



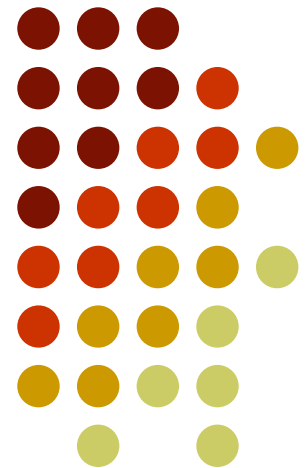
# Dyadic International



## Biofuels Presentation



**Annual Global Investment Conference**  
**September 12-15, 2010**





# Safe Harbor Statement

Certain statements contained in this presentation are forward-looking statements. These forward-looking statements involve risks and uncertainties that could cause Dyadic's actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by such forward-looking statements. Except as required by law, Dyadic expressly disclaims any intent or obligation to update any forward-looking statements.



# Dyadic International



- ❖ A global biotechnology company
- ❖ Founded in 1979 by Mark A. Emalfarb
- ❖ Uses patented and proprietary technologies to discover, develop, manufacture and sell enzyme products and solutions
- ❖ Applications in bioenergy, biopharmaceutical and industrial enzyme markets
- ❖ Manufacturing enzymes since 1994
- ❖ Publicly traded since 2004 (DYAI)
- ❖ Headquartered in Jupiter, Florida, USA
- ❖ R&D arm located in the Netherlands





# Dyadic International



## Biofuels

Develop and manufacture fuels & chemicals from agricultural feedstocks



Ethanol



Chemicals



## Biopharmaceuticals

Focus on developing and producing antibodies and other therapeutic proteins



Pharmaceutical Biotech



Animal Feed



Pulp & Paper<sub>4</sub>

## Enzymes

Develop, manufacture and market enzymes and other biological products for a variety of industrial uses



Textiles



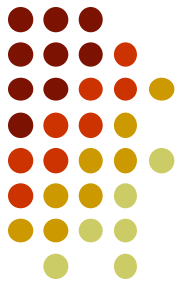
Food



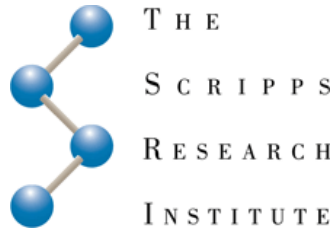
Animal Feed



Pulp & Paper<sub>4</sub>



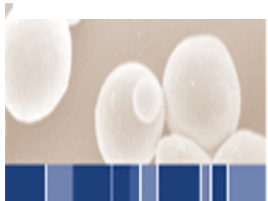
# Science Collaborations



Moscow State University



Savannah River National Laboratory

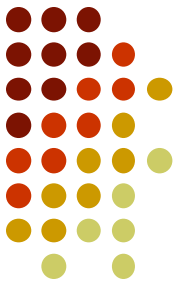




# Scripps Collaboration

- ❖ **One of the world's largest and most reputable biomedical research organizations**
  - ❖ Dr. Richard Lerner, President of Scripps and Chairman of Dyadic's Scientific Advisory Board
- ❖ **Sequenced and annotated C1 genome (2005-2008)**
- ❖ **Re-sequenced and re-annotated C1 genome (2009-2010)**
  - ❖ Expanding knowledge of C1 genetics
  - ❖ Provides information and knowledge to improve C1 Technology Platform – to do more for less at higher yields.
  - ❖ Provides new product candidates and enzyme catalysts to improve manufacturing processes
  - ❖ Enter new markets





# Dyadic Netherlands



## ❖ **Dyadic's Research & Development Subsidiary**

- ❖ 18 employees – 6 with Ph.D.'s
- ❖ Participation in a number of funded international projects
- ❖ Member of the Industrial Platform of the Kluyver Centre for Genomics of Industrial Fermentation
- ❖ Partner in The Eurofung Project (European scientific and industrial network on fungal research)

## ❖ **Management Team**

- ❖ Wim van der Wilden, Ph.D (ETH Zurich Switzerland) – General Manager
  - ❖ Former Director of R&D, Gist-brocades/DSM
  - ❖ Former Director Biotechnology Division, TNO Quality of Life
- ❖ Jan Wery, Ph.D. – Science Director
  - ❖ Former Sr. Scientist, Netherlands Organization for Applied Scientific Research

## ❖ **Located in Wageningen, the Netherlands**

- ❖ Wageningen University and Research Institutes
- ❖ Centre of excellence for Life Sciences research



# Dyadic Netherlands



## ❖ Core competencies in:

- ❖ Molecular Biology
- ❖ Fermentation Technology
- ❖ Enzymology
- ❖ Gene Expression

## ❖ Collaborates with Strategic Partners to:

- ❖ Provide on-site research and development capabilities
- ❖ Assist partners in producing customized C1 fungal strains for the manufacture of large quantities of diverse enzymes and proteins at higher yields and lower costs





# Dyadic's C1 Platform Technology

## A Robust Versatile Enzyme-Producing Fungal Expression System

- ❖ Offers significant advantages over other microbial and cell culture-based systems
- ❖ Programmable (annotated and sequenced genome)
- ❖ Commercially Scalable
- ❖ Proven test results on wide range of feedstocks
- ❖ Performs well under broad pH and temperature conditions
- ❖ Higher Yields
- ❖ Lower Costs
- ❖ Patent protected
- ❖ Broad platform capabilities in biofuels validated through partnerships with key players





# Biofuels Partners



- ❖ Biofuels partner of Royal Dutch Shell



- ❖ Non-exclusive license agreement

- ❖ Covers use of C1 expression system for large-scale production of enzymes in biofuels, chemical and pharmaceutical intermediate production



# Biofuels Partners

## ABENGOA

- ❖ One of the largest ethanol producers in the world
- ❖ R&D program led to non-exclusive license agreement
- ❖ Covers use of C1 expression system for large-scale production of enzymes for use in manufacturing of biofuels, power and chemicals
- ❖ Currently focused on enzymes for lignocellulosic bioethanol production
- ❖ Biomass Pilot Plant (US) in 2007 - 0.02 Mgal/yr capacity
  - ❖ Objective: competitive process with grain ethanol culture-based systems
- ❖ Biomass Demonstration Plant (Spain) in 2008 – 1.3 Mgal/yr capacity
  - ❖ Objective: demonstrate commercial-scale process systems
- ❖ Commercial Plant (US) in 2012
  - ❖ Objective: production at a cost line competitive



# Dyadic's International Initiative

## The Abraham Group LLC

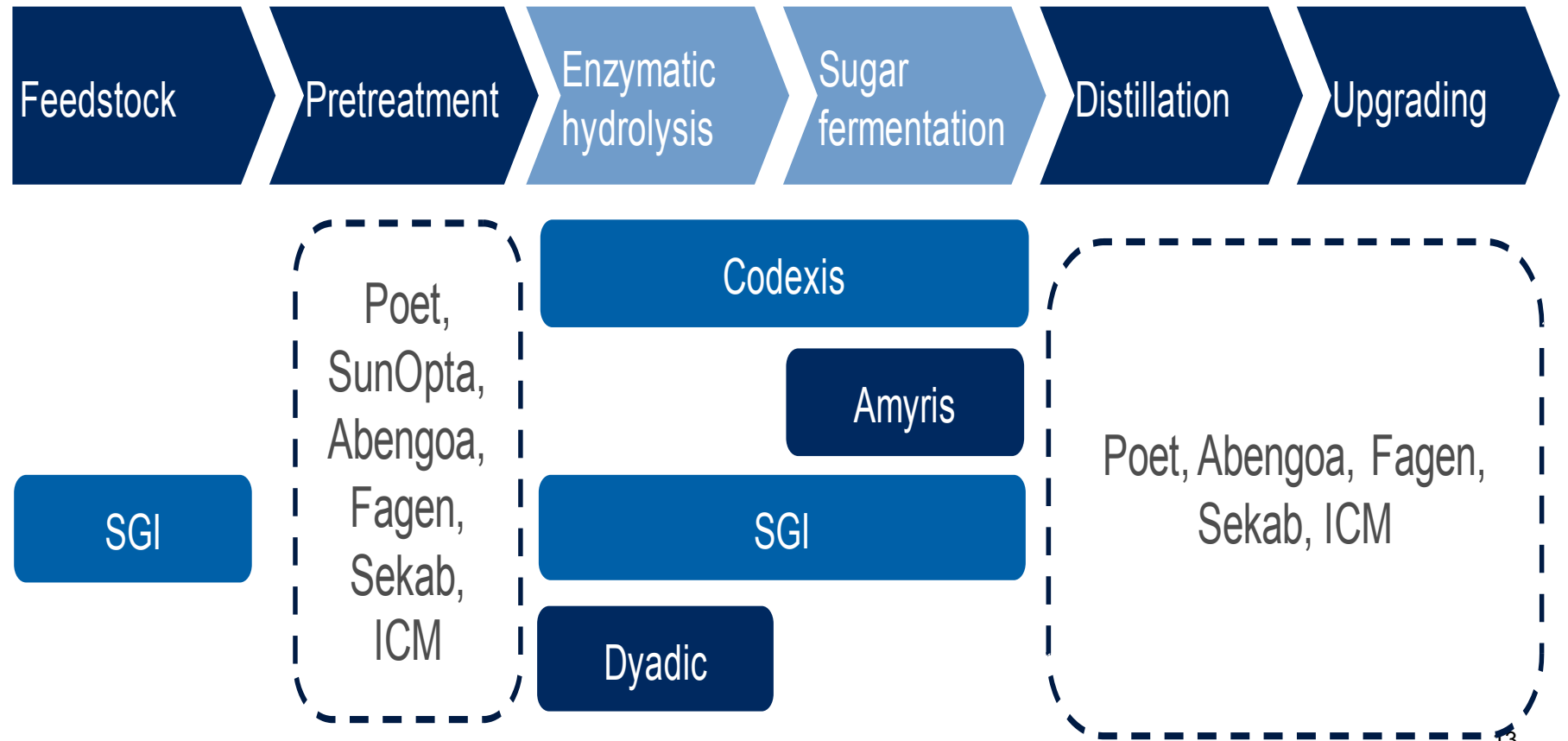
- ❖ Influential consulting firm led by former U.S. Secretary of Energy, Spencer Abraham
- ❖ Engaged by Dyadic for strategic advice and transactional assistance
- ❖ Will communicate advantages of Dyadic's C1 platform technology and its R&D capabilities to major international energy groups committed to cellulosic ethanol and other forms of sustainable energy





# 2<sup>nd</sup> Generation Biofuels

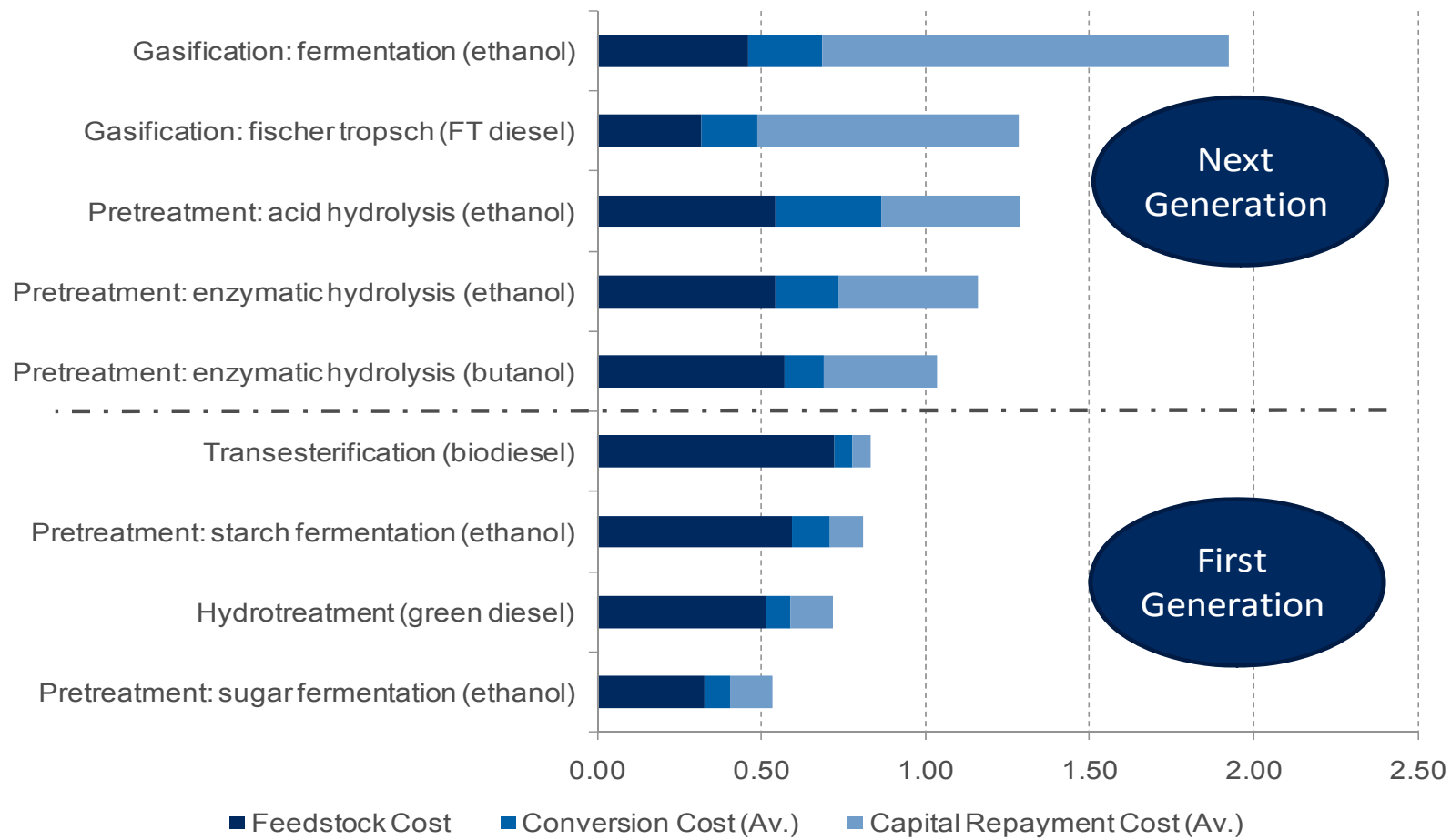
## Value Chain





# Bioenergy Pathways Comparison

(2010: \$ per litre)



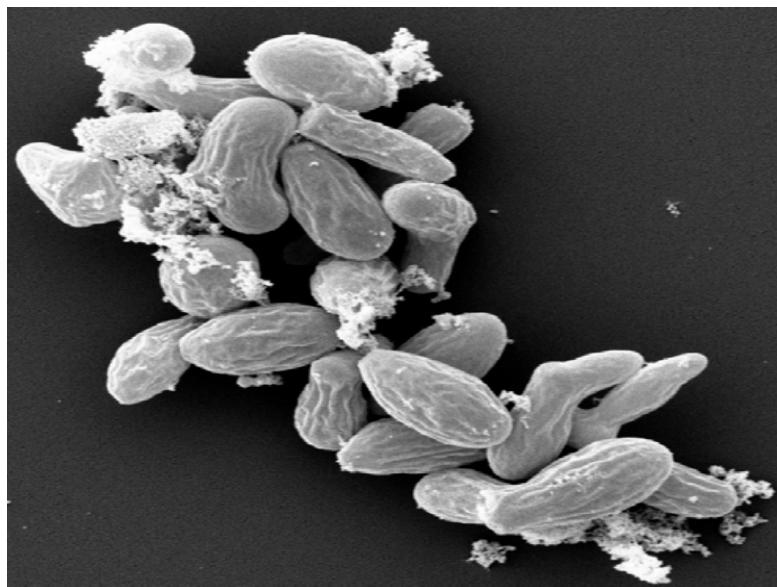
Source: Bloomberg New Energy Finance



# C1 Technology Platform

## *Chrysosporium lucknowense*\* (C1)

System for gene discovery, expression and protein production



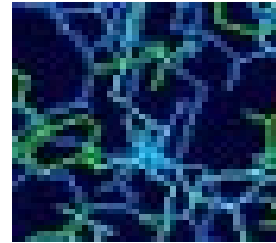
- ❖ A fungus isolated from alkaline soil in Eastern Russia
- ❖ Platform for enzyme and protein production
  - Favorable fermentation characteristics
  - High yields
- ❖ Highly versatile
  - Can be used to produce a growing number of enzymes or proteins

*\*Agency Response Letter GRAS Notice No. GRN 000292, CFSAN/Office of Food Additive Safety: The C1 strain was initially deposited with the International Depository of the All Russian Collection of Microorganisms of the Russian Academy of Sciences, and was assigned Accession Number VKM-3500D and classified as Chrysosporium luckowense based on morphological characteristics and subsequently reclassified as M. thermophila based on genetic tests.*



# Applications

- ❖ **Biofuels**
- ❖ **Biopharma**
- ❖ **Chemicals**
- ❖ **Animal Feed**
- ❖ **Agriculture**
- ❖ **Food**
- ❖ **Cosmetics**
- ❖ **Nutraceuticals**



Source: EPA



# C1 Attributes

## From Gene to Product in a Single Host Strain

### From Promise to Product in 5 Steps:

- 1. Gene discovery**
  - ❖ Access the full spectrum of biodiversity
  - ❖ Robotic high-throughput screening
- 2. Gene expression**
  - ❖ Functional expression to identify genes
- 3. Characterization**
- 4. Optimization**
- 5. Commercial Manufacturing**
  - ❖ Commercial scale-up to 150,000L





# C1 Attributes

**FERMENTATION**

**Inexpensive fermentation media  
Wide pH and temperature range tolerated**

**SCALABILITY**

**Scalable to 150,000 L**

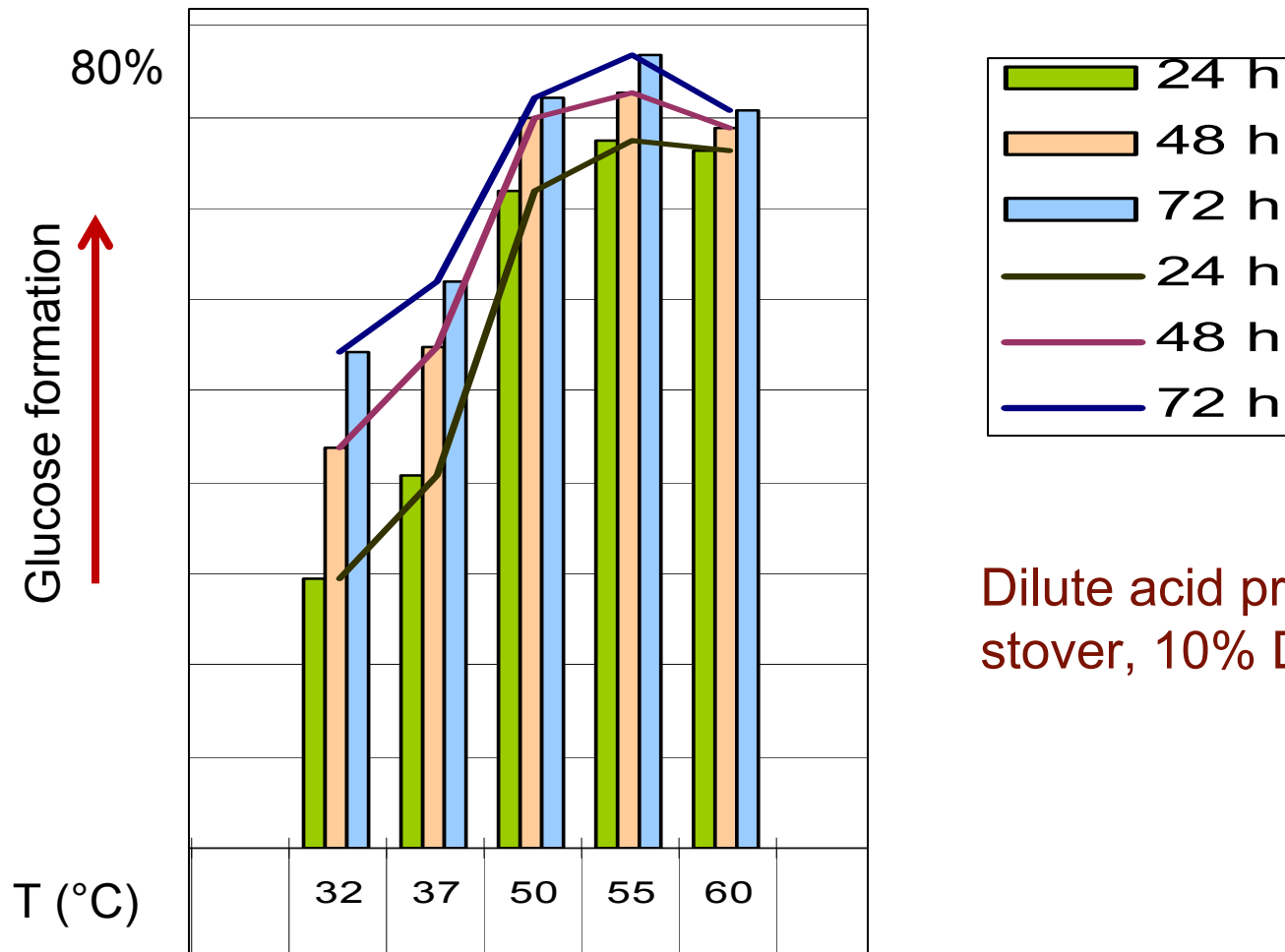
**PURIFICATION**

**Target protein secreted into media  
under low viscosity**

**Lower cost, greater yield  
Improved productivity**



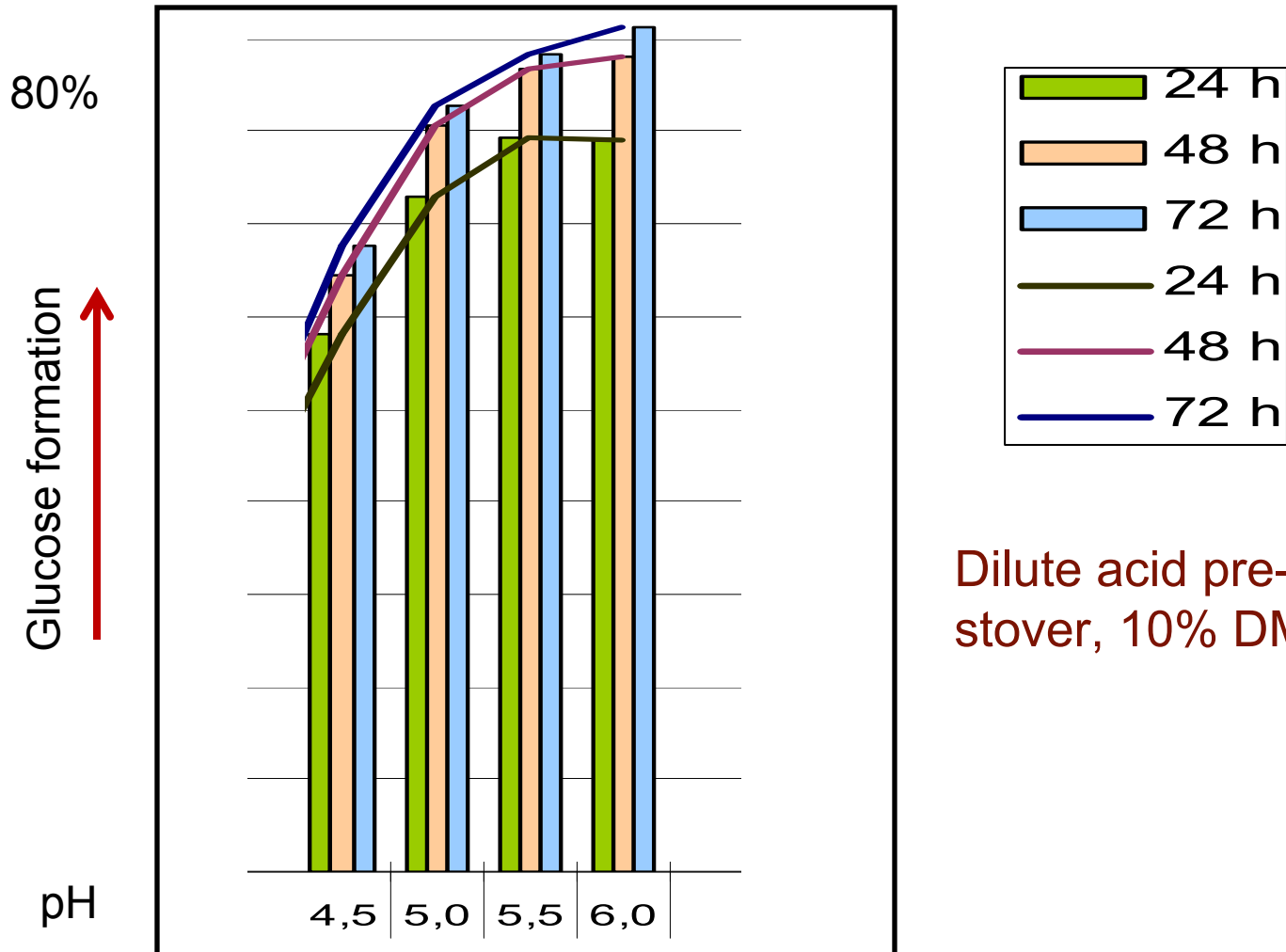
# Broad Temperature Range



Dilute acid pre-treated corn stover, 10% DM



# Broad pH Range



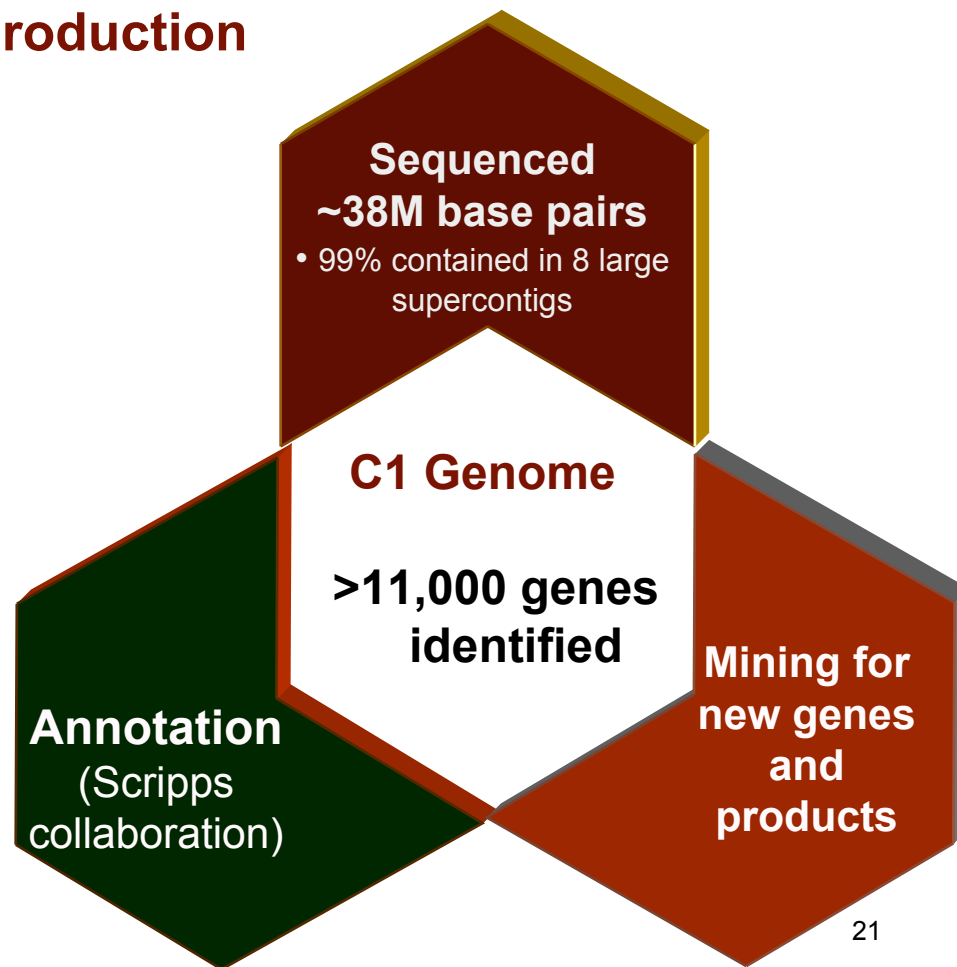
Dilute acid pre-treated corn stover, 10% DM



# C1 Gene Discovery

Over 200 genes encoding putative carbohydrate-active enzymes useful in ligno-cellulosic ethanol production

- ❖ Cellobiohydrolases
- ❖ endo-/exo- $\beta$ -glucanases
- ❖ endo-/exo-xylanases
- ❖ xyloglucanases, mannanases, arabinases, galactanases
- ❖ pectinases (pectin-/pectate lyases, polygalacturonases, etc.)
- ❖  $\alpha$ -amylases, glucoamylases
- ❖ glycosidases ( $\alpha$ -/ $\beta$ -glucosidases,  $\alpha$ -/ $\beta$ -xylosidases,  $\alpha$ -/ $\beta$ -galactosidases,  $\alpha$ -L-arabinofuranosidases,  $\alpha$ -/ $\beta$ -mannosidases)
- ❖ ferulic acid esterases, cutinases, esterases, polyesterases





# Strong Intellectual Property

- ❖ 5 issued U.S. patents
- ❖ Broad claims blocking use of C1
- ❖ 10 pending U.S. patent applications
- ❖ 60 issued foreign patents
- ❖ 34 pending foreign applications

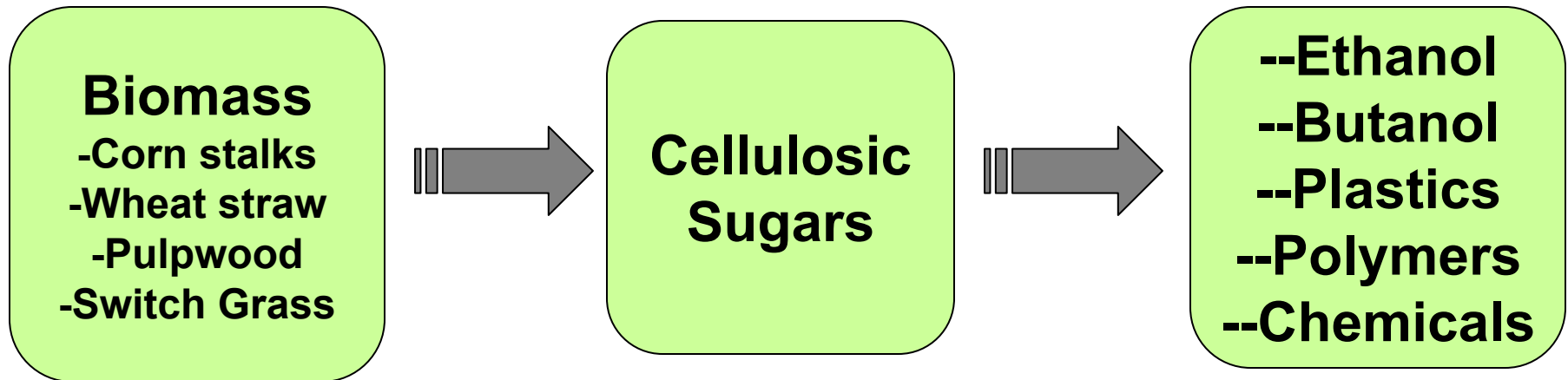




# Biofuels Process

## Development of Proprietary Enzymes for Cellulosic Ethanol Production

Discovery and optimization of new enzymes to improve performance and lower costs



**\*\*Renewable\*\***

**\*\*Environmentally friendly\*\***



# Biofuels Process

Uses (examples):

- Enzymes
- Fuels
  - ethanol
- Chemicals
  - chemical intermediates
  - polymers
  - solvents
  - plastics
  - textiles
  - adhesives
  - paints
  - dyes, pigments, ink
  - detergents
  - lubricants
  - etc.
- Food, Feed, Fiber



## Biomass Feedstocks

- Corn
- Corn stover
- Trees
- Grasses
- Agricultural crops
- Agricultural Residues
- Municipal waste



## Conversion Processes



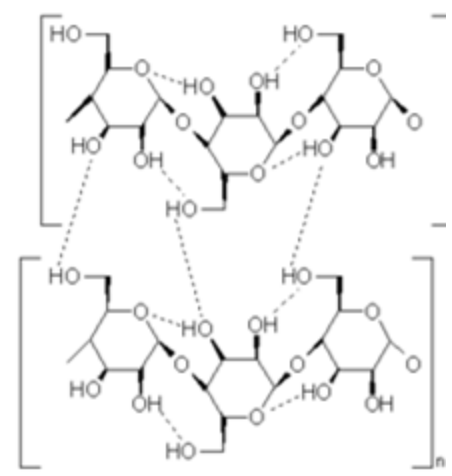
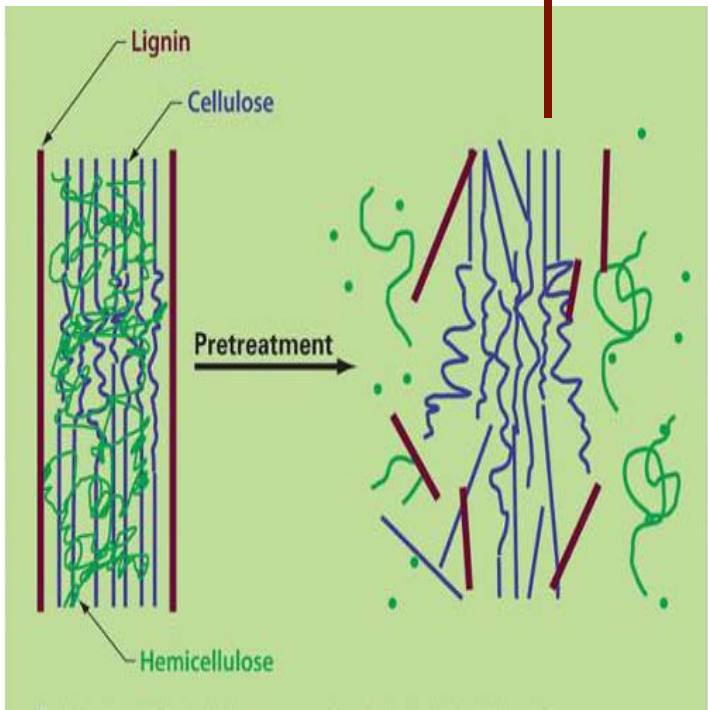
**Dyadic Technology:**

- Enzyme Hydrolysis
- Strain optimization
- Metabolic engineering
- Large scale production

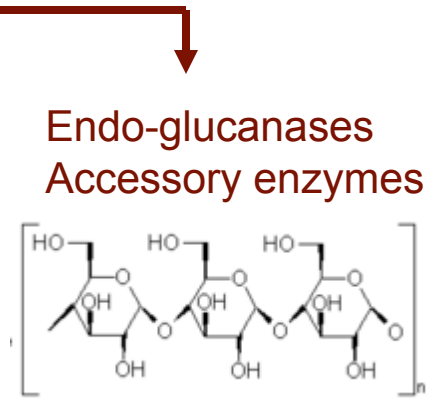


# Enzymes for Biomass Saccharification

Cellulases

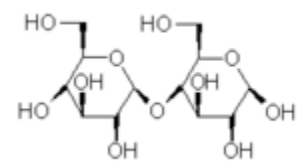


Crystalline cellulose



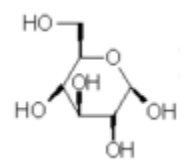
oligomers

Exo-glucanases/  
cellobiohydrolases



cellobiose

B-glucosidase



glucose

Fermentation

ETHANOL



# Matching Enzyme Activity to Substrates



DDG's



Corn Fiber



Corn Stover



Wheat Straw



Wood pulp

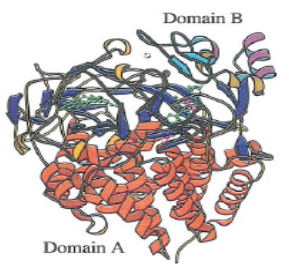
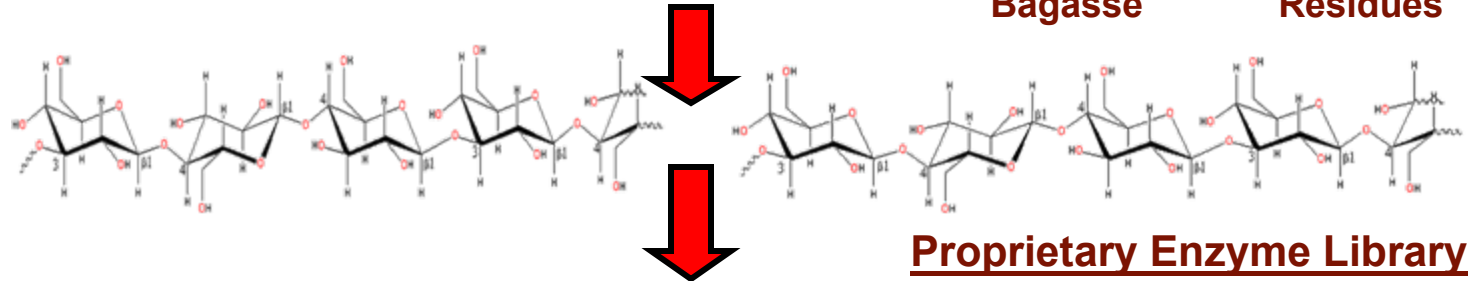


Sugar Cane Bagasse

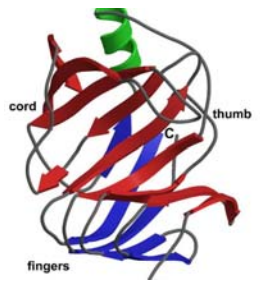


Agric. Residues

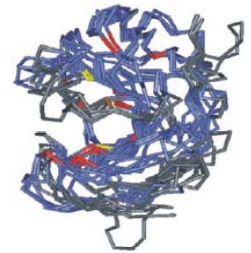
Alternative Pretreatments



EG's



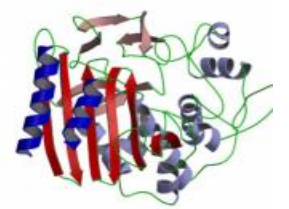
CBH's



$\beta$ G



Xylanases



Esterase + Accessory Enzymes

High Yield of Fermentable C5's and C6's



# Enzyme Development for (hemi-) Cellulosic Biofuels

## Enzyme Mixtures for Cellulosic (C6) Substrates

**Pretreated wheat straw**



**Pretreated wheat straw (20% TS w/w)**



**Hydrolysed wheat straw**



Buffer →

C1-enzyme mixture →

Cellulose: 50-55%  
Hemicellulose: 5%

Insoluble cellulose (≈100g/kg)

Glucose (≈100g/kg)



# C1 vs. *Trichoderma*

**C1 vs. the Lignocellulolytic Potential of C1 and *Trichoderma reesei***  
(the main industrial source for biofuel enzymes, e.g. Accellerase™)

	<b>C1</b>	<b><i>T.reesei</i>**</b>
Cellulases	~ 55	~ 35
Cellulose binding domains (CBM1)	~ 46	~11#
Xylanases	~ 11	~ 5
Arabinofuranosidases/arabinases	~ 14	~ 3
Esterases (Axe, Fae)	~ 10	~2#

\*\* From the JGI database

# Based on literature and JGI database searches

**C1 is a rich source of lignocellulolytic enzymes!**



# C1 vs. The Competition

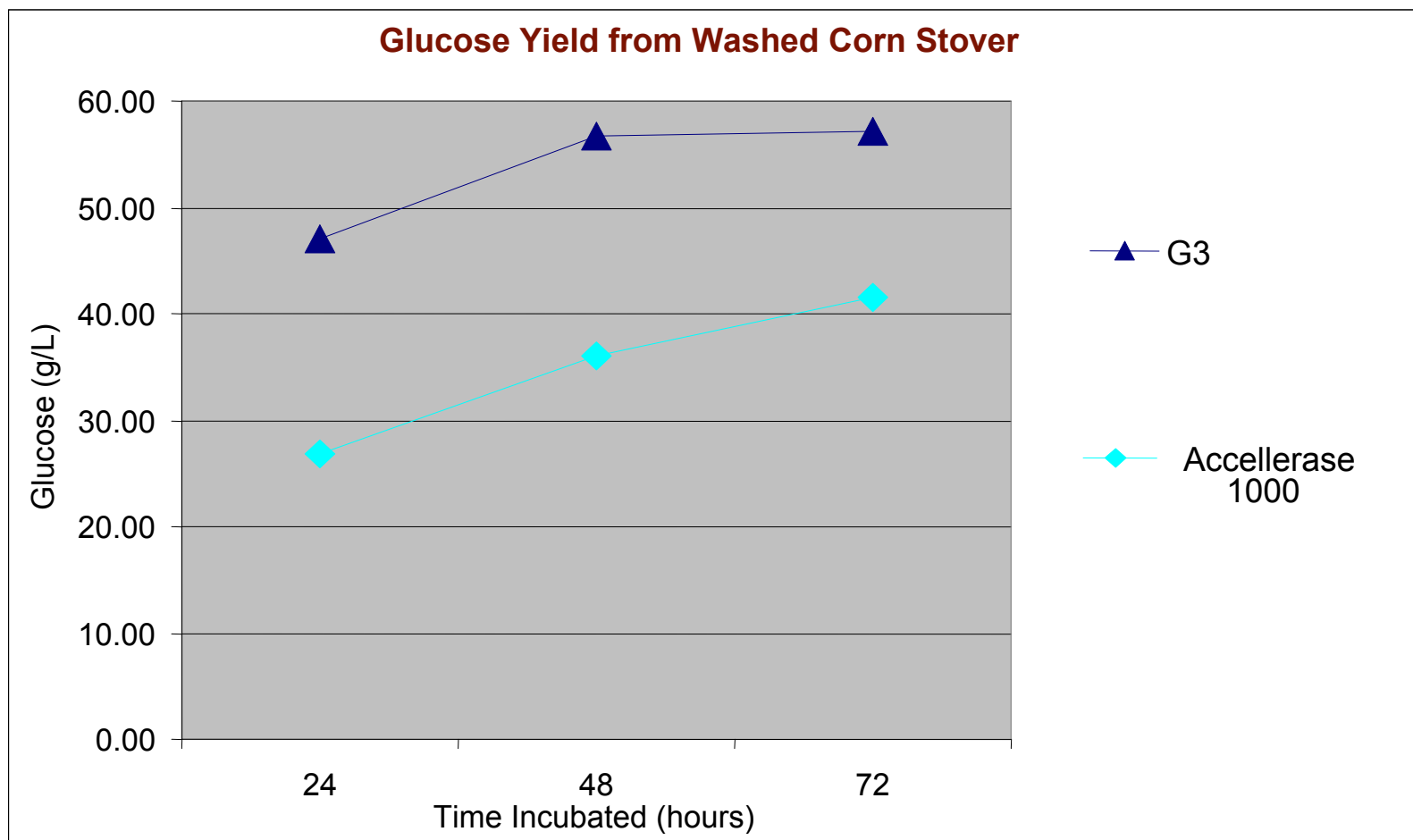
Independent Confirmation of C1 vs. Genencor's Accellerase™ 1000\*

Enzyme Preparation	Protein dose (mg protein/g total solids) **	Temp (°C)	pH	Total Solids (% wt)	Glucose productivity (g/Lh <sup>-1</sup> )***	Relative Glucose productivity (%)
<b>Dyadic C1</b> (Chrysosporium sp)	20	50	5.0	10	0.86	97
	20	50	6.0	10	0.89	100
<b>Accellerase™ 1000</b> (Trichoderma reesei)	20	50	5.0	10	0.73	82
	20	50	6.0	10	0.46	52

\*Substrate: pretreated hardwood, \*\*protein measured by BCA, \*\*\* 96h reaction

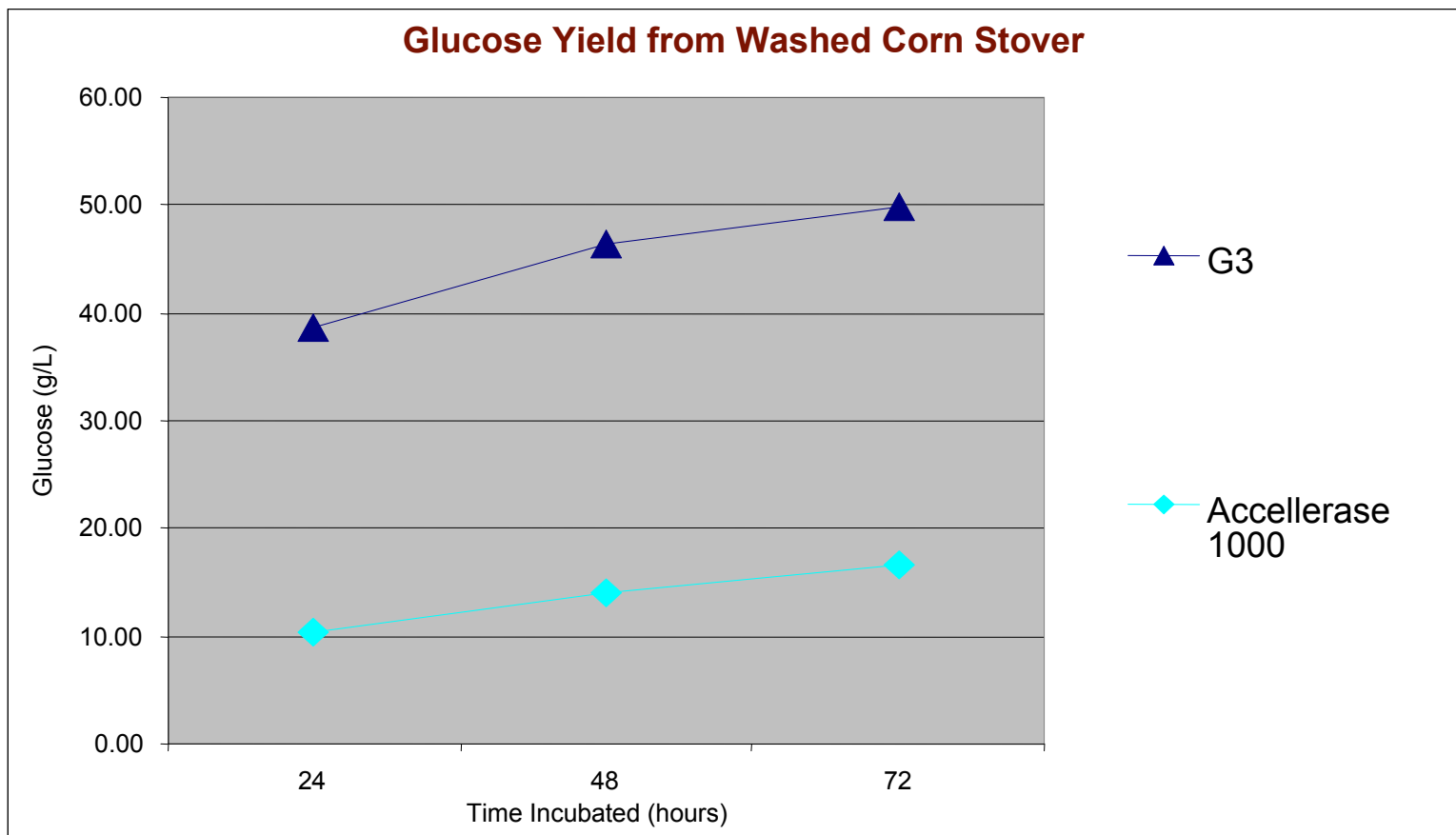


# C1 vs. Accellerase™ 1000 at pH 5





# C1 vs. Accellerase™ 1000 at pH 6

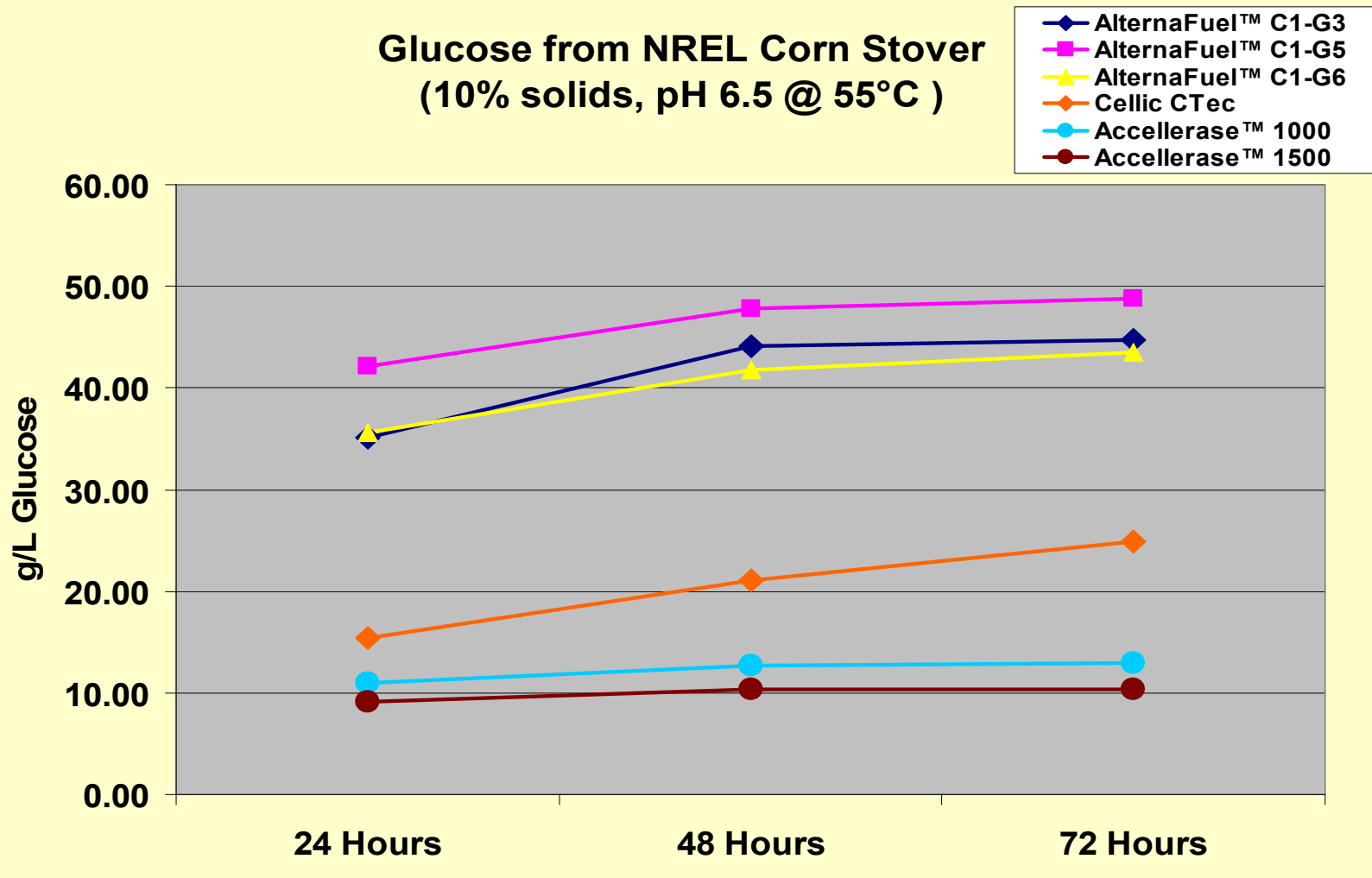


**Accellerase at pH 6 is ~ 50% less effective than at pH 5  
C1 enzymes provide broader operating conditions**



# C1 vs. Genencor & Novo at pH 6.5

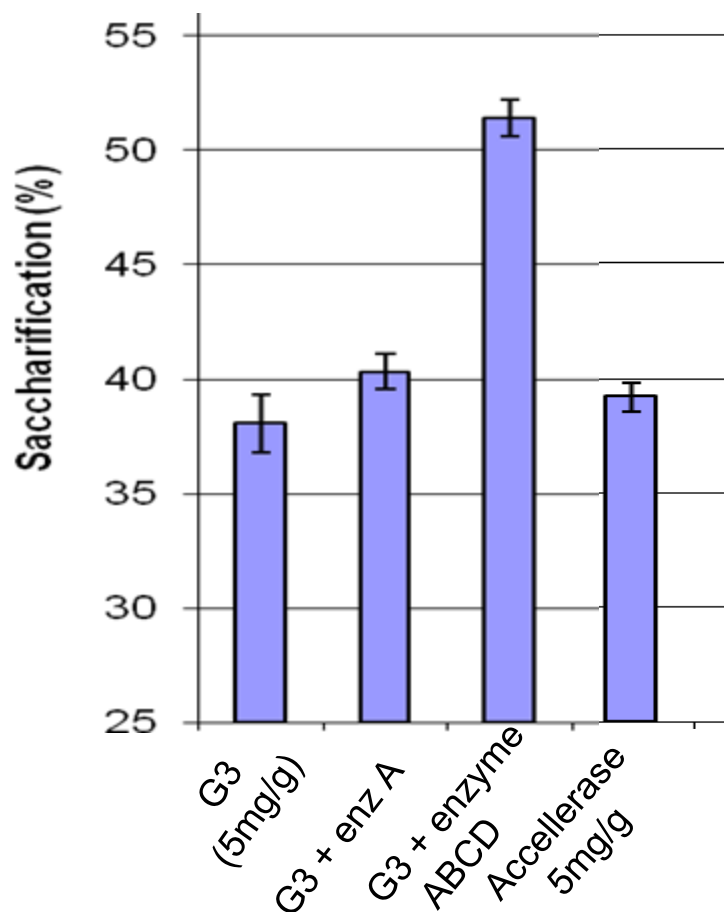
Glucose from NREL Corn Stover  
(10% solids, pH 6.5 @ 55°C)





# C1 Strain Improvement

Substrate: Pretreated corn stover



**G3: An improved C1 strain for Biofuel Production**

**Further improvements:**

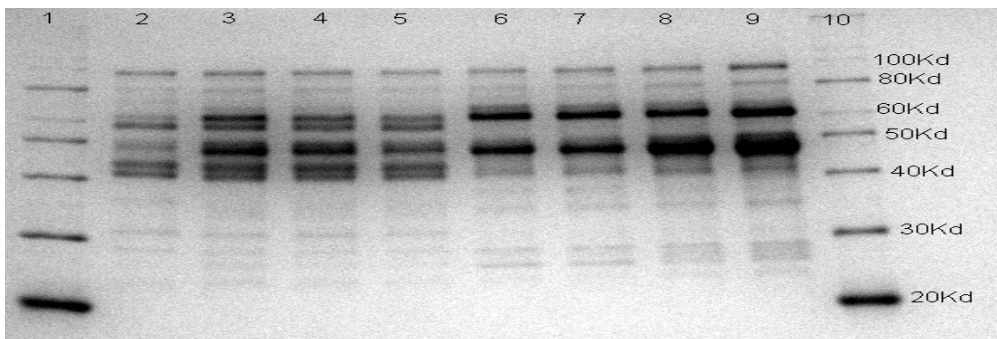
- ❖ **Addition of distinct C1 cellulases to increase saccharification efficiency**
  - ❖ discovery of new enzymes (genomics)
  - ❖ directed evolution of key single enzymes
- ❖ **A single C1 strain producing optimized mix of enzymes**
- ❖ **Improve production yield from enzyme mixtures**



# Productivity Improvement

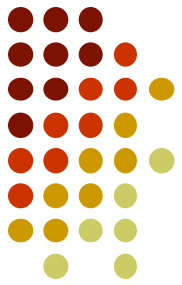


Ferm. No.	Strain	Medium Density	AzoCMC U/g	BCA Protein g/l
EG2-17	UV18#100.fΔalp1	1x	728	n/a
EG2-18	EG2#65	1x	3,054	n/a
EG2-124	EG2#65	1.86x	3,507	68
EG2-125	EG2#257	1x	5,456	77
EG2-126	EG2#257	1.86x	6,457	86
14 Liters	EG2#257	1.86x	8,625	102



**Protein production by optimized strain = 102g/L**

**~ 2X improvement over current strain**



# U.S. Management Team



<u>Name</u>	<u>Title</u>	<u>Experience</u>
Mark A. Emalfarb	President and CEO Chmn. of the Board/Founder	Dyadic International, Inc.
Anne E. Whitehead, Esq.	Executive Director Strategic Alliances	Shearman & Sterling Skadden Arps
Adam J. Morgan, Esq.	VP General Counsel & Bus. Dev. / Secretary	Advance Publishers, L.C. Rexall Sundown, Inc.
Michael J. Faby, CPA	VP Finance	Perry Slingsby Systems PricewaterhouseCoopers
Richard H. Jundzil	VP Operations	Genzyme Corporation
Thomas M. O'Shaughnessy	VP Sales & Marketing	Hexion Specialty Chemicals Occidental Chemical/GE



# Scientific Advisory Board



<u>Name</u>	<u>Title</u>	<u>Experience</u>
Richard Lerner, MD	Chairman	President of the Scripps Research Institute
Carlos Barbas, Ph.D	Advisor	Scripps Research Institute, Chair in Molecular Biology and Chemistry
Arnold Demain, Ph.D	Advisor	Fellow at Charles A. Dana Institute for Scientists Emeriti/MIT /Merck
Arkady Sinitsyn, Ph.D	Advisor	Head of Dept. of Enzymology, Moscow State University (Russia)
Cees van den Hondel, Ph.D	Advisor	Professor of Fungal Genetics, Leiden University



# Recent Publications



- ❖ Hinz, S.W.A., Pouvreau, L., Joosten, R., Bartels, J., Jonathan, M.C., Wery, J., Schols, H.A. (2009). Hemicellulase production in *Chrysosporium lucknowense* C1. *J. Cereal Sci.*, 50: 318-323
- ❖ Kühnel, S., Hinz, S.W.A., Pouvreau, L., Wery, J., Schols, H.A., Gruppen, H. (2010) *Chrysosporium lucknowense* arabinohydrolases effectively degrade sugar beet arabinan *J. Biores. Technol.*, DOI: 10.1016/j.biortech.2010.05.070
- ❖ Yvonne Westphal, Stefan Kühnel, Pieter de Waard, Sandra W. A. Hinz, Henk A. Schols, Alphons G. J. Voragen, Harry Gruppen. Branched arabino-oligosaccharides isolated from sugar beet arabinan. 2010. *Carbohydrate Research* (2010) article in press.



# Summary



## ❖ **Patented Technology Platform with Vast Potential**

- ❖ C1 fungus-based expression system offers significant advantages over microbial and cell culture-based systems
  - ❖ One-stop shop, same organism from discovery to production
  - ❖ Broad operating conditions, lower costs
  - ❖ Scalability and high yields

## ❖ **Strategy to leverage platform into biofuels**

- ❖ Platform capabilities have been validated in industrial enzymes and biofuels through partnerships with key players

## ❖ **Experienced management & scientific teams**



# Thank You

For more information about Dyadic and its  
C1 Platform Technology, please contact:

**Adam J. Morgan**

**Vice President General Counsel  
& Business Development**

**Tel: 561-743-8333, ext. 23**

**Email: [amorgan@dyadic.com](mailto:amorgan@dyadic.com)**



**Wim van der Wilden**

**General Manager, Dyadic Netherlands**

**Tel: 31-317-465-454**

**Email: [wvanderwilden@dyadic.nl](mailto:wvanderwilden@dyadic.nl)**

