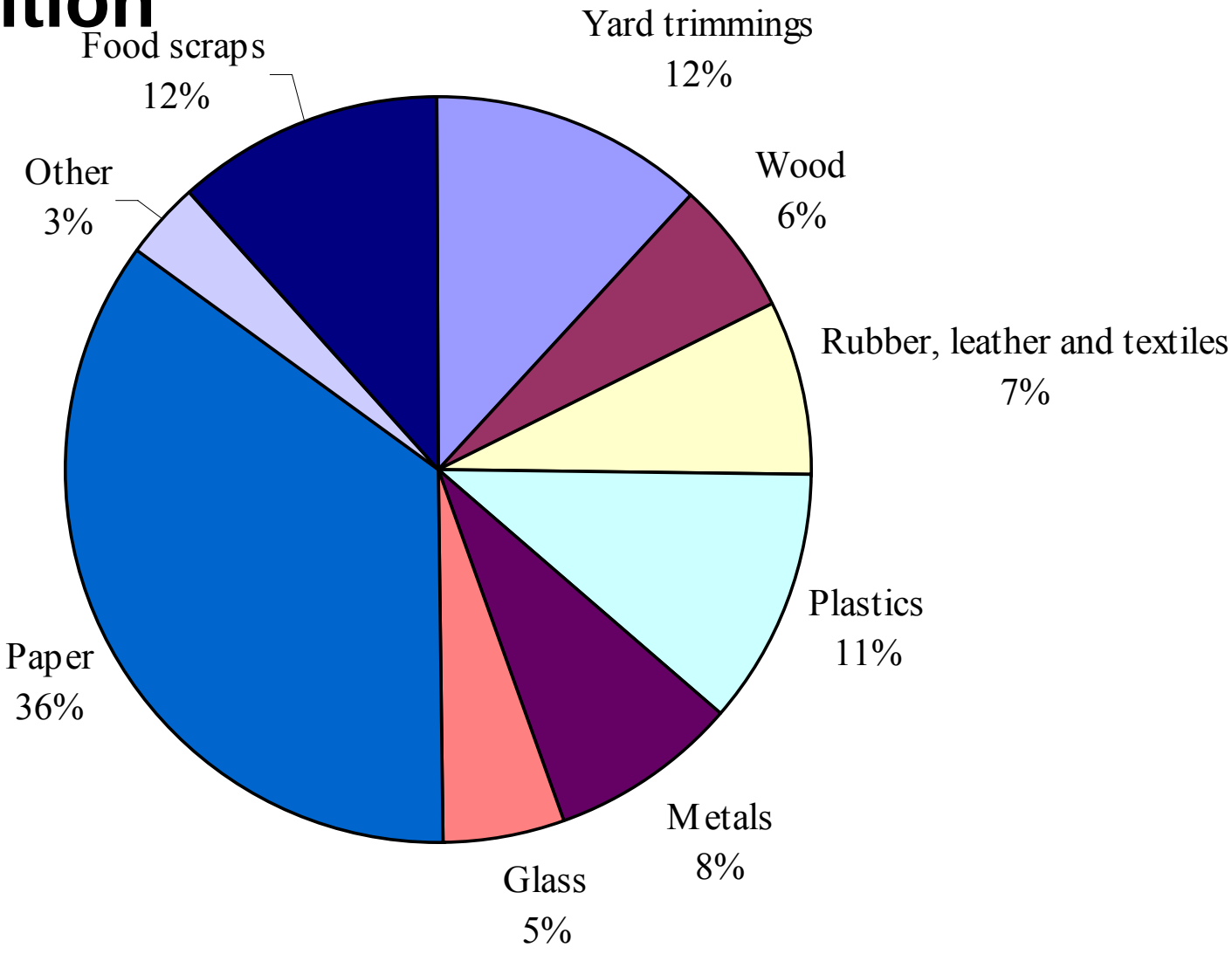
Kevin Holtman, William Orts, De Wood

ISSUES:

- **Straw varies with seasons**
- **Aging ⇔ harvest time is once per year**
- **Moisture and storage are challenging**
- **Transportation ⇔ Low density**
- **Supply is not near highest demand.**

Convert Municipal Solid Waste (MSW) to Ethanol

Composition

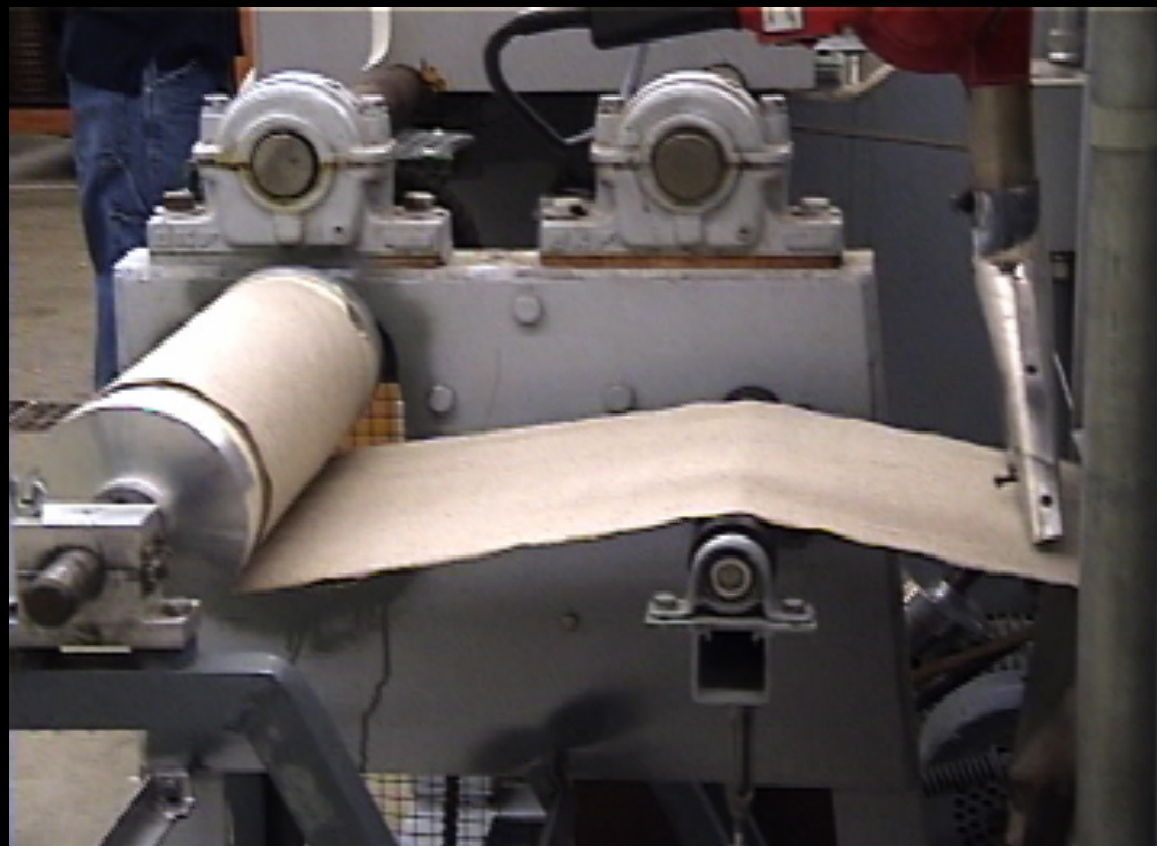


Biomass Pretreatment:

A compressed hot water treatment allows straw to be hydrolyzed relatively easily.

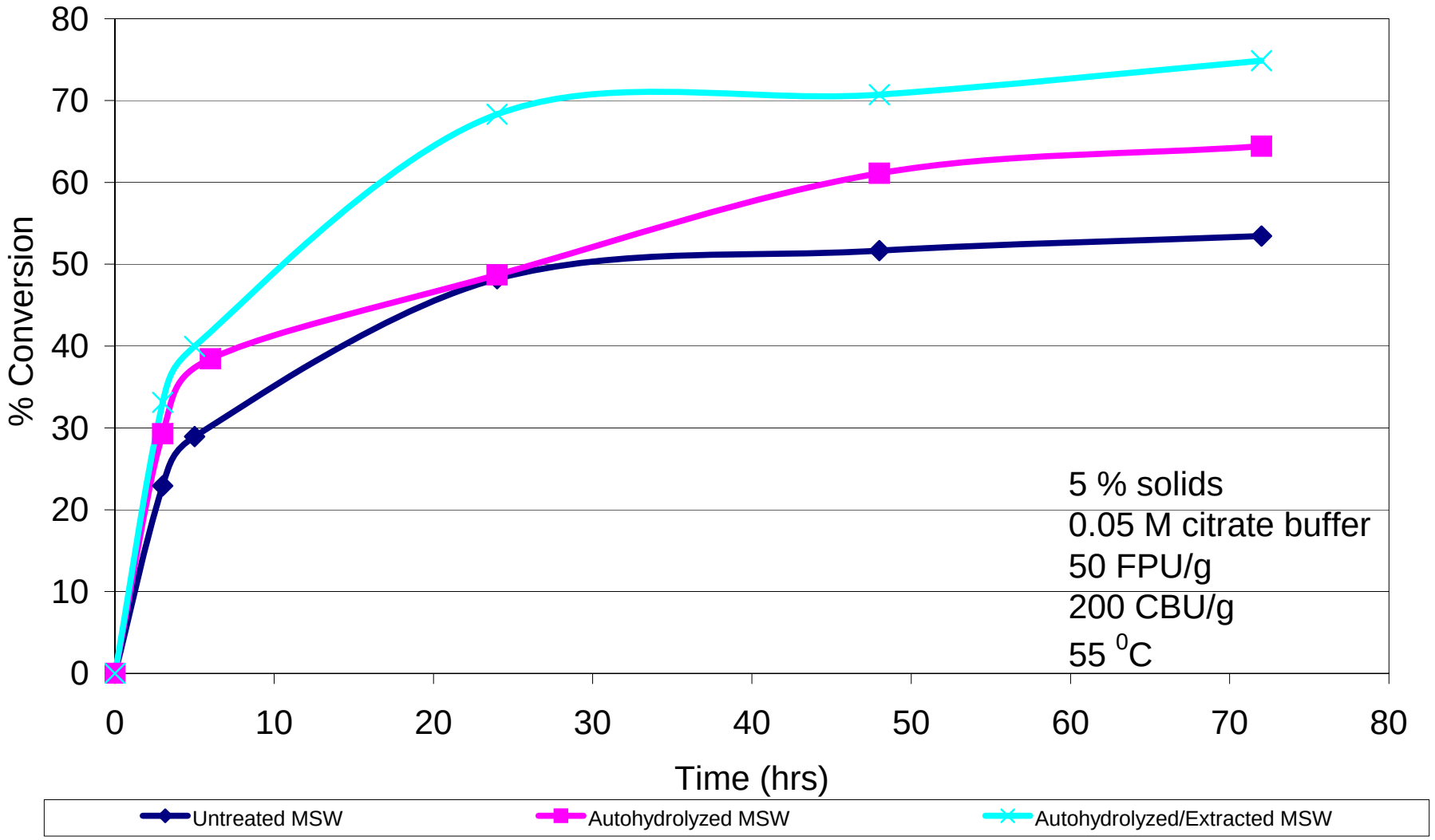
Cellulose-to-Ethanol Biorefinery ⇔ CR^3

Processed paper from recovered fiber

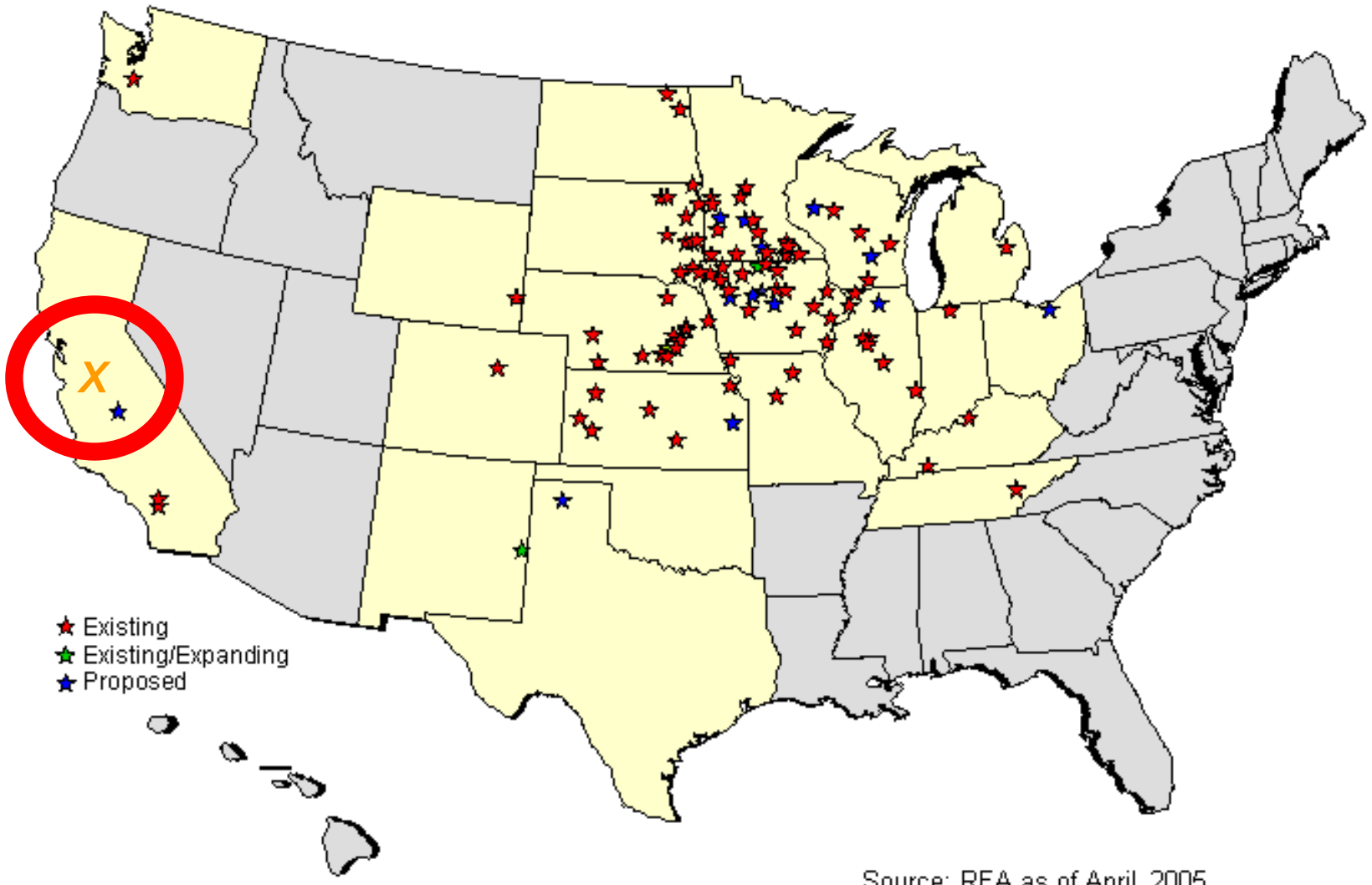


Biomass ⇔ MSW and ag-waste processing plant in Salinas

Enzymatic hydrolysis of MSW

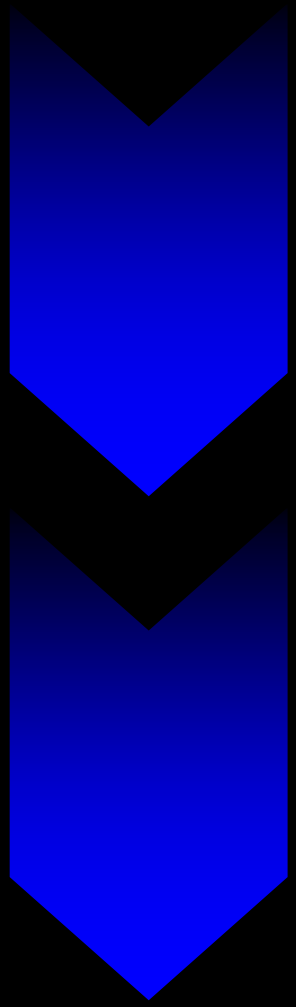


U.S. ETHANOL MANUFACTURING LOCATIONS



Source: RFA as of April, 2005

MSW as a Platform for Biomass-to-Ethanol Biorefinery



- MSW ⇔ 236 million tons/year in U.S.
- 35 - 45% paper and paperboard products
- Will reduce landfill volume by >40%
- In MSW, paper is already fractionated
- Can produce other co-products
  ⇔ Pulp ⇔ Methane⇔ Syngas
- Can readily mix in Ag-derived waste

Alcohols Recovery from Aqueous Mixtures

Richard Offeman, George Robertson

Low-energy alternatives to distillation

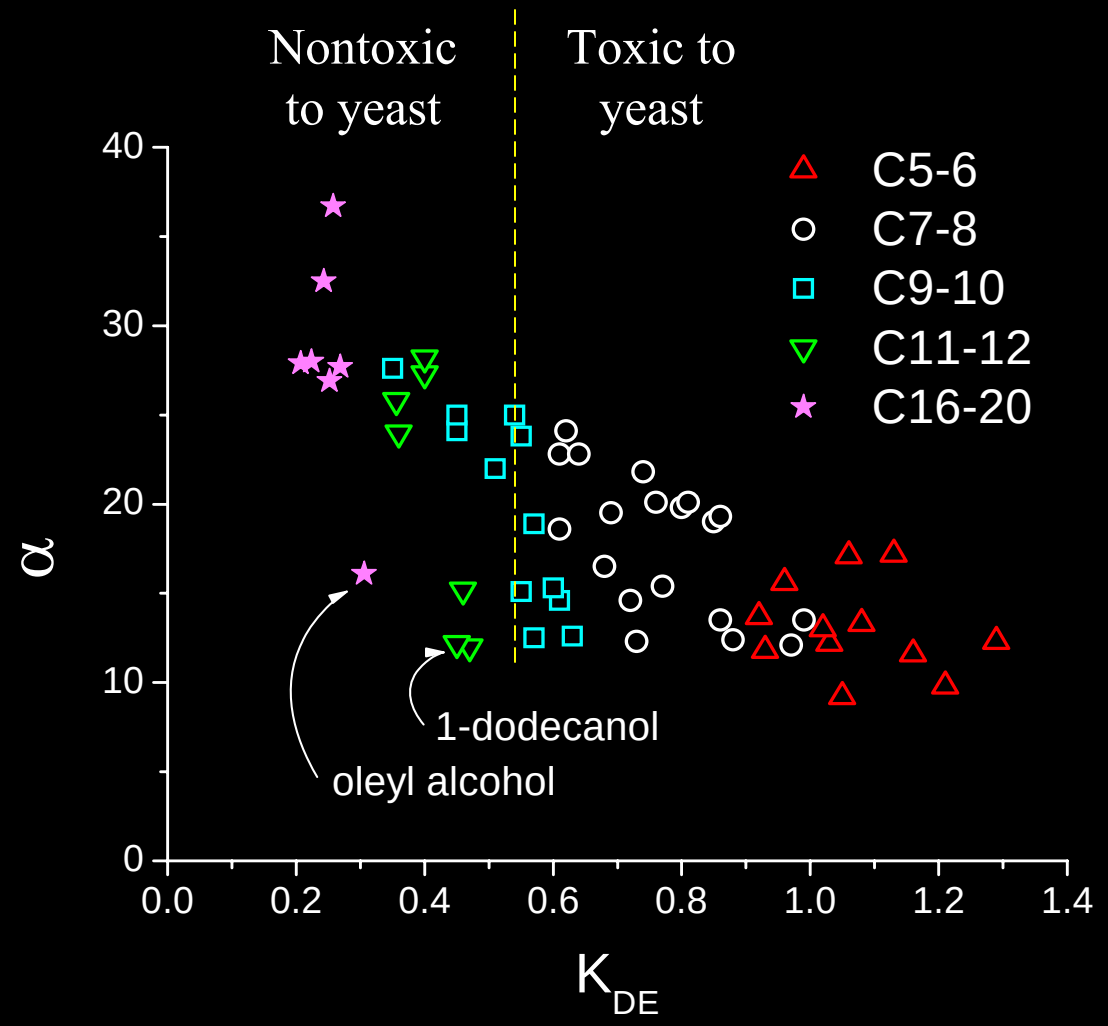
- **Solvent extraction, Membrane permeation**

Lower Energy Requirements

Overcome Product Inhibition

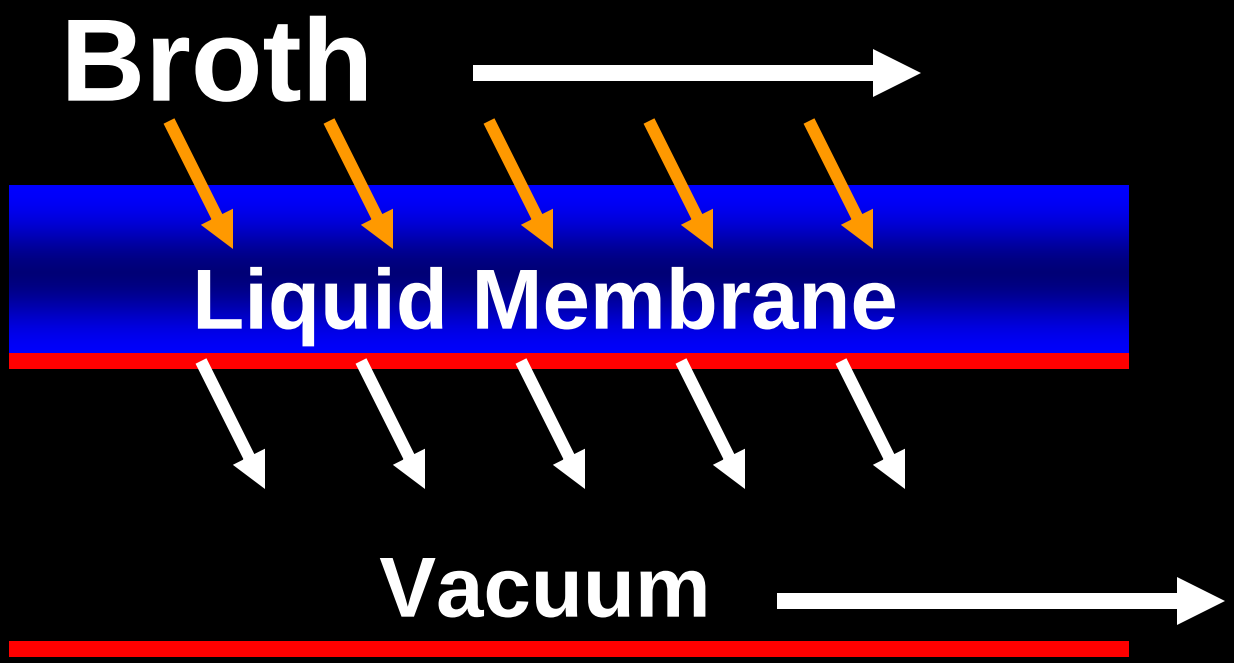
Continuous Fermentation?

Ethanol Extraction Using Alcohol Solvents



Found extraction solvents with double the separation ability of the standards

Ethanol Recovery from Aqueous Mixtures

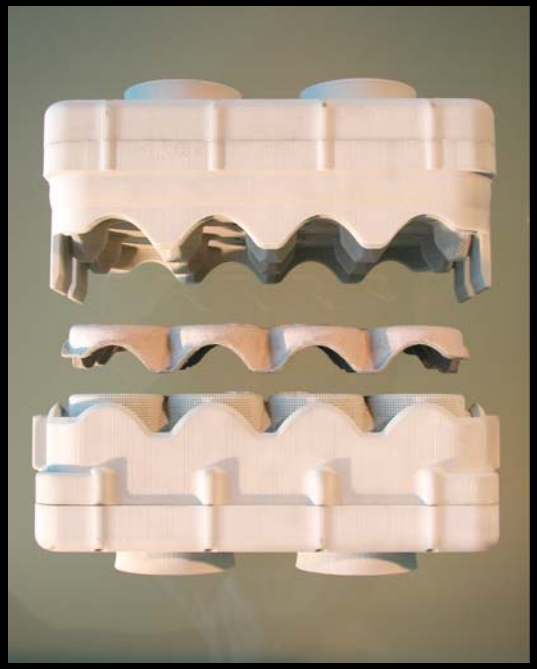


Hybrid extraction/membrane system

- Invention disclosure submitted
- Collaboration with MTR, Menlo Park

Straw Biomass Utilization:

Straw-based packaging



Summary:

MSW is a Platform for Biomass-to-Ethanol 'Athletic' Biorefinery

MSW is consistent, de-lignified, and can be mixed with ag-waste

New research in enzymes and microbes should lower costs

Energy crop improvements will increase yield and lower lignin, improving ethanol production

Bioproduct Chemistry & Engineering

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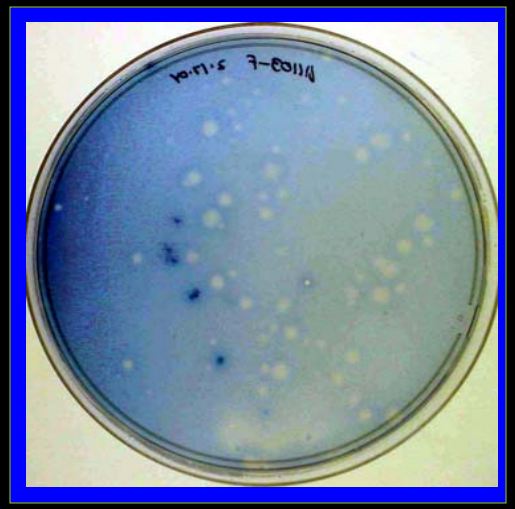
Bor-Sen Chiou
Greg Glenn
Kevin Holtman
Syed Imam
Charles Lee
Rick Offeman
Bill Orts
George Robertson
Mike Smith
Kurt Wagschal
Dominic Wong
De Wood

Ethanol from straw

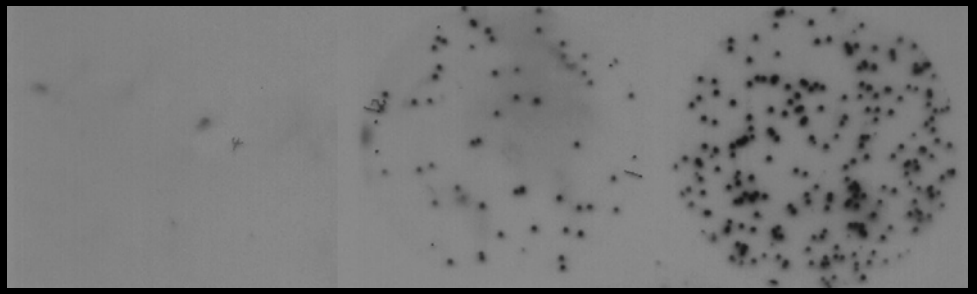
Thank you.

High-throughput Gene Screening

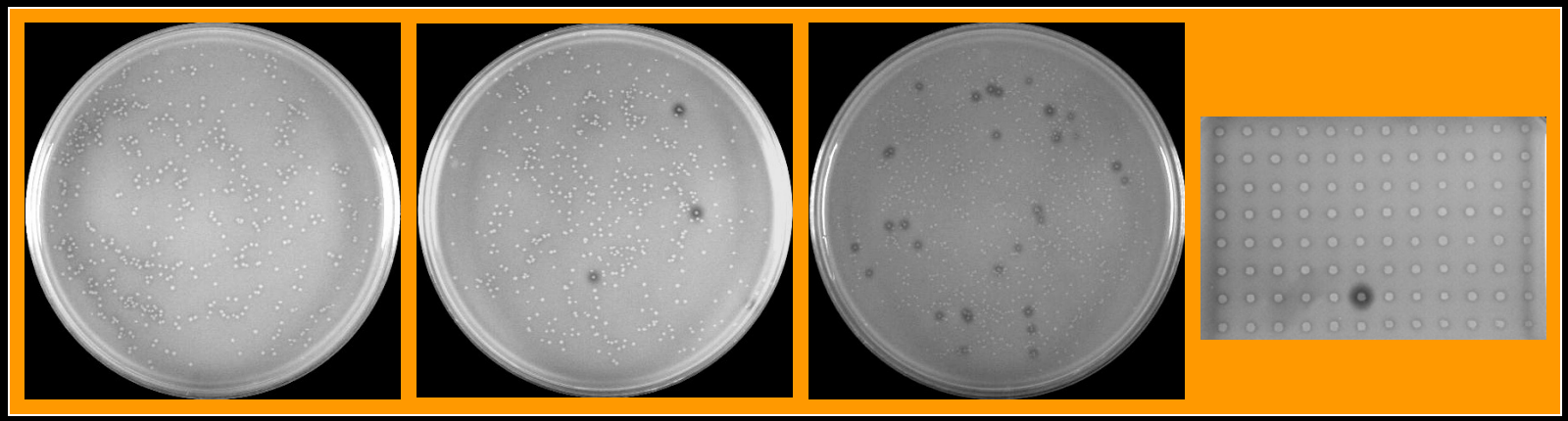


Screening for xylanase genes from metagenomic libraries

Screening for amylase genes from cDNA libraries



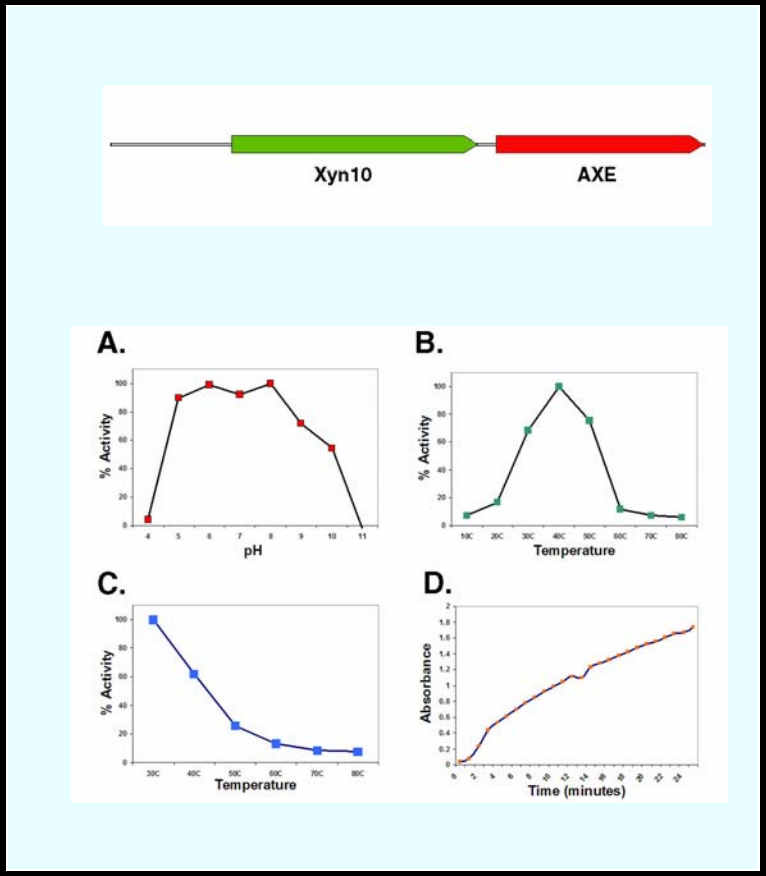
Screening for feruloyl esterase genes from metagenomic libraries



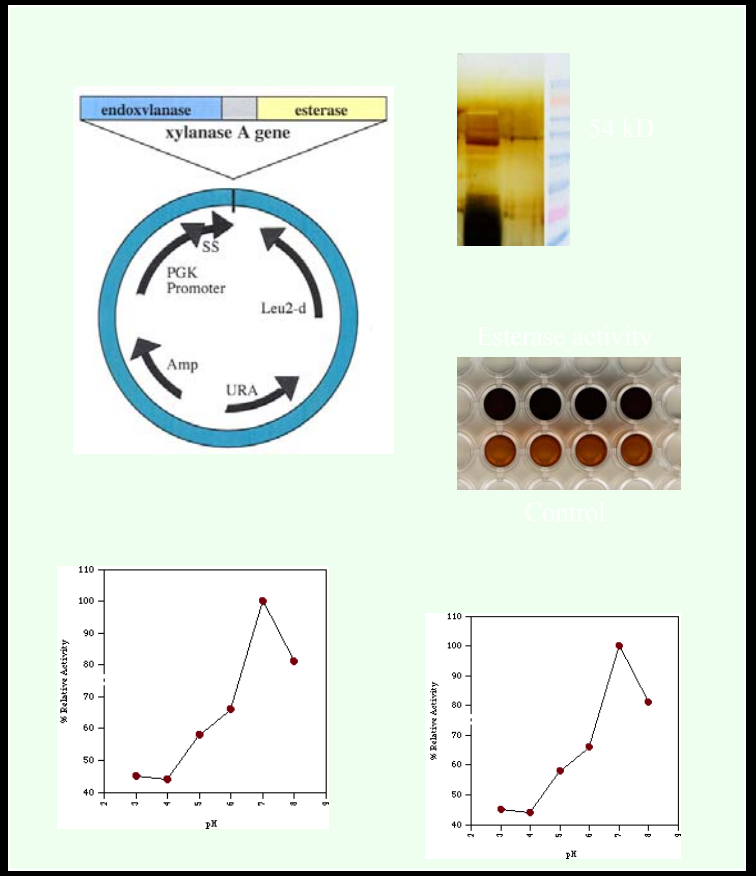
Recent Research Developments

I. Bifunctional Xylanase-Acetylxylan Esterases

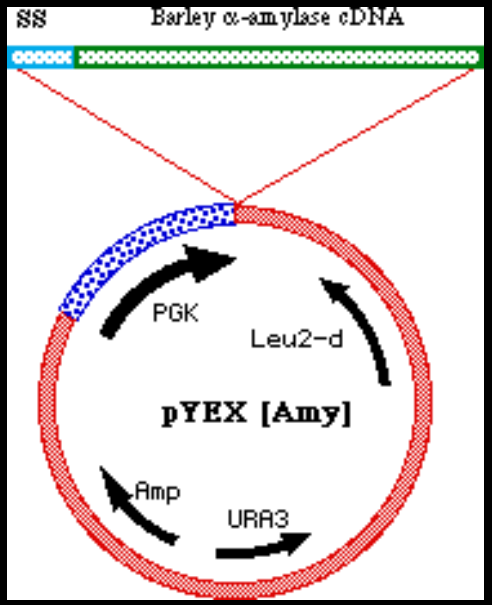
Xyn10-Axe gene



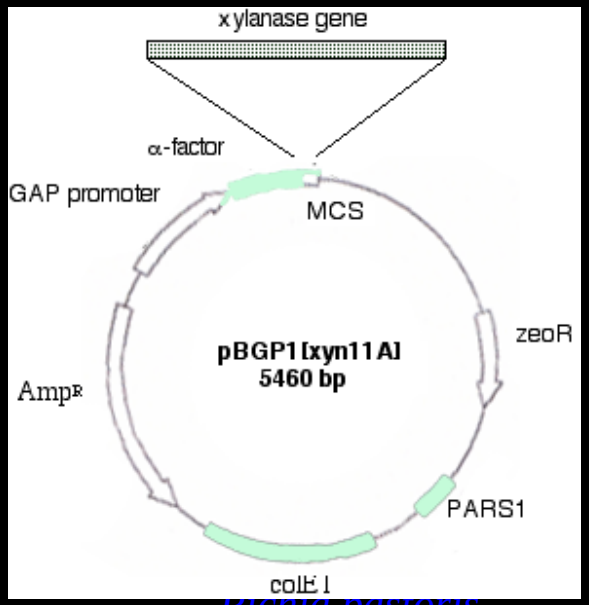
XynA-esterase gene



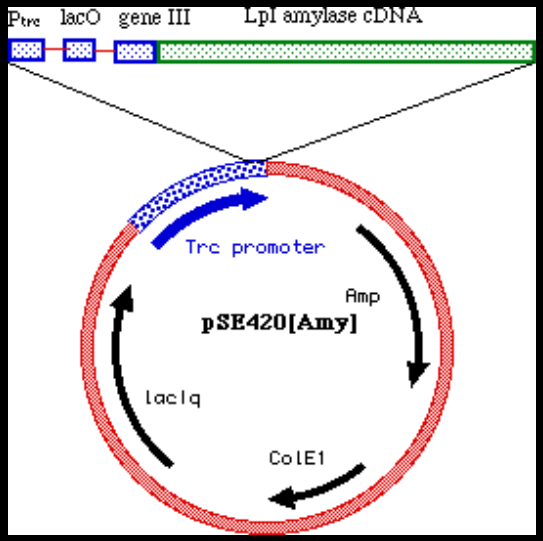
Gene-Vector constructs



Saccharomyces cerevisiae



Pichia pastoris



E. coli

- ***Mature proteins (correct N-terminus)***
- ***High level protein synthesis***
- ***Secretion of recombinant protein to culture medium***

Recent Research Extension

Two CRADAs

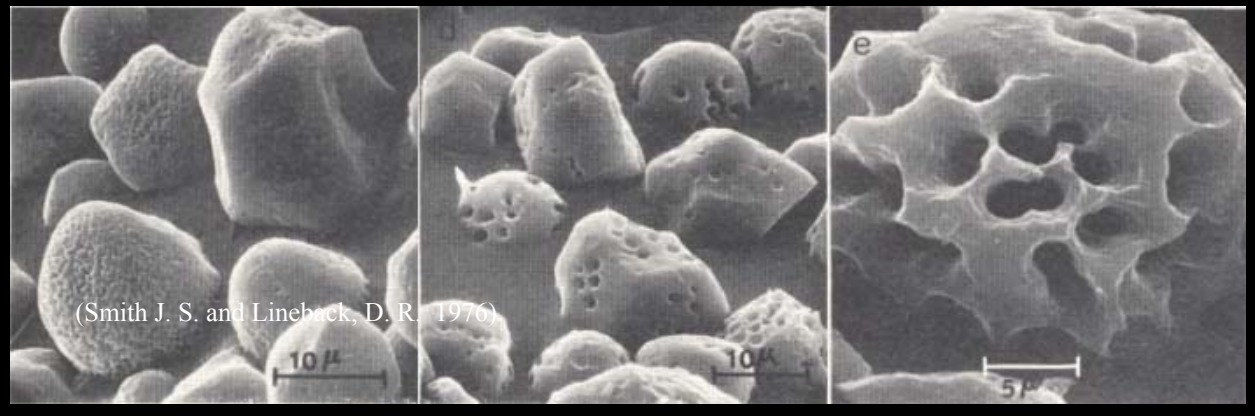
one postdoc
one technician

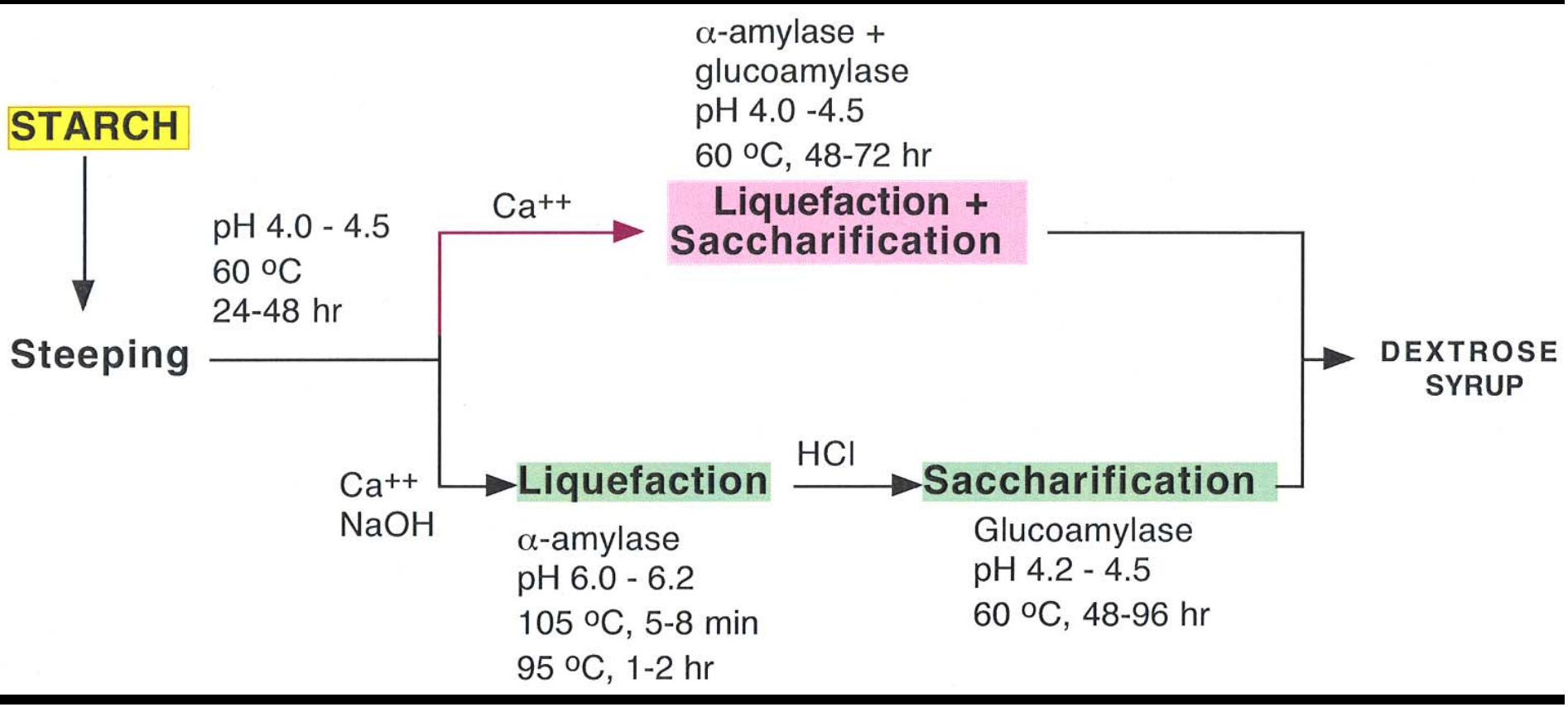
Two Patent Applications

Two Patent Disclosures

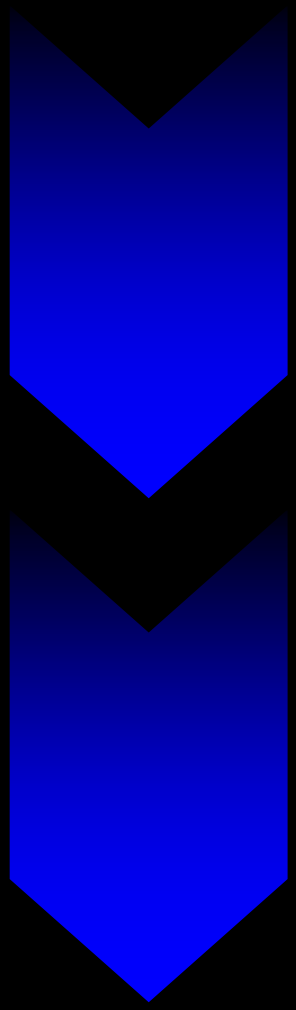
Collaborations: UC Davis, University of Kentucky……..

Starch - Cold Hydrolysis



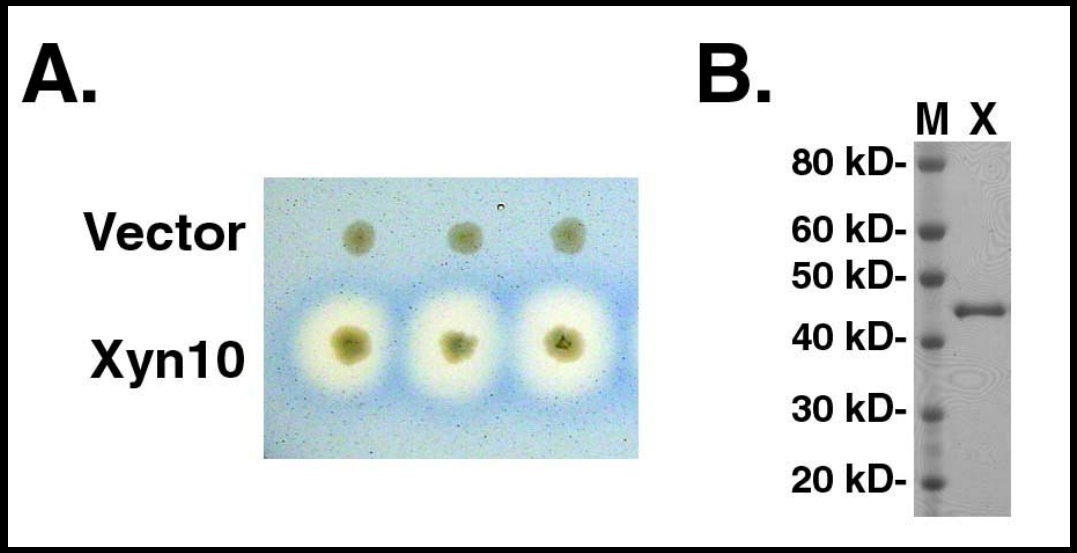


Biomass-to-Ethanol: Why it will work!



- Enzyme costs are greatly reduced
- Oil companies are participating
- Investors are appearing
- Models of “success” ⇔ US corn-to-ethanol, Brazil, Sweden
- Flex fuel cars are here

Discovering Genes Encoding Novel Lignocellulolytic Enzymes

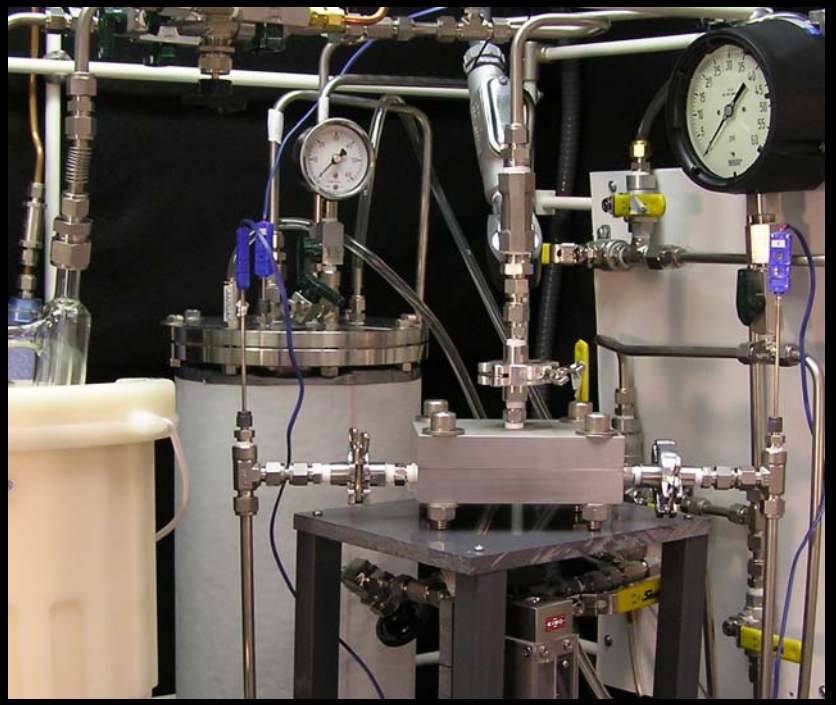


20 Novel xylanase genes.

2 Novel β-xylosidase genes.

2 Novel esterases.

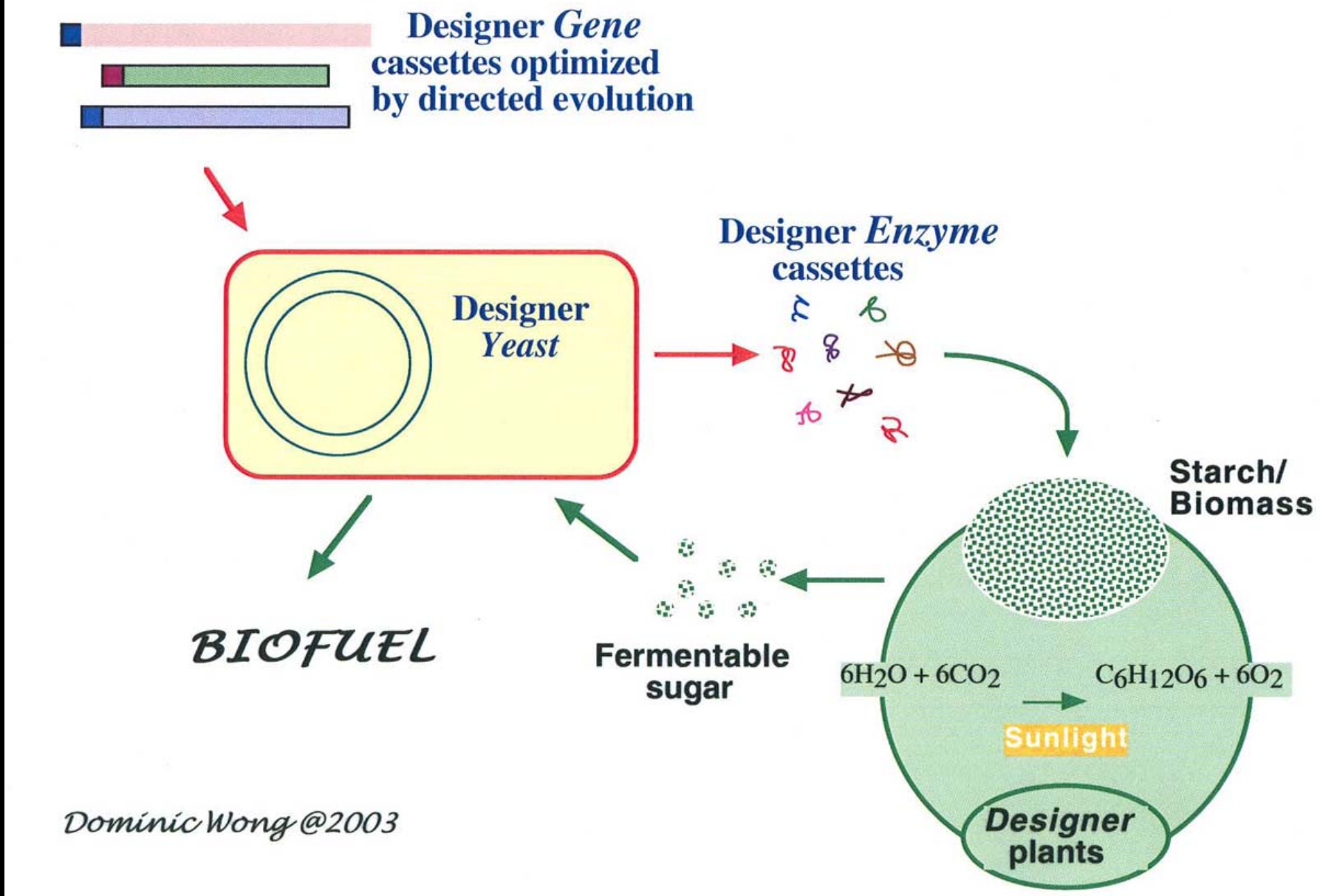
Ethanol Recovery by Membrane Permeation



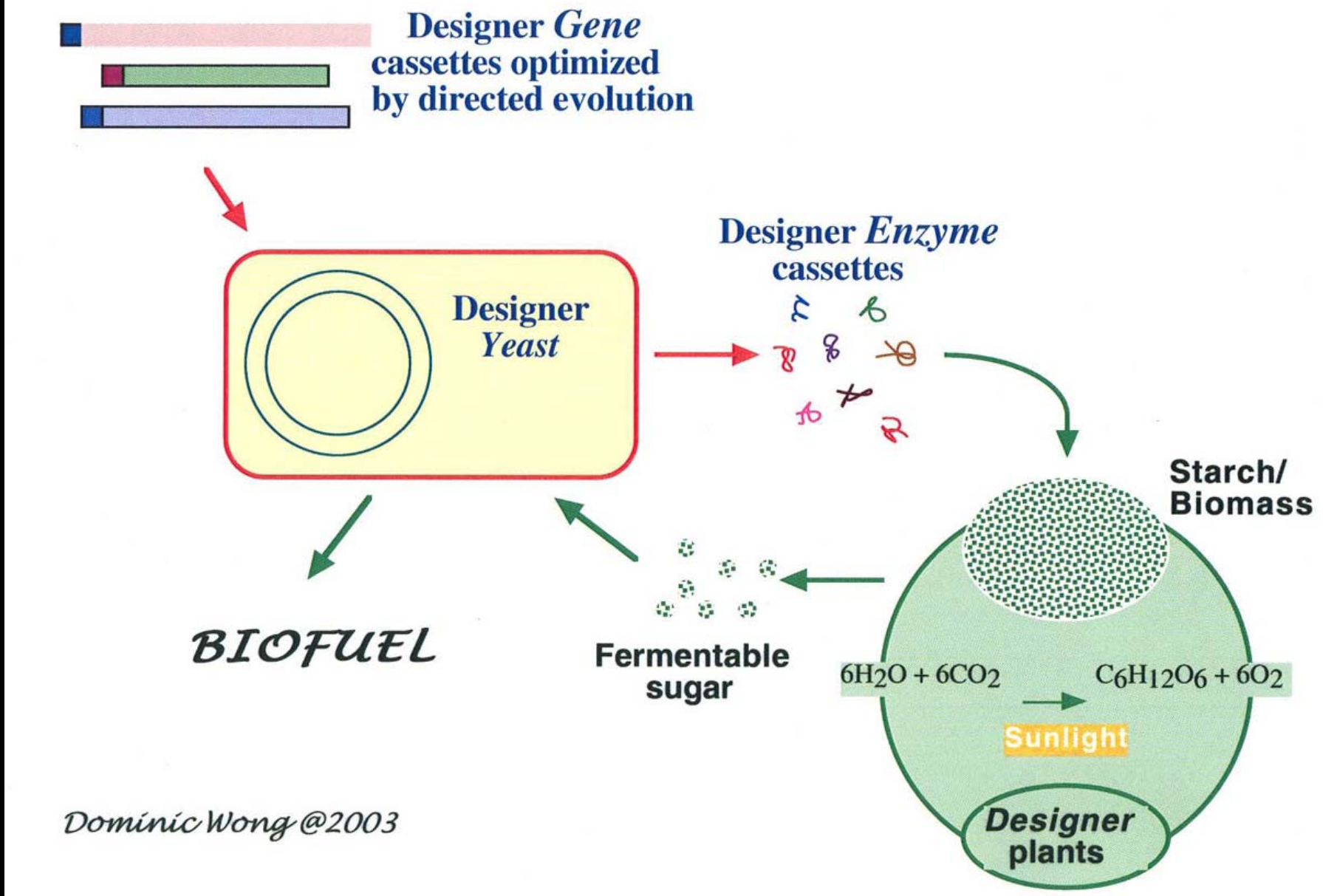
Approaches

- Pervaporation
  - **Mixed matrix membranes**
    - CRADA with Chevron
  - **Block copolymers** (defined domain structures)
    - Helios (LBNL, UCB, …) collaboration
  - **Supported liquid membranes**
    - Patent application for novel module design
- Hybrid processes

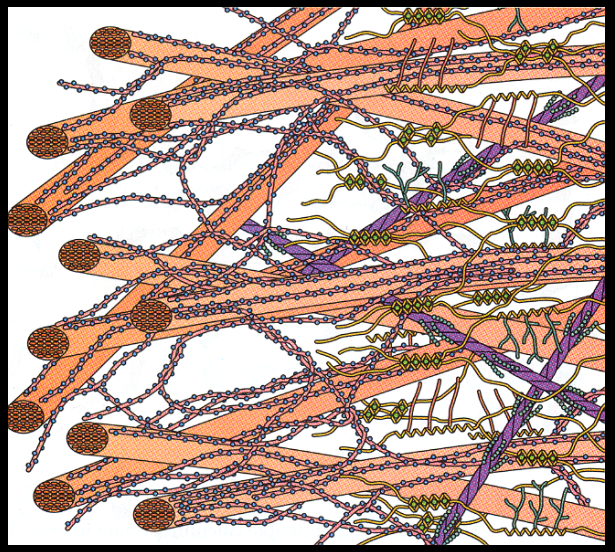
One-Pot Conversion

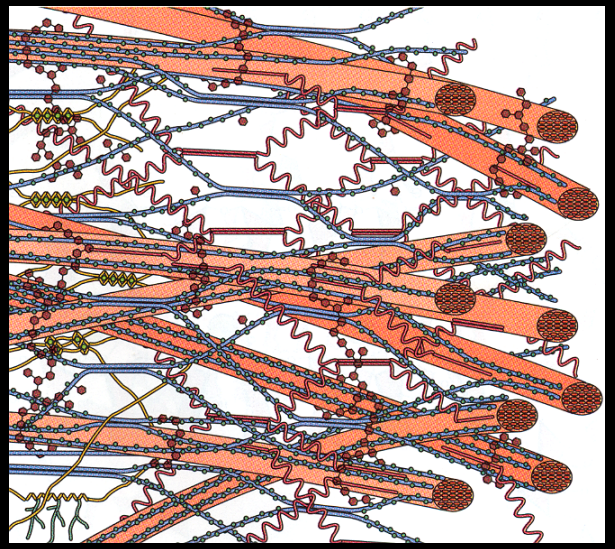


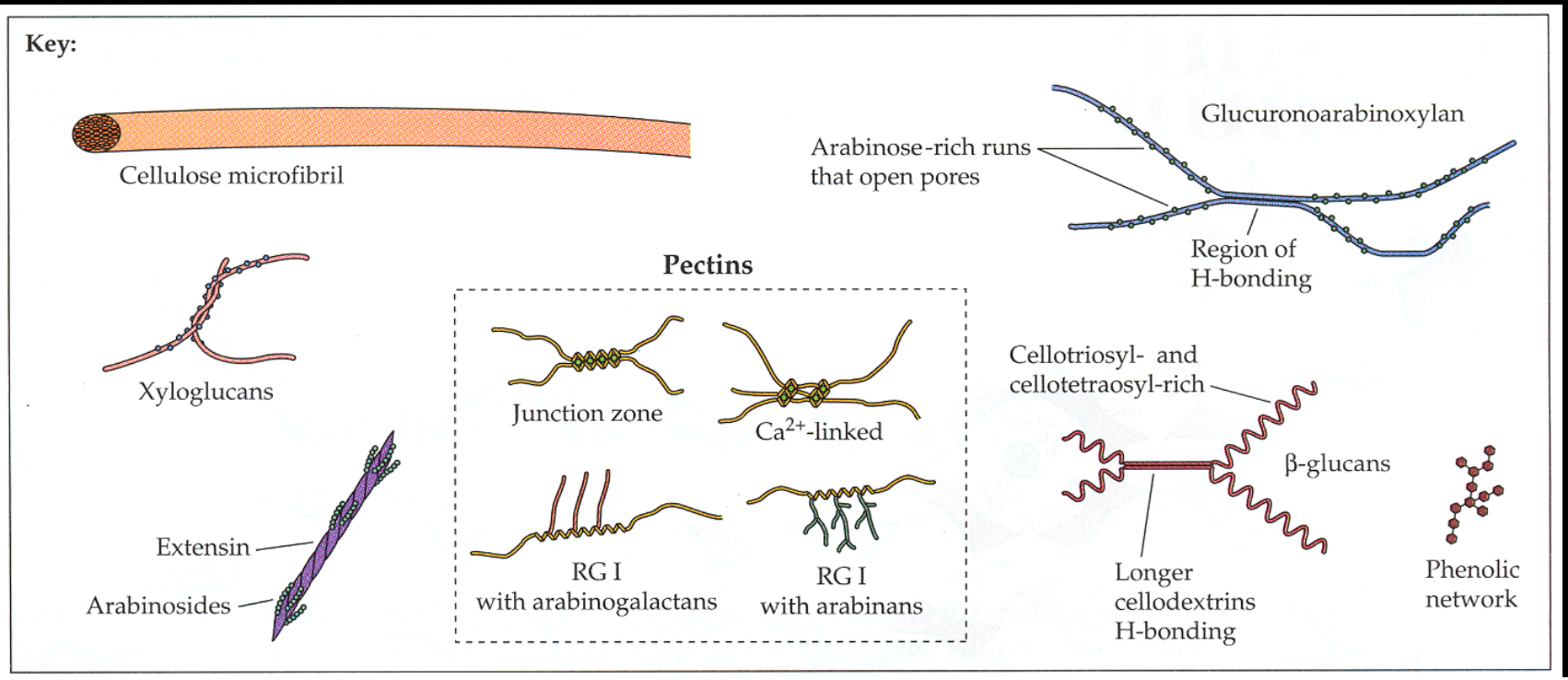
One-Pot Conversion



Cell wall structures







Cellullose Isolation from MSW and Ag-Waste

- The fraction sent through 3-5mm screens and washed is rich in cellulose!!