UNIVERSITY of GUELPH

CHANGING LIVES
IMPROVING LIFE

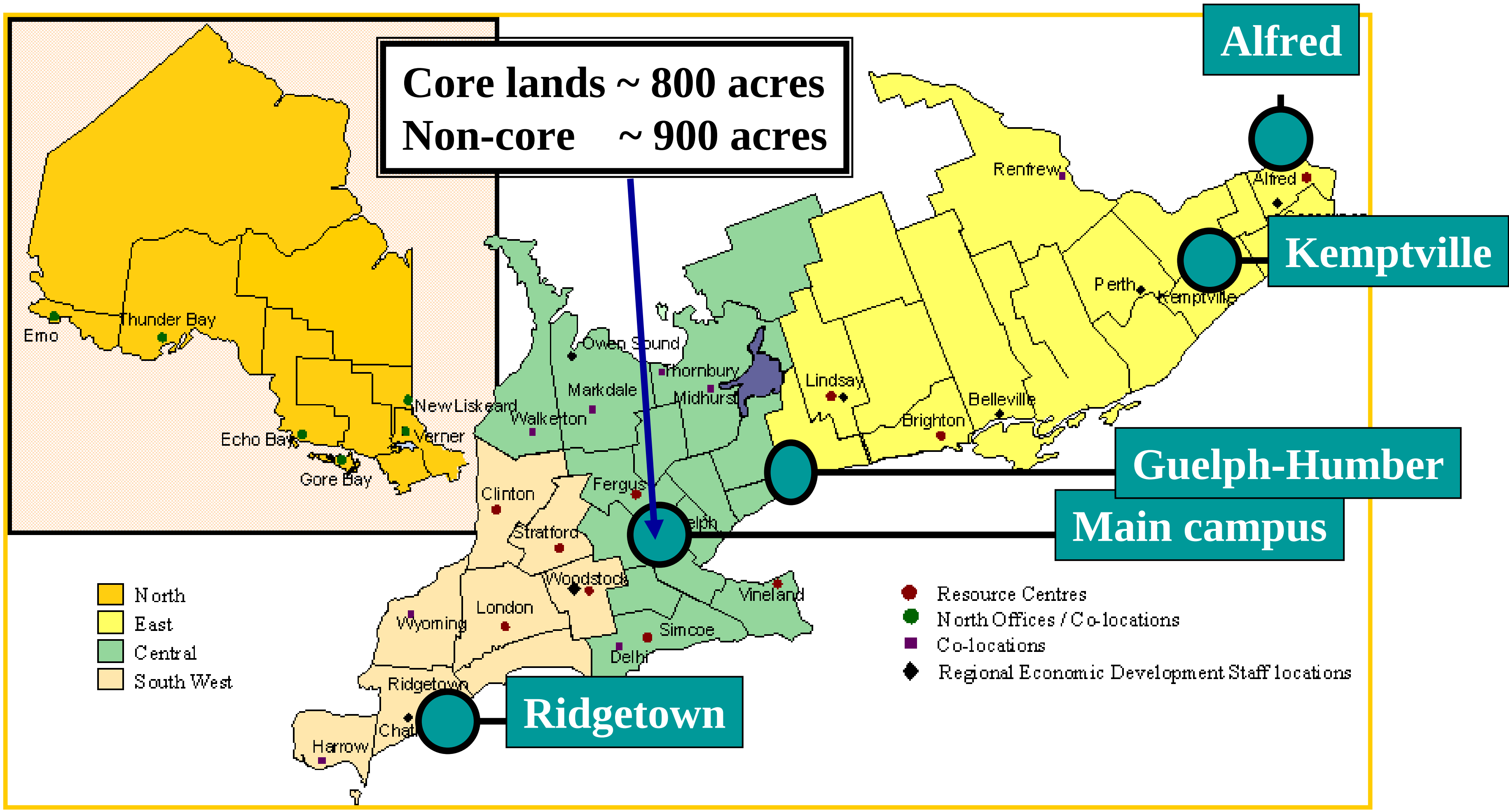
Biomass/Cellulosic Energy Research at Guelph

Transforming Biomass for the Bioeconomy

Steven N. Liss, PhD
Associate Vice-President, Research Services
University of Guelph, Guelph, Ontario Canada

University of California – *System-Wide Technology Transfer Forum April 3, 2008*
Canada   Canadian Consulate General, San Francisco
Hyatt Regency San Francisco Airport, Burlingame, California

Large Geographic Spread



Core lands ~ 800 acres
Non-core ~ 900 acres

Alfred

Kemptville

Guelph-Humber

Main campus

Ridgetown

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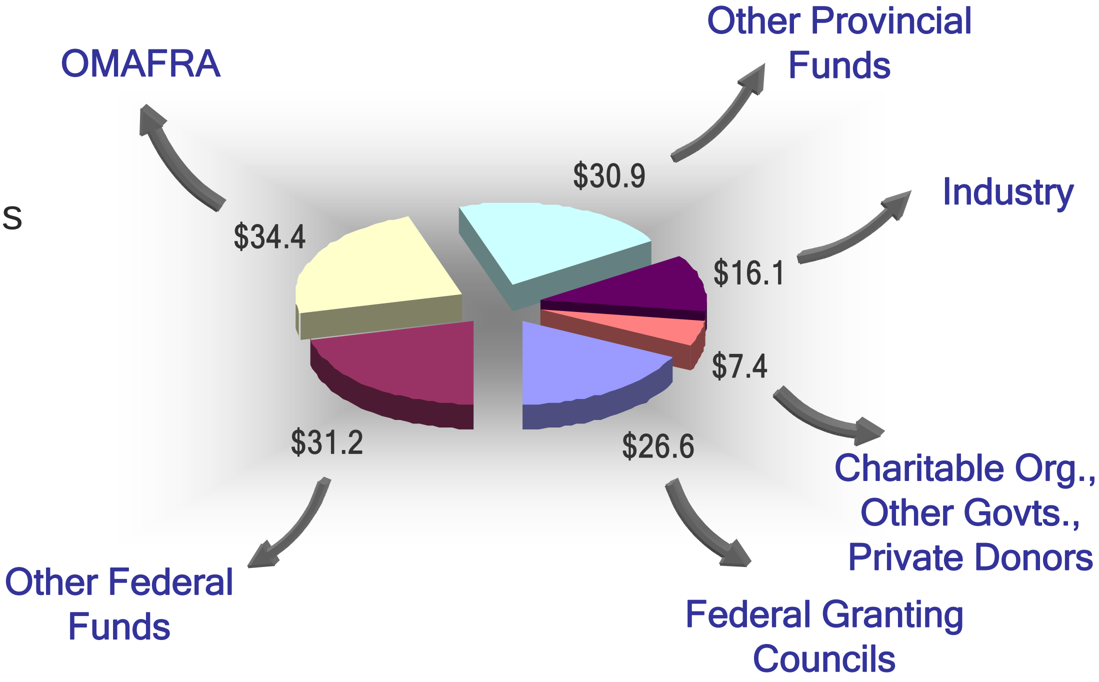
Facts and Figures

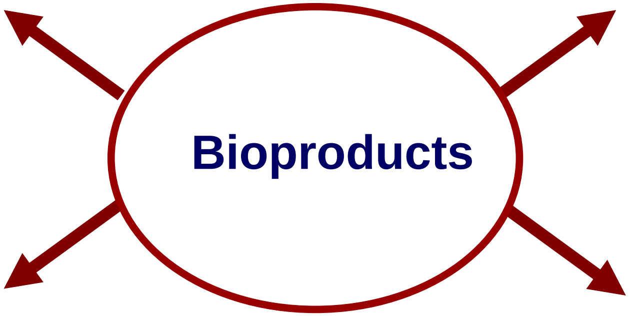
- **Institutional revenue**
  - ~$ 510 million
- **Research support**
  - ~ $145 million
- **Undergraduate students**
  - 16,000 - Guelph campus
  - 2,500 - Guelph-Humber
- **Diploma students**
  - 1,000 - Regional campuses
- **Graduate students**
  - 2,000 - Guelph campus
- **Distance Education**
  - > 51,000 registrants
- **Faculty**
  - ~800
- **Staff**
  - **~1,900**



- UG/OMAFRA partnership returns $3 for every $1 received in the form of various impacts. The annual economic impact exceeds $1.15 billion

Deloitte and Touche LLP UG/OMAFRA Impact Study 2007

Agriculture, Life Sciences, Physical and Engineering Sciences



Energy

Biodiesel from crop oils

Ethanol from ligno-cellulose

Biogas, cogeneration

Food and Agri-Forestry

Crop breeding/biotech

Livestock nutrition

Production systems, environment

Health and Nutrition

Omega 3 in dairy products, etc.

Probiotics in eggs and milk

Clinical trials on functional foods

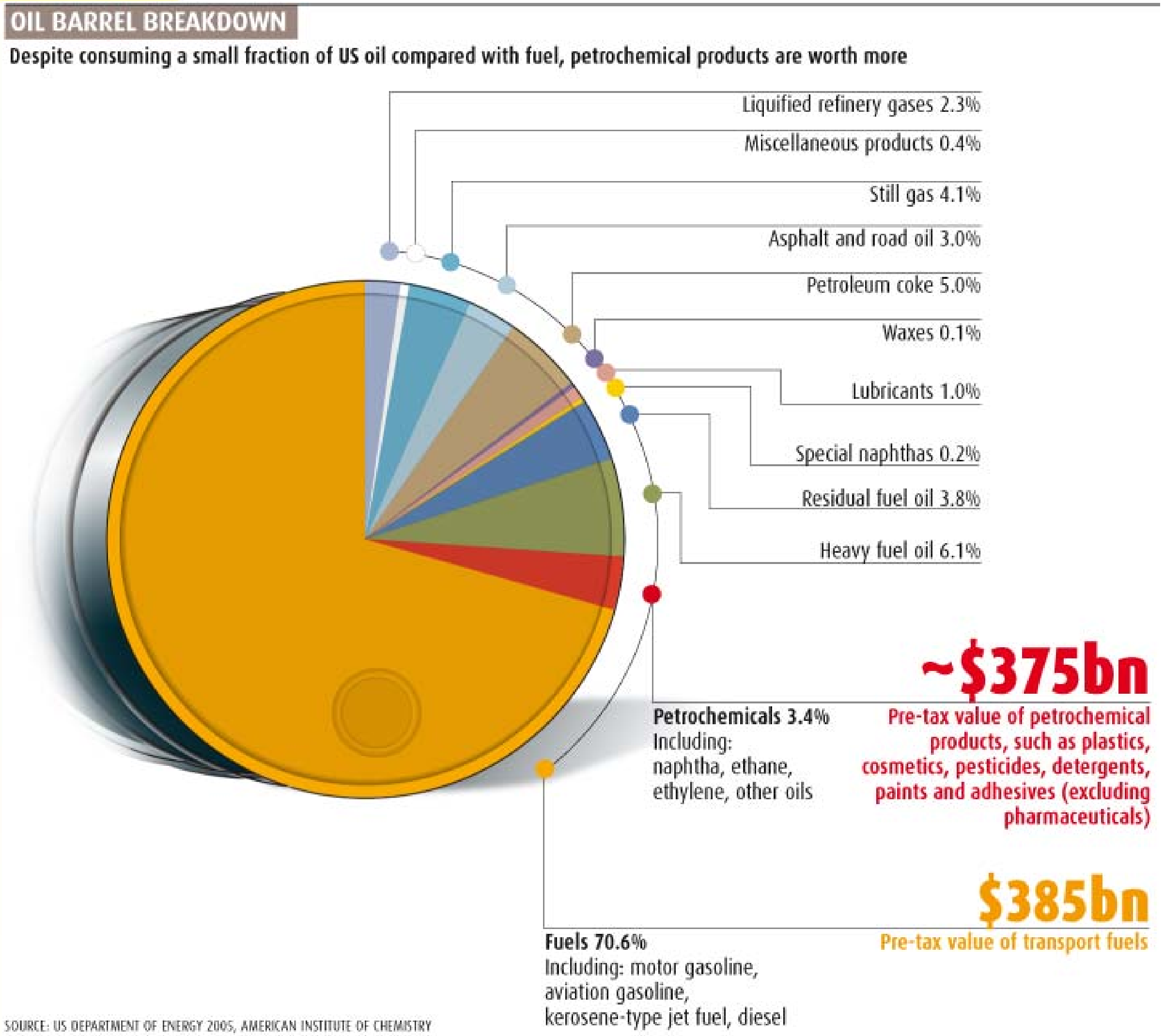
Industrial-Manufacturing

BioCar (Premier’s Research Chair)

Polylactic Acid

Construction materials

Producing Chemical Feedstock from Biomass



OIL BARREL BREAKDOWN

Despite consuming a small fraction of US oil compared with fuel, petrochemical products are worth more

SOURCE: US DEPARTMENT OF ENERGY 2005, AMERICAN INSTITUTE OF CHEMISTRY

Source: New Scientist 2007

- Only ~ 3.4 % of all crude oil processed ends up in petrochemicals – over 70 % is used as fuel, the remaining goes towards asphalt, coke, bitumen, wax, and other products.
- **In spite of this, these chemicals are worth as much as the fuel !**
- Because the total volume of material is comparatively small there is little competition with food production …

Ontario BioCar Initiative

Goal

Accelerate the use of biomass in automotive materials, while maintaining or improving value

Focus

Replacement of petroleum-based products by biochemicals and high content bio-fibre materials, from the field and forests

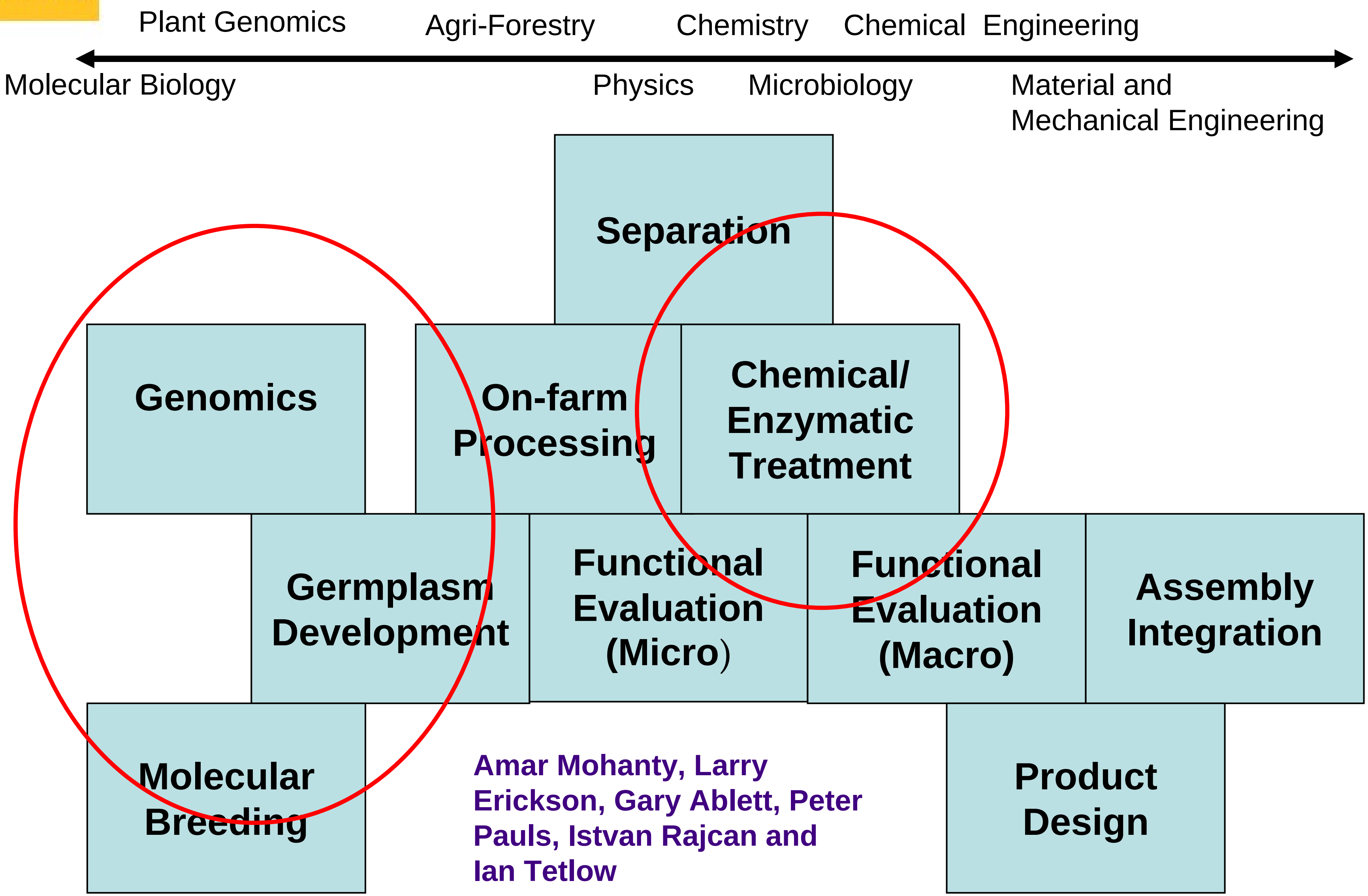
Ontario BioCar Initiative

UNIVERSITY of GUELPH

VELUT ARBOR ÆVO

University of Waterloo

WINDSOR



Plant Genomics Agri-Forestry Chemistry Chemical Engineering

Molecular Biology Physics Microbiology Material and Mechanical Engineering

Separation

Genomics

On-farm Processing

Chemical/ Enzymatic Treatment

Germplasm Development

Functional Evaluation (Micro)

Functional Evaluation (Macro)

Assembly Integration

Molecular Breeding

Product Design

Amar Mohanty, Larry Erickson, Gary Ablett, Peter Pauls, Istvan Rajcan and Ian Tetlow

Project
Genes to Fields: Corn Biotechnology Capacity for Ontario

Background

- Corn is one of the world's most important food crops
- More recently, corn became the foundation of a new "green" economy (renewable corn-based ethanol and industrial polymers)
- Need to to make corn production more profitable and sustainable
- Annual corn production in Ontario is worth $1 billion

Goal

Test genes that hold the potential to:

1. **increase yields of corn food/fibre,**
2. **combat farmer losses caused by Ontario’s unpredictable/short-growing seasons, and**
3. **reduce agricultural greenhouse gas emissions.**

Project
Genes to Fields: Corn Biotechnology Capacity for Ontario

Approach

Corn domestication for adaptation in Northern climates can be accelerated by using modern genetic resources

Established partnership with Syngenta, a leading agbiotech company

$9M funding from the Ontario Ministry for Research and Innovation, Syngenta, NSERC and the University of Guelph

Research Team

Steven Rothstein (lead PI)
Joseph Colasanti
Elizabeth Lee
Manish Raizada
Matthijs Tollenaar

Lignocellulosic biomass conversion

- Two primary processes:
  - Thermochemical conversion
  - Biochemical conversion (Bioconversion)
- Potential substrates:
  - Agricultural and forestry residues
  - Waste paper and newsprint
  - Dedicated short rotation plants and crops

Four steps in bioconversion

1. Lignocellulosic substrate pretreatment
   - By chemical and physical methods

→ 2. Enzyme hydrolysis (*Anthony Clarke*)
   - Cellulases, xylanases & debranching enzymes

→ 3. Microbial fermentation (*Hung Lee*)
   - Yeasts and bacteria

4. Co-product development & product recovery

Lignocellulosic biomass conversion is technically feasible, but process economics are poor based on today's technology

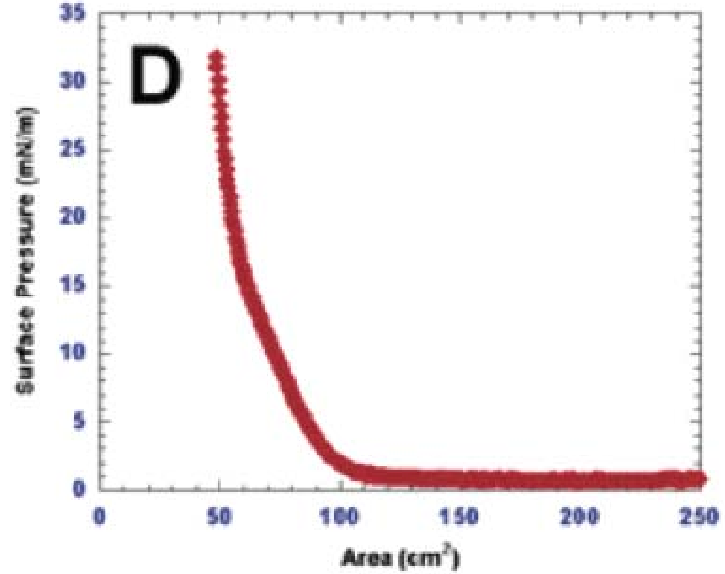
Advancing the Development of Cellulase

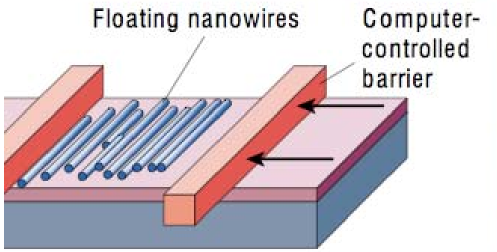
- **Goal:** *Increase the overall efficiency of the process using genetically modified derivatives of the enzymes (cellulases and cellobiohydrolases) which will include alterations to the carbohydrate-binding modules and their associated tethers.*

- **Key Objectives and Approach**
  - Gain an insight into the binding of cellulolytic enzymes to crystalline cellulose.
  - Both wild-type an engineered enzymes with modified carbohydrate binding modules are being investigated by atomic force microscopy as they bind to and degrade this cellulose.
  - Bacterial cellulose at the moment as a model system to layer it onto a gold surface using the Langmuir-Blodgett technique in an aqueous environment - this has not
    been done before and Dr. Clarke's group has succeeded.

Langmuir-Blodgett

- Logs on a river
  - Use cellulose dispersion
- Order
- Reproducibility

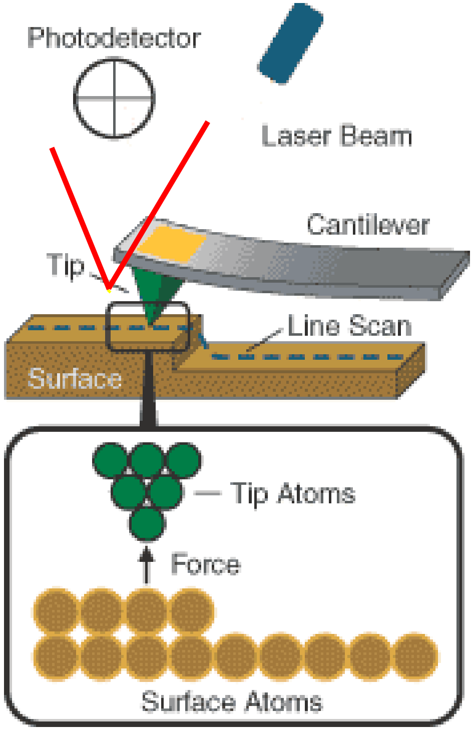


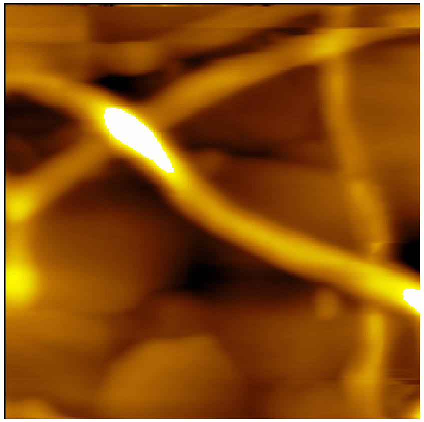


Yang. Nature 425 (2003) 243

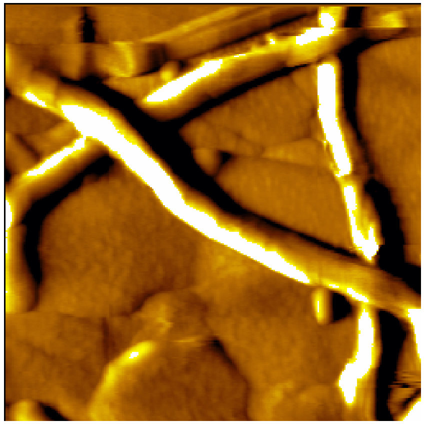
Atomic Force Microscopy

- Soft contact mode
  - Interaction between tip and sample
  - Constant contact
  - Detection modes:
    - Topography
    - Deflection
    - Friction





Topography

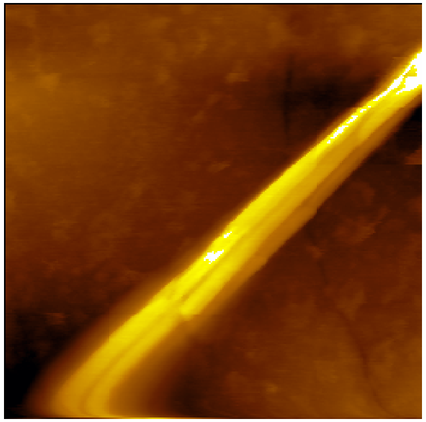


Deflection

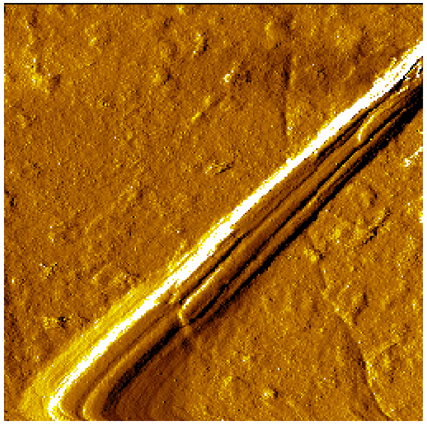
molec.com

Atomic Force Microscopy

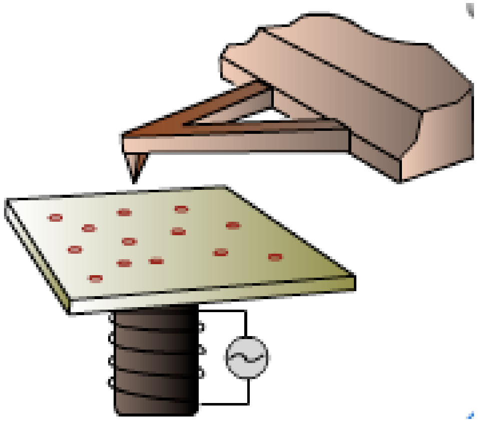
- MAC mode
  - Tip oscillated by applied magnetic field
  - Intermittent contact
  - Ideal for soft samples in liquid environments
  - Detection modes:
    - Topography
    - Amplitude
    - Phase



Topography



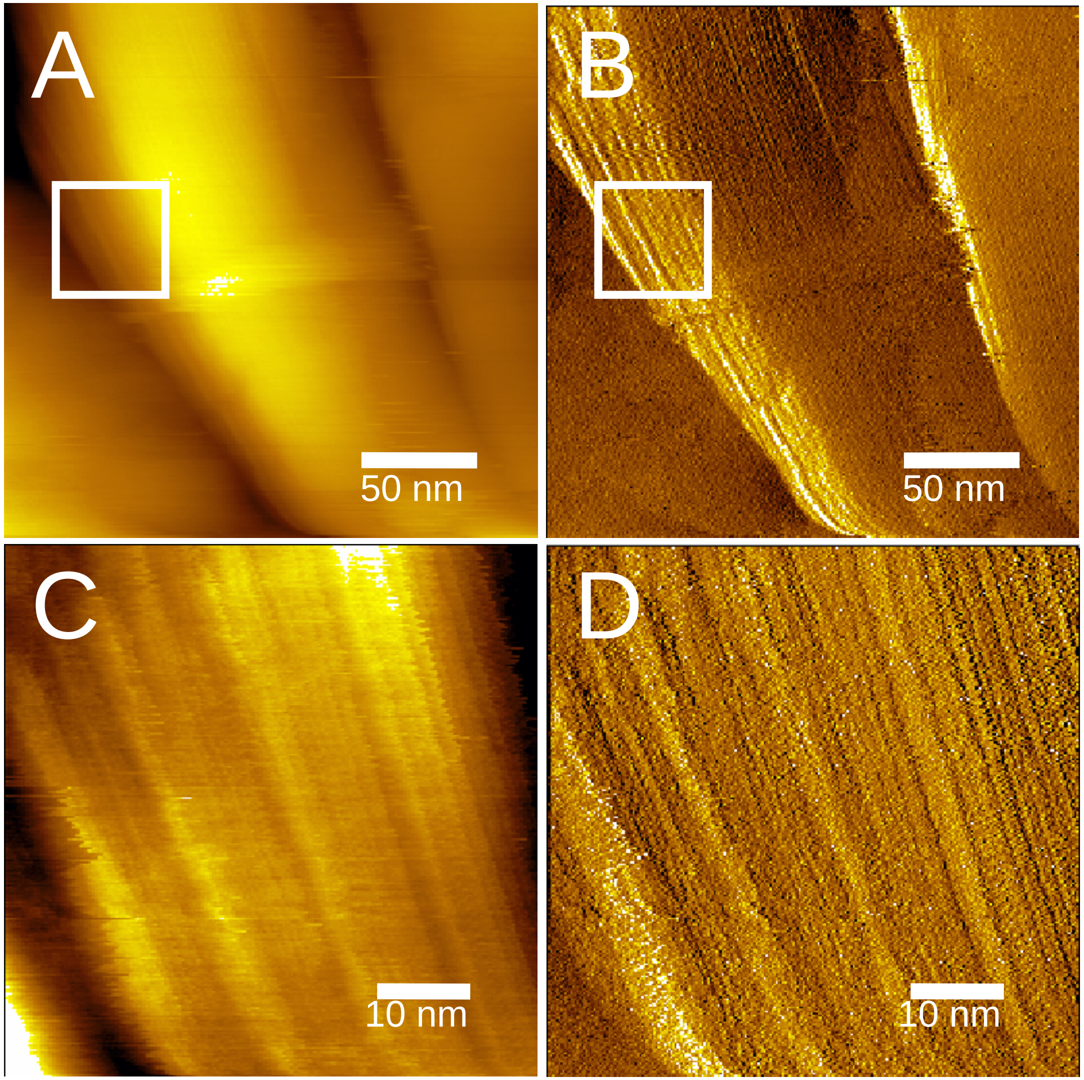
Amplitude



molec.com

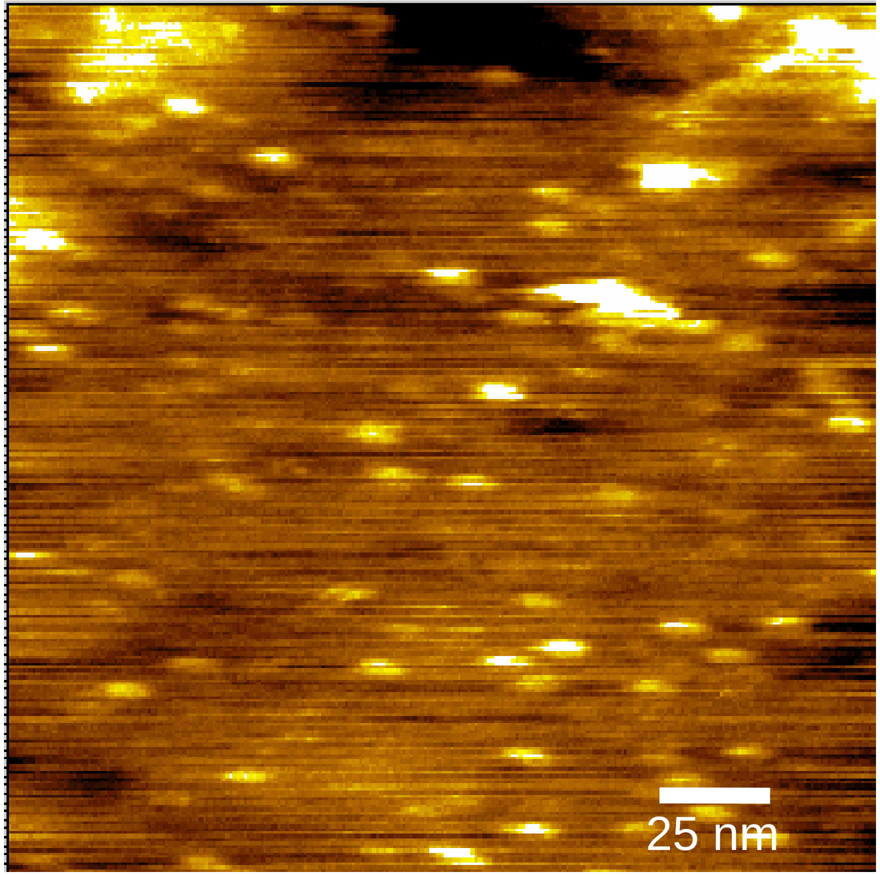
AFM High Resolution

- Soft contact mode
- Cellulose fibers are made up microfibrils
- Microfibrils 4 nm in diameter



Cellulomonas fimi CenA

- Image CenA on gold to determine size and shape
- MAC mode



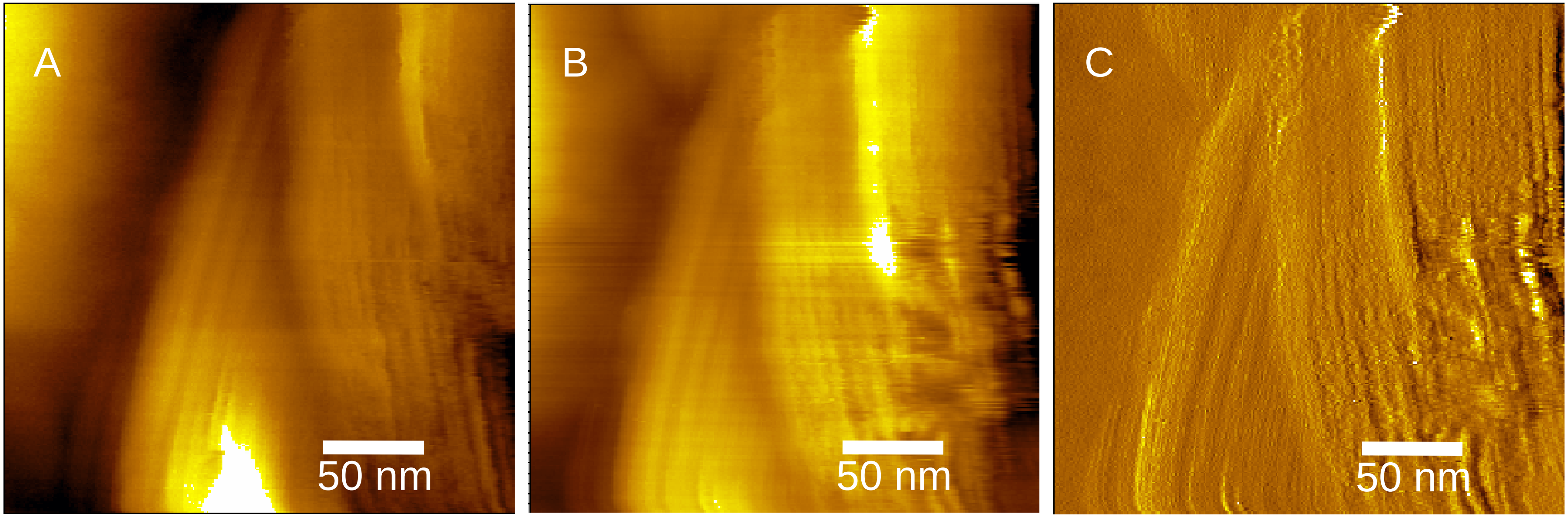
CenA is a bacterial cellulase (beta-1,4-glucanase) comprised of a globular catalytic domain joined to an extended cellulose-binding domain (CBD) by a short linker peptide.

In Situ Degradation with CenA

- MAC mode

Topography

Amplitude

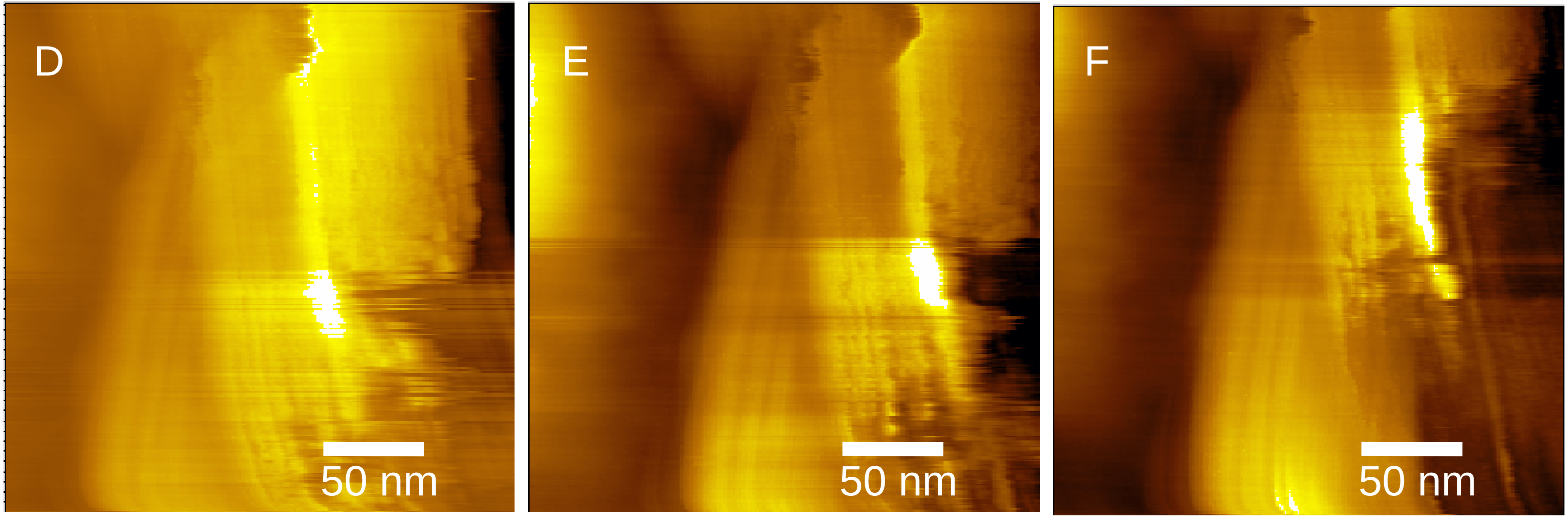


Before enzyme

3 minutes after

In Situ Degradation with CenA

- MAC mode



15 minutes 30 minutes 60 minutes

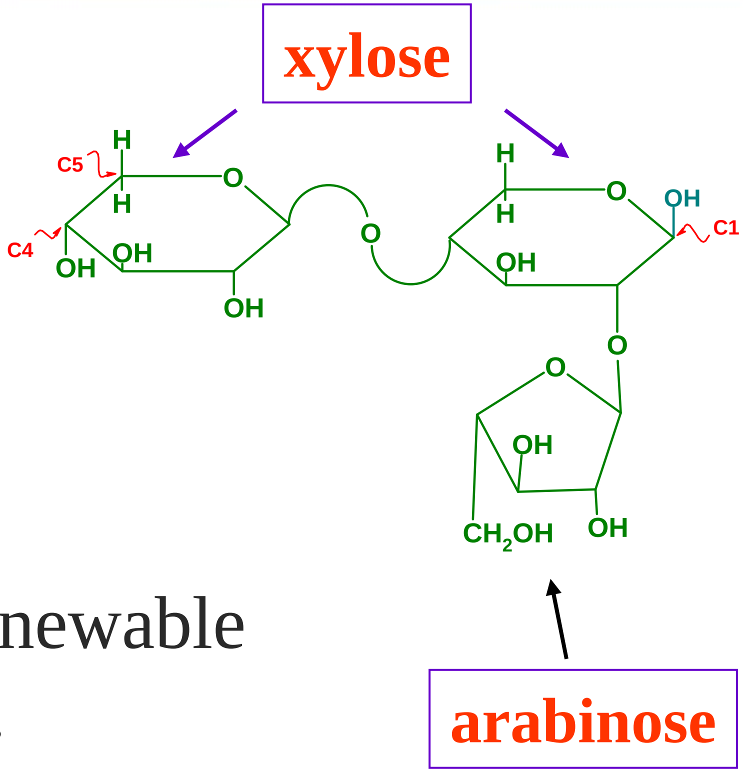
Pentose-Fermenting Yeasts

- **Two main lines of research**

1. **Biochemistry and physiology of pentose-fermenting yeasts**
   - Researcher: **Hung Lee** (U of Guelph)

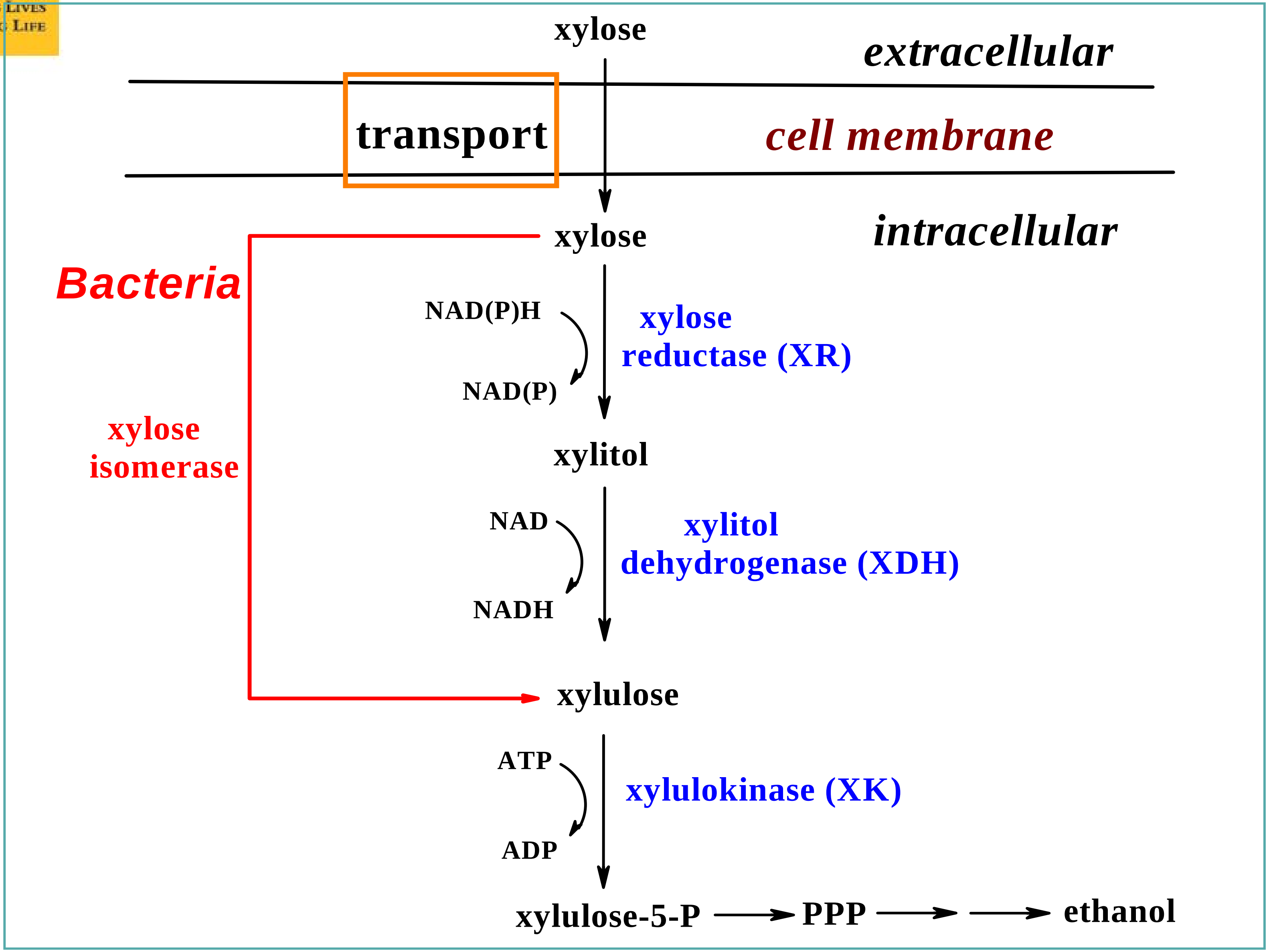
2. **Genetic improvement of pentose-fermenting yeasts**
   - Researchers: **Hung Lee** (U of Guelph), **Vince Martin** (Concordia U) & **Jack Trevors** (U of Guelph)
   - Partners: **Mike Paice** (FPInnovations – Paprican division) & **Benji Ahvazi** (Tembec)

Pentoses constitute 10-40% of total structural sugars in plant biomass



- **D-xylose**.
  - The second most abundant renewable sugar in nature, after glucose.
- **L-arabinose** is much less abundant than xylose.
- Lignocellulosic biomass sugars also include the hexoses **glucose, mannose and galactose**.

Xylose metabolism in yeasts and fungi



xylose

extracellular

transport

cell membrane

xylose

intracellular

Bacteria

xylose isomerase

NAD(P)H

NAD(P)

xylose reductase (XR)

xylitol

NAD

NADH

xylitol dehydrogenase (XDH)

xylulose

ATP

ADP

xylulokinase (XK)

xylulose-5-P → PPP → → ethanol

Biochemical studies on xylose reductases

- Focused on the structure-function relationships of yeast xylose reductases.
  - This enzyme is essential for growth of yeasts on xylose
  - This enzyme is inducible by xylose and repressible by glucose and mannose.
- Experimental approaches include site-directed mutagenesis, chemical modification and fluorescence spectroscopy.

Key findings on yeast xylose reductases

- The following 4 catalytic residues were found to be essential for activity: Asp44, Tyr49, Lys78 & His111.
- Lys270 in the IPKS motif was found to be essential for cofactor binding.
- Future studies will examine the structure-function relationships of yeast **xylitol dehydrogenase** and **xylulokinase**.

Genetic improvement of native pentose-fermenting yeasts

- The primary objective is to improve the performance of pentose-fermenting yeasts in industrially relevant lignocellulosic hydrolysates.
- Focus on *Pichia stipitis* for now.
    - Start with UV mutagenesis.
    - Followed by genome shuffling.

Target(s) of genetic improvement

- **Improved tolerance to pretreatment derived inhibitors.**
    - Lignocellulosic substrate being used for selection is a waste pulping liquor known as spent sulfite liquor (SSL) from Tembec.
    - The pulping process generates many inhibitors which adversely affect microbial fermentation.
    - The main inhibitors are acetic acid (from hardwoods), furfurals, hydroxymethyl furfurals, and phenolic compounds.

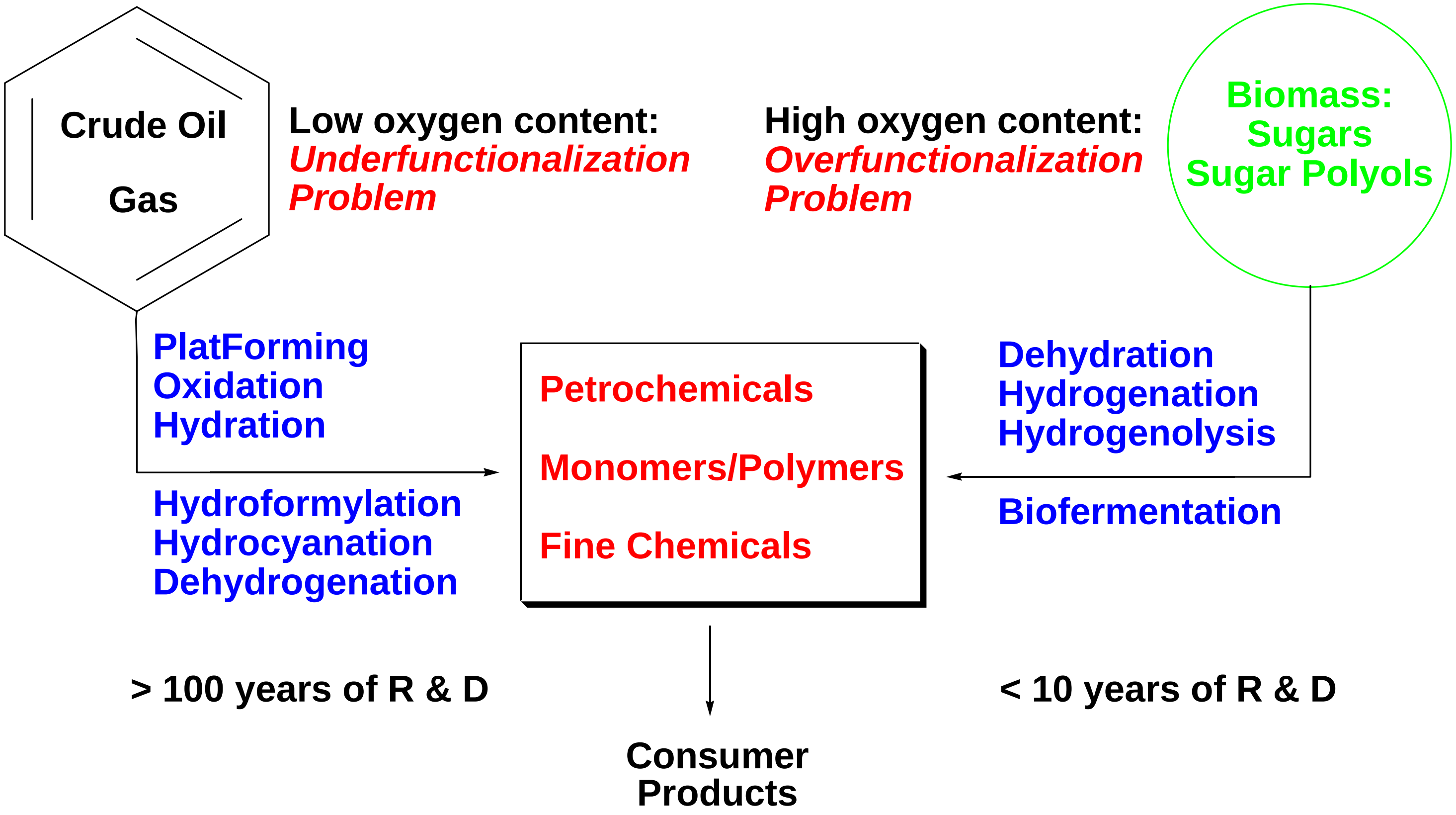
Improved mutants of *Pichia stipitis*

- **Selection of improved mutants are based on the following characteristics relative to the wild type (WT)**
  - Ability to grow at higher concentrations of hardwood SSL.
  - Ability to grow well on glucose and xylose as the sole carbon source in defined medium, based on growth rate and growth yield measurements.
  - Ability to ferment glucose and xylose individually to ethanol in defined medium in good yield.
  - Better able to ferment a glucose-xylose mixture.

Future targets for genetic improvement

- Simultaneous utilization of glucose and xylose
- Improved ethanol tolerance
- Improved acetic acid tolerance
- Genome shuffling to merge all the improved traits
- Genetic improvement of *Pachysolen tannophilus* and *Candida shehatae*.

The Deoxygenation Challenge



Crude Oil
Gas

Low oxygen content:
Underfunctionalization Problem

High oxygen content:
Overfunctionalization Problem

Biomass:
Sugars
Sugar Polyols

PlatForming
Oxidation
Hydration

Hydroformylation
Hydrocyanation
Dehydrogenation

Petrochemicals

Monomers/Polymers

Fine Chemicals

Dehydration
Hydrogenation
Hydrogenolysis

Biofermentation

> 100 years of R & D

< 10 years of R & D

Consumer
Products

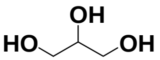
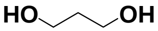
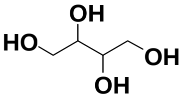
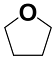
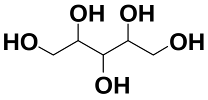
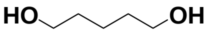
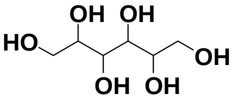
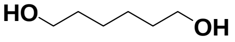
Starting from highly functionalized/oxygenated biomass will require a very different chemical approach than starting from coal/oil/gas …

Lit.: M. Schlaf, *Dalton Trans.* **2006**, 4645 - 4653.

Carbon Sources I: Polymers from Sugar Alcohols

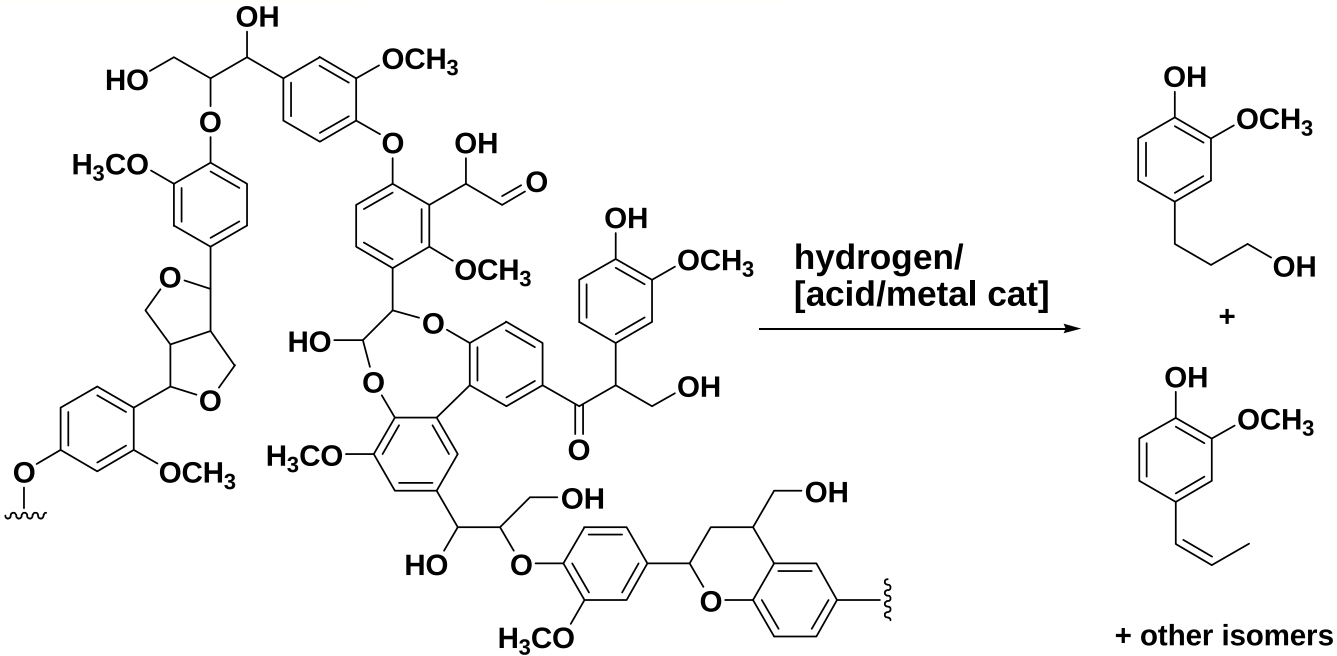
Est. Production 2007 [t/a]:

C_3	> 1 × 10^6 (and growing → Bio-Diesel glycerol glut …)
C_4	? – produced by Cargill as a non-caloric sweetener
C_5	> 25,000 (Xylose) > 30,000 (Xylitol)
C_6	> 5,000,000 (glucose) > 600,000 (sorbitol) > 130,000,000 (sucrose)

Biomass	$[H^+]$; - n H_2O [cat.; +n H_2(g)	α,ω-diol	Application
 n= 3: Glycerol (Waste By-product of Bio-Diesel production)	⟹	 1,3-propanediol	Sorona^{TM} (DuPont)/ Corterra^{TM} (Shell)
 n=4: Erythritol (Biofermentation of Cornsugar/starch)	⟹	 THF	Lycra^{TM} / Industrial Solvent
 n=5: Xylitol (Acid digestion/pyrolysis of hemi-cellulose, e.g. corn cobs)	⟹	 1,5-pentanediol	Thermoplastic Polyurethanes Polyesters
 n=6: Glucitol (Sorbitol) (Hydrogenation of glucose from corn/beet/cane sugar)	⟹	 1,6-hexanediol	Polyurethanes Polyesters Potential Nylon-6,6 precursor

Sugar alcohols can provide linear C_3 to C_6 building blocks – ideally α,ω-diols for direct application in polyesters, polyamides and polyurethanes.

Carbon Sources II: Aromatics from Lignins



Typical composition of lignin
(non-sulfonated form)
(Source: Lignin Institute)

- **Propyl catechols could serve as platform for epoxy and other resins.**
- **Combination of aquoeus acid digestion with simultaneous hydrogenation/hydrogenlysis could break up lignin framework.**
- **Steric access to C-O bonds and catalyst deactivation *coking* would not be a problem with homogeneous systems.**

Carbon Sources III: Engine Fuel from Bio-Oil

- **Obtained by pyrolysis of ligno-cellulosics at ~ 500 °C.**
- **High viscosity, acidity, water and acid content (15 % w/w formic + acetic acid).**
- **Hydrogenation upgrade using acid- and water-tolerant iron-based homogeneous catalysts and removal of water formed (by distillation) would lower acid and oxygen content.**
  → 3rd generation bio-Diesel
- **Iron catalyst stays in fuel to act as combustion catalyst, i.e., does “double duty” !**

200 tons/day

Guelph Plant

A sample of bio-oil obtained by the DYNAMOTIVE process.

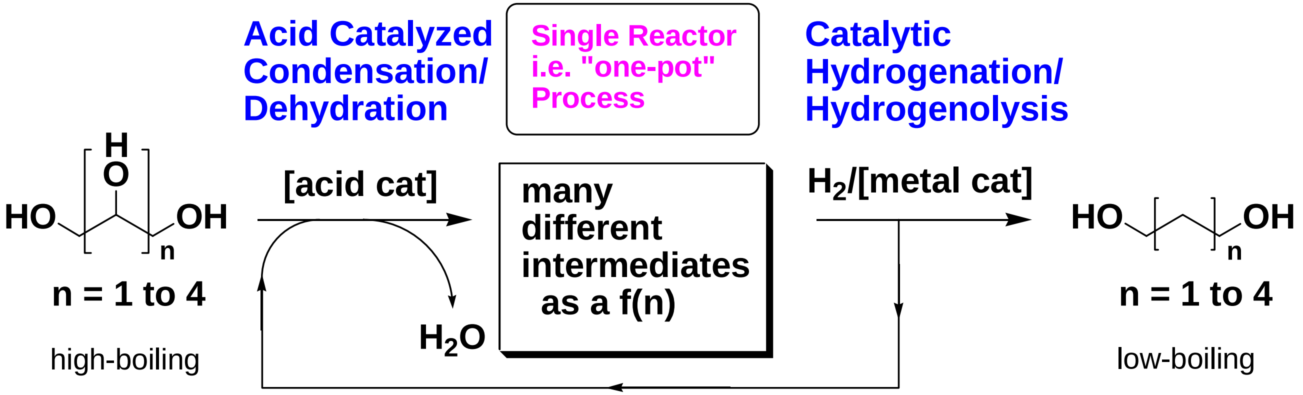
Acidity: pH ~ 2

Oxygen content: ~ 50 % w/w

Water content: ~ 20 % w/w

Not suitable as engine fuel “as is”.

Strategy or How to deoxygenate stuff ?



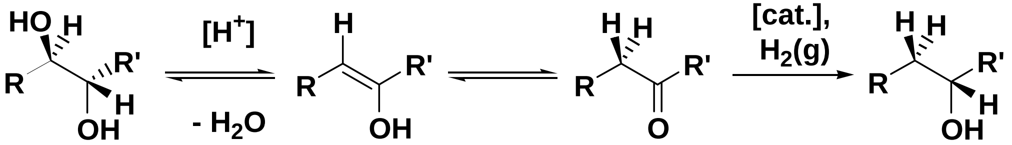
Net reaction: - n H_2O + n $H_2(g)$ = - n O

- **Controlled Acid (Brønstedt or Lewis) Catalyzed Dehydration. (*carbocations → thermodynamic bias for loss of secondary OH*)**
- **Hydrogenation and/or Hydrogenolysis of C=O, C=C, C-O & O-C=O.**
- **Thermodynamically “downhill” for all reactions.**
- **Iterative repetition in single reactor if required.**
- **Requires water- and acid-tolerant homogeneous ionic hydrogenation/hydrogenolysis catalysts.**
- **Advantage: no coking of catalyst surface with highly polar substrates**

The Four Reactions for Catalytic Deoxygenation

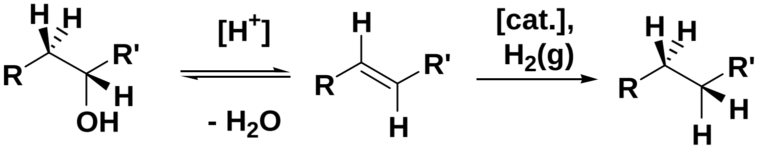
- Regardless of the source or target of the biomass deoxygenation the chemistry always involves only four reaction types.
- All are thermo-dynamically feasible and/or have been demonstrated with homogeneous catalysts.
- Key: catalysts must tolerate acid, water and fairly high temperatures.
- Top choice: highly chelated and robust electron poor group 8 metal (Fe, Ru) complexes.

1) Dehydration of *vic*-diols and hydrogenation of the resulting C=O double bond:



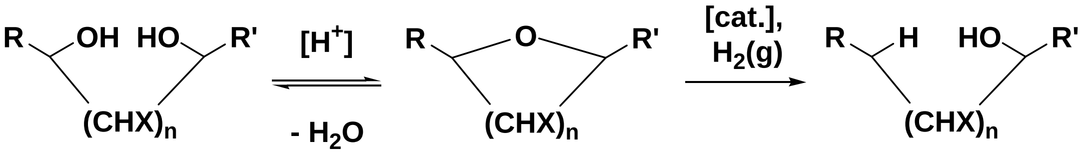
R = alkyl, hydroxy alkyl; R' = H, alkyl, hydroxy alkyl

2) Dehydration of alcohols and hydrogenation of the resulting C=C double bond:



3) Condensation of alcohols to oxacycles and hydrogenolysis of the resulting ether:

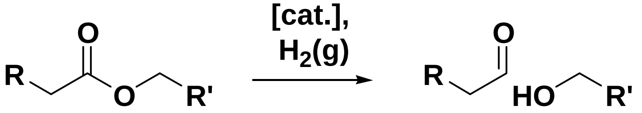
a) Direct Hydrogenolysis (thermodynamically possible but kinetically difficult)



X = H, OH, alkyl, aryl; n = 2,3,4

b) Easier: Rehydration, Loss of Water, Hydrogenation ⟹ Reactions 1 or 2

4) Hydrogenolysis of Esters

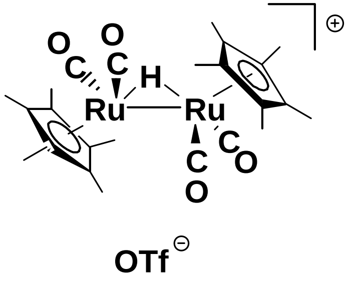


⟹ Reactions 1 or 2

Schlaf Research Group, Department of Chemistry, University of Guelph 2008

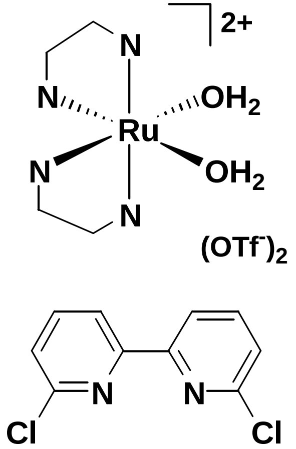
Types of Catalysts under Development @ Guelph

Time, money spent and # of grad students frustrated →

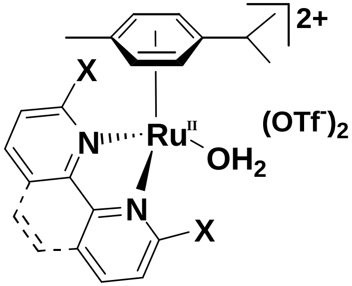


BNL/DuPont Catalyst - deactivated by water.

1



2



X = H, NH_2

3

coming to a patent server near you ... (hopefully)

4

Catalyst	1 (1998)	2 (2004)	3 (2006)	4 (2008)
Temp. Limit [°C]	< 150	< 150	< 125	> 200
Diol ?	Yes	Yes	Yes	Yes
Alkanes from diols ?	No	Yes	Yes	Yes
Glycerol ?	< 1%	No	No	Maybe

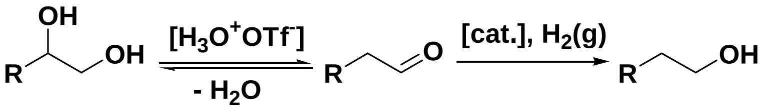
1) US 6,555,717 & US 6,462,206. 2) Xie, Z.; Schlaf, M. *J. Mol. Catal. A: Chem.* **2005**, *229*, 151-158. 3) Dykeman, R. R.; Luska, K. L.; Thibault, M. E.; Jones, M. D.; Schlaf, M.; Khanfar, M.; Taylor, N. J.; Britten, J. F.; Harrington, L. *J. Mol. Catal. A: Chem.* **2007**, *277*, 233-251. 4) Thibault, M.E. & Schlaf, M.; Pending …

Example of a Catalyst in Action (Diol Model System)

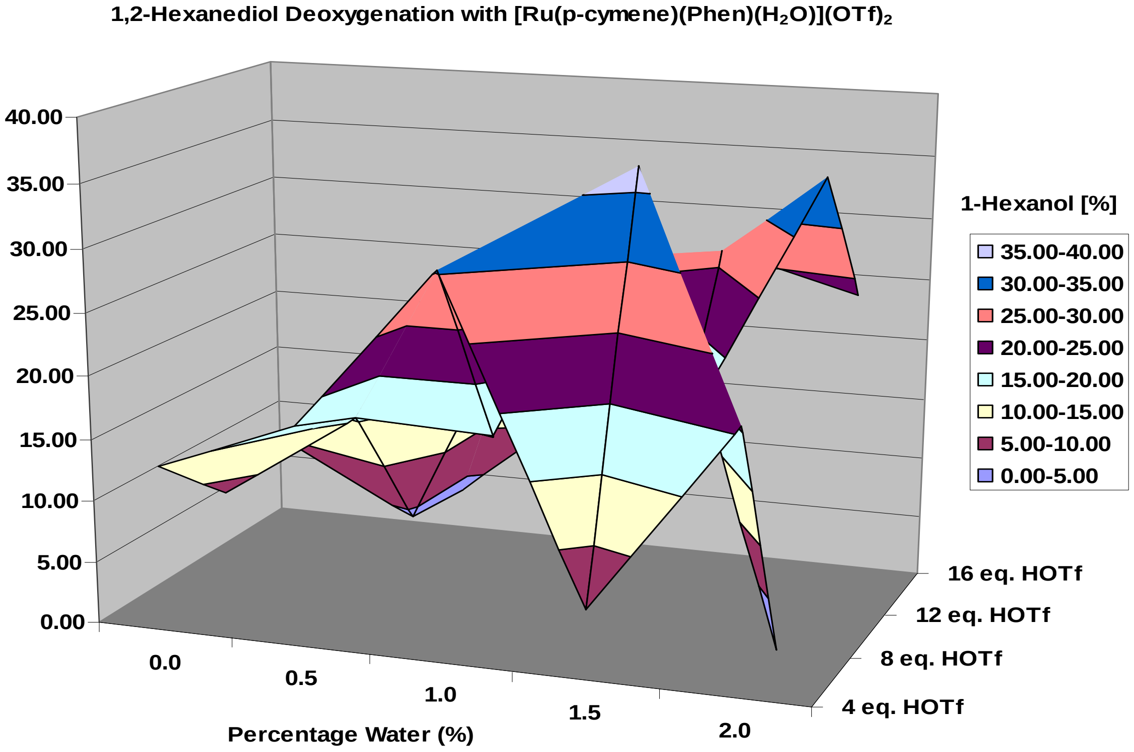
24 well parallel reactor from HEL Group (UK)

Allows rapid screening of:
- various catalysts and/or substrates.

- influence of two inter- or independent reactant concentrations on the reaction outcome.



R = CH_2OH or CH_3 or C_4H_9



Reaction 1: Yield of 1-hexanol from 1,2-hexanediol with Ru-catalyst **3** as a function of acid/water concentration.

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- www.uoguelph.ca/research/
- www.uoguelph.ca/research/bdo/index.shtml