



Dyadic[®] International

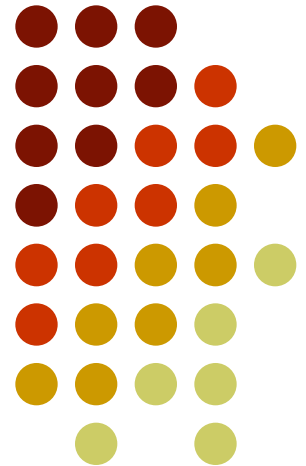


Continuing Enzymatic Advancements

ADVANCED BIOFUELS
LEADERSHIP CONFERENCE



CAPITAL HILTON • WASHINGTON, DC • APRIL 19-21, 2011





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



Biofuels

Provides technology to enable the development and manufacture of fuels & chemicals from agricultural feedstocks



Ethanol



Chemicals



Biopharmaceuticals

Provides technology to enable the development and manufacture of antibodies and other therapeutic proteins



Pharmaceutical Biotech



Enzymes

Develops, manufactures and markets enzymes and other biological products for a variety of industrial uses



Textiles



Food



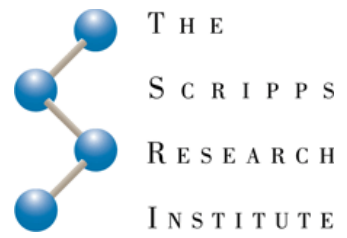
Animal Feed



Pulp & Paper₃



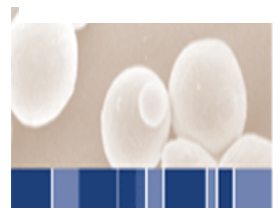
Scientific Collaborations



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
- ❖ **Annotated sequenced C1 genome (2005-2010)**
- ❖ **Re-annotated re-sequenced 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 Senior Scientist, Netherlands Organization for Applied Scientific Research (TNO)

❖ 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 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 International Initiative

The Abraham Group LLC

- ❖ Influential consulting firm led by former U.S. Secretary of Energy, Spencer Abraham
- ❖ Secretary Abraham also serves as non-executive Chairman of AREVA, Inc. and as a member of the Board of Directors of Occidental Petroleum
- ❖ Pursuing a global strategy to 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





Biofuels Partners

ABENGOA

- ❖ One of the largest ethanol producers in the world
- ❖ R&D program led to non-exclusive Dyadic C1 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
- ❖ Established the Enzyme Development Technological Program
- ❖ Commercial Plant (US) in 2012
 - ❖ Objective: production at a cost line competitive



Biofuels Partners (Cont.)



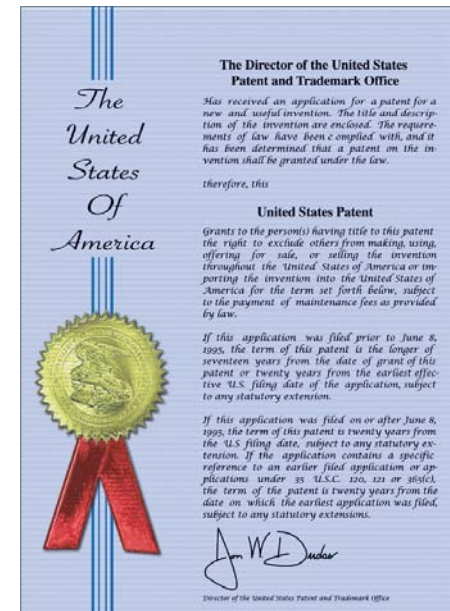
- ❖ **2008 - Non-exclusive Dyadic C1 license agreement**
- ❖ **Biofuels partner of Royal Dutch Shell/Cosan**





Strong Intellectual Property

- ❖ 10 issued U.S. patents
- ❖ Broad claims blocking use of C1
- ❖ 10 pending U.S. patent applications
- ❖ 74 issued foreign patents
- ❖ 23 pending foreign applications



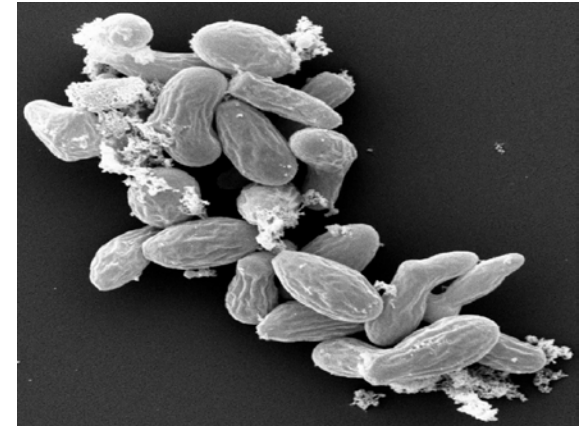


The C1 Technology Platform

*Chrysosporium lucknowense** (C1)

System for gene discovery, expression and protein production

- ❖ A fungus isolated from alkaline soil in Russia
- ❖ Sequenced and annotated (large enzymatic potential)
- ❖ Versatile genetic tools developed (programmable)
- ❖ Mature platform for enzyme and protein production
 - ❖ Favorable fermentation characteristics
 - ❖ High yields, low costs
- ❖ Highly versatile
 - ❖ Can be used to produce a growing number of enzymes or proteins
- ❖ Broad platform capabilities in biofuels and biochemicals



*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 lucknowense* based on morphological characteristics and subsequently reclassified as *M. thermophila* based on genetic tests



Dyadic's Licensing Model vs. Commercial Enzyme Sale Model

- ❖ Proprietary Ownership
- ❖ Customized C1 Fungal Strains
 - ❖ Feedstock
 - ❖ Pre-treatment
 - ❖ Fermentation agents and process
 - ❖ Broad operating conditions (pH and temperature)
 - ❖ *Trichoderma*-based enzymes in state of patent/legal conflict
- ❖ Tax and accounting flexibility allows for treatment of licensing fee as capital expense or operating expense
- ❖ Elimination of commercial enzyme production and transportation costs



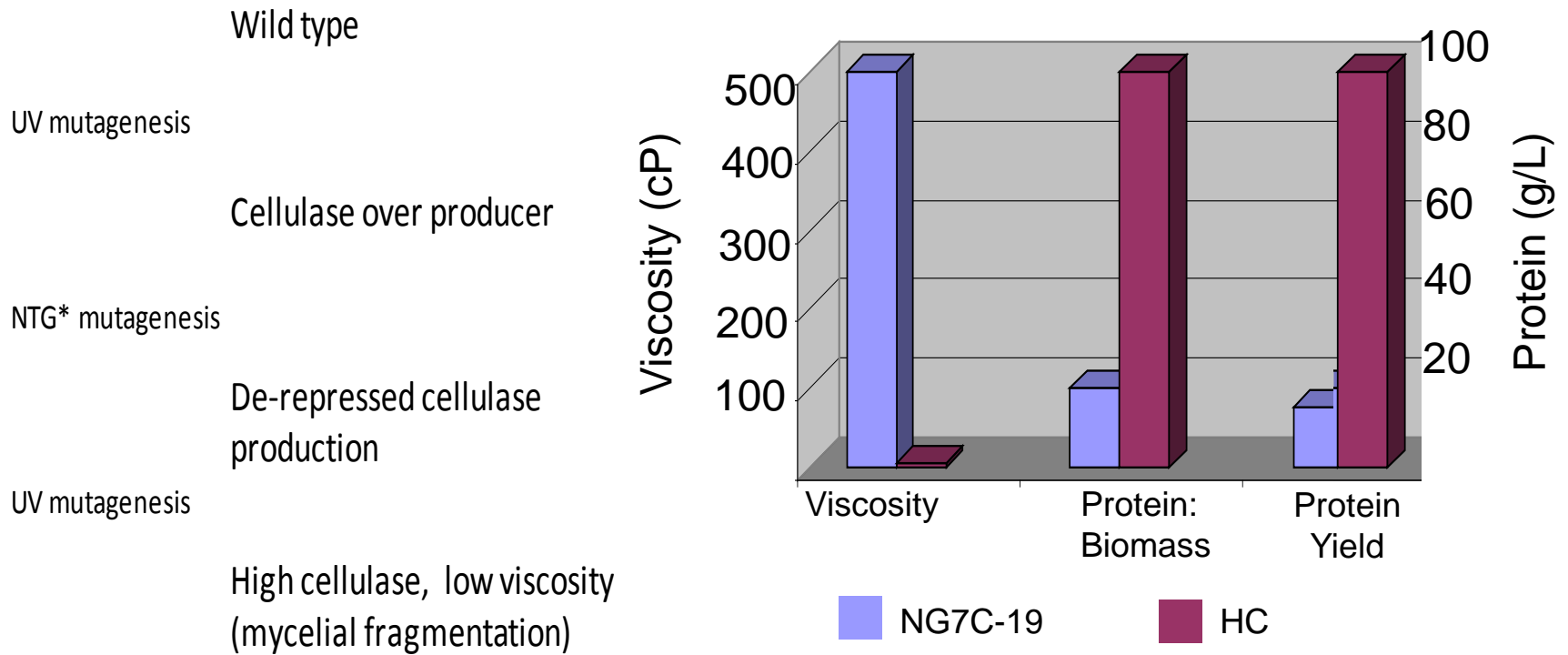
On-Site vs. Off-Site Production

<u>Enzyme Production Costs/Benefits</u>	<u>Purchasing Enzymes Offsite</u>	<u>Producing Enzymes Onsite</u>
Fermentation process	<ul style="list-style-type: none"> •30 -50% of the cost of production 	<ul style="list-style-type: none"> •No markup of fermentation costs
Cell separation process	<ul style="list-style-type: none"> •Cost of equipment and additives •Loss of approximately between 5-10% of enzyme activity •Loss of approximately 5-15% of total enzyme quantity 	<ul style="list-style-type: none"> •No cell separation process required •No loss of enzyme activity/quality •No loss of enzyme quantity
Ultra-filtration (concentration) process	<ul style="list-style-type: none"> •Cost of equipment and replacement filter cartridges 	<ul style="list-style-type: none"> •No ultra-filtration required •No stabilizers or other additives required
Transportation	<ul style="list-style-type: none"> •Shipping costs and delivery time •Potential loss of activity from heat 	<ul style="list-style-type: none"> •No shipping costs or delivery time
Forecasting/Inventory	<ul style="list-style-type: none"> •Longer lead times •Higher inventory levels and warehousing costs 	<ul style="list-style-type: none"> •Reduced lead times •Lower inventory levels and warehouse costs
<ul style="list-style-type: none"> •Ownership •Customization •Improvements 	<ul style="list-style-type: none"> •Customer owns the product but not the process •Customer reliant on supplier to customize and improve product and lower cost of goods 	<ul style="list-style-type: none"> •Proprietary process and product •Programmable system •Control your own destiny



The C1 Technology Platform

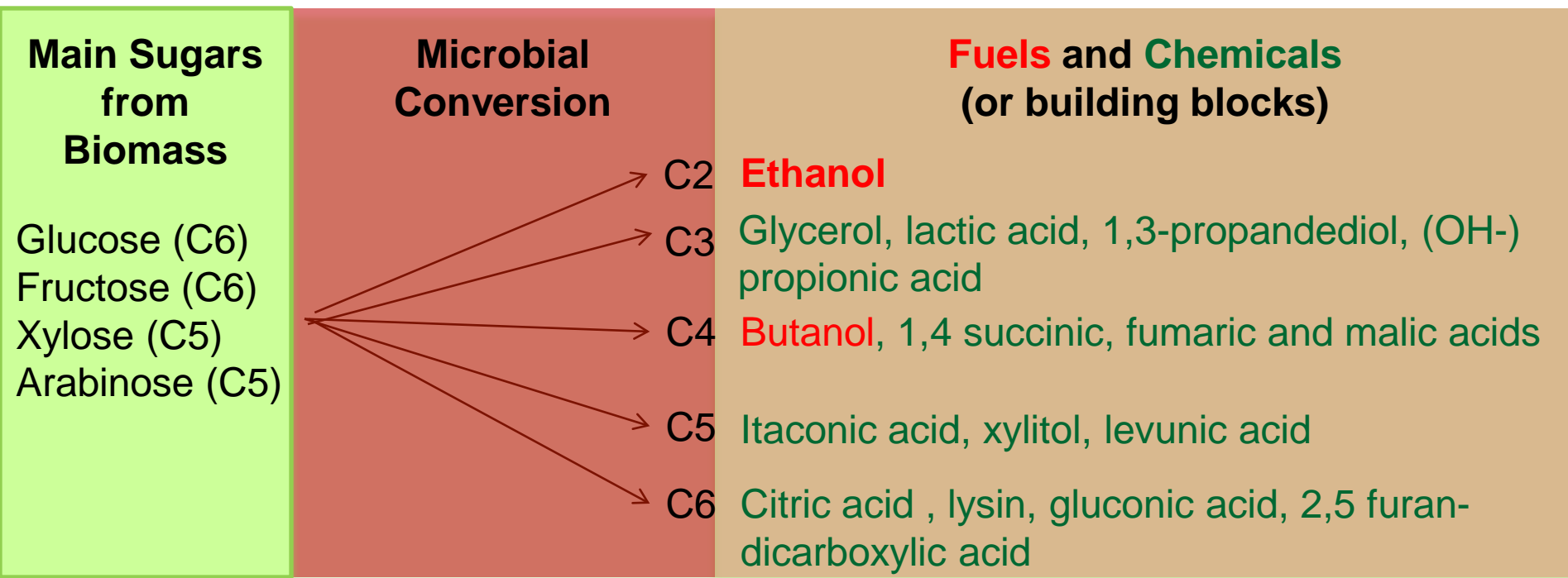
Development of Protein Hyper-producing Strains





Sugar-based Fuels and Chemicals

Replacing Chemicals Now Derived from Fossil Oil with **Sugar-based** Fuels and Chemicals





Sustainable Fuels and Chemicals

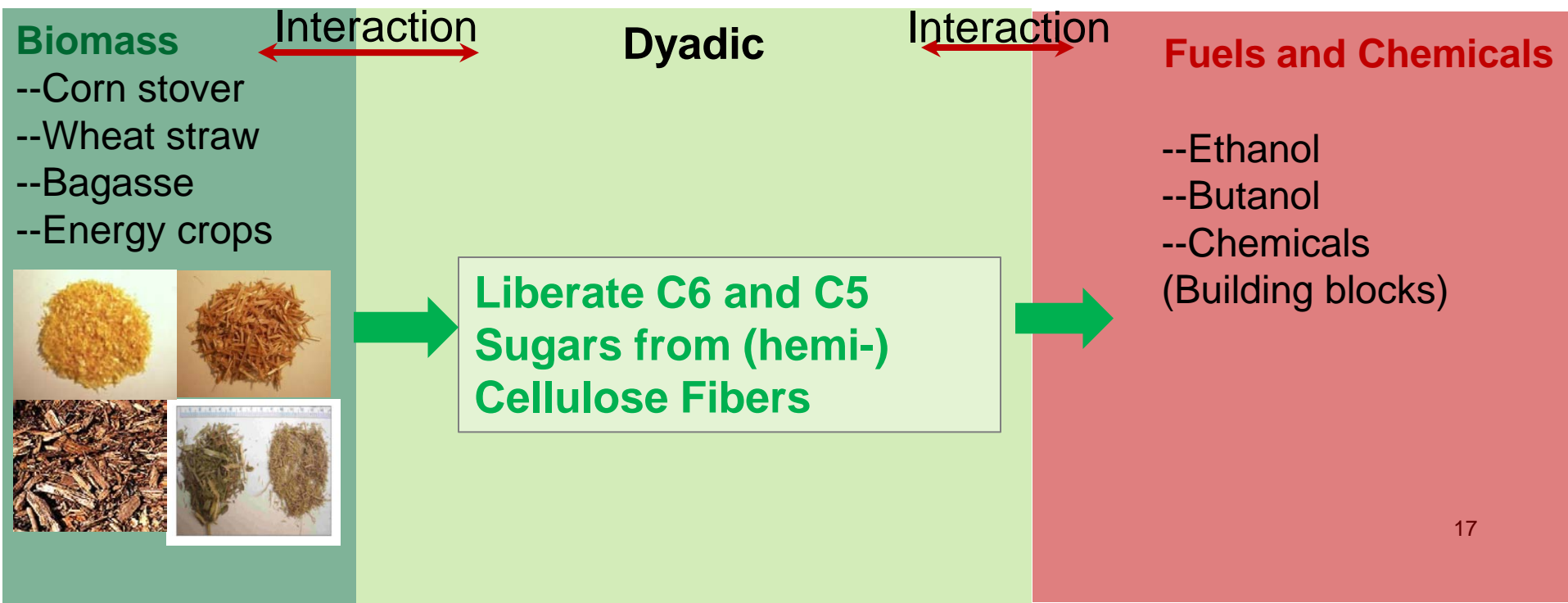
Production from (hemi-) Cellulosic Sugars

Three important processes need to be integrated:

1. Thermo/Chemical Pretreatment

2. Enzyme treatment

3. Microbial conversion





C1 vs. *Trichoderma*

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

Genes encoding	Number in C1	Number in <i>T.reesei</i> *	Biomass Fiber
Endo-glucanases, Cellobiohydrolases, β -glucosidases	~ 55	~ 35	Cellulose
Cellulose binding domains (CBM1-type)	~ 46	~11#	
Xylanases/Xylosidases	~ 13	~ 5	
Arabinofuranosidases/arabinases	~ 14	~ 3	Hemi-cellulose
Esterases (Axe, Fae)	~ 13	~2#	

*From the JGI database

Based on literature and JGI database searches

C1 is a Rich Source of Lignocellulolytic Enzymes!



Biomass Degrading Potential of C1

Carbohydrate Active Enzymes (CAZy) Revealed from Automated Annotation

CAZy Enzymes in C1	Number in C1
Glycoside hydrolases (GH)	175
Carbohydrate esterases (CE)	18
Polysaccharide Lyases (PL)	7
Glycosyl Transferases (GT)	55
Carbohydrate Binding Domains (CBM)	75 (72 part of GH-enzymes)
Total (no-overlap)	258



Exploitation of C1's (Hemi-) Cellulolytic Potential

- ❖ Collection of about 70 functional single (hemi-) cellulase expressing strains have been obtained
- ❖ Detailed biochemical and mechanistic studies performed*
 - ❖ Endo/exo-glucanases, Cellobiohydrolases, β -glucosidases, endo/exo-xylanases, acetyl-esterases, ferulic acid esterases, arabinofuranosidases, β -xylosidases
- ❖ Great diversity of activities discovered--Important for synergy and versatility
- ❖ Ongoing activity in collaborative projects

 **This information can be used to develop highly efficient biofuel enzyme mixtures**

* Hinz, S.W.A., Pouvreau, L., Joosten, R., Bartels, J., Jonathan, M.C., Wery, J., Schols, H.A. (2009). J. Cereal Sci., 50: 318-323.

Kühnel, S., Hinz, S.W.A., Pouvreau, L., Wery, J., Schols, H.A. Gruppen, H. (2010) J. Biores. Technol., DOI: 10.1016/j.biortech.2010.05.070



Experimental C1 Biofuel Enzymes in Industrial Conditions

In specially designed reactors:

- ❖ High biomass loadings possible (>20% DM)
- ❖ Controlled pH, Temperatures
- ❖ Combined (hybrid) saccharification and yeast fermentation (SSF) possible
- ❖ Online ethanol measurements

0.5h



1h



2h



5h



48h



20% solids
100 g/L glucan

Fast viscosity reduction and formation of glucose

~ 90 g/L glucose



Enzyme Development for (Hemi-) Cellulosic Biofuels

Enzyme Mixtures for Cellulosic (C6) Substrates

Pretreated Wheat Straw



Cellulose: 50-55%
Hemicellulose: 5%

Buffer →

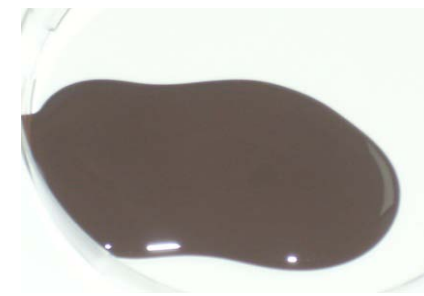
Pretreated Wheat Straw
(20% TS w/w)



Insoluble Cellulose (≈100g/kg)

C1 Enzyme Mixture →

Hydrolysed Wheat Straw

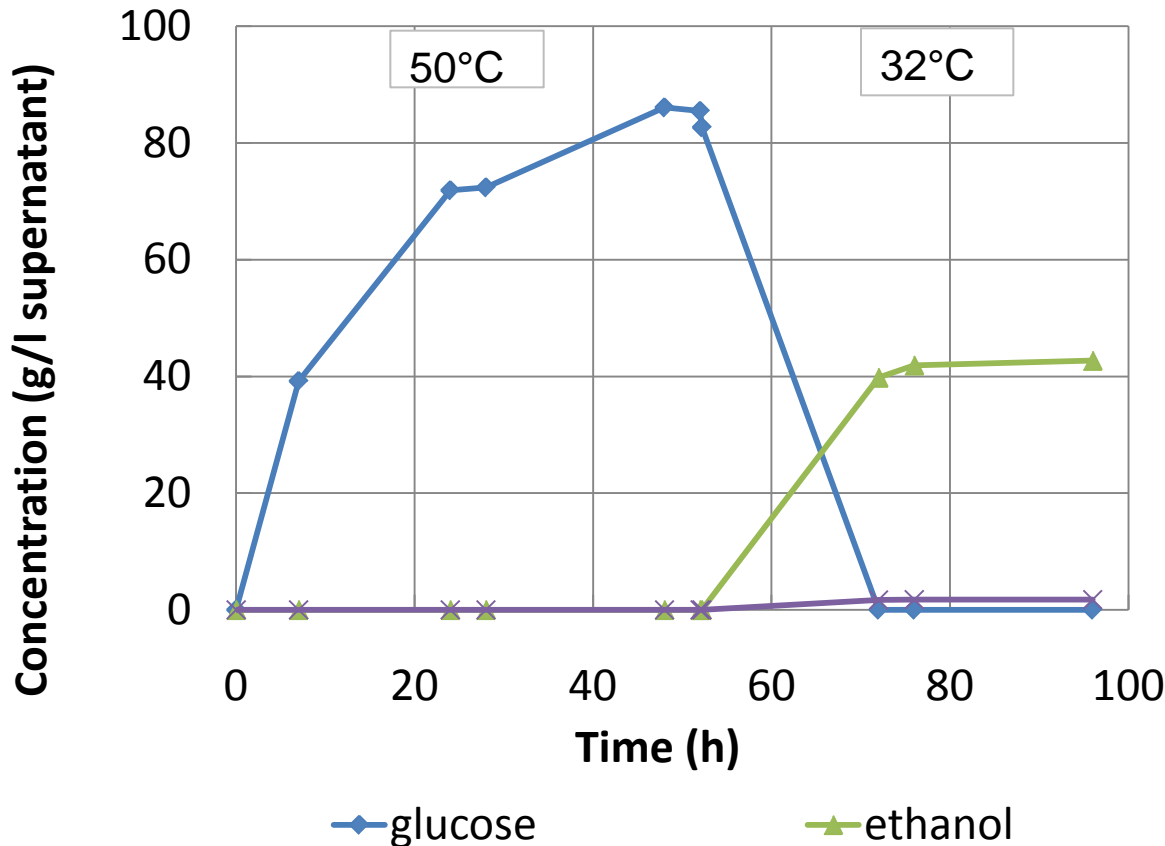


Glucose (≈100g/kg)



Hybrid Saccharification and Fermentation by G3

Example: Dilute acid pre-treated wheat straw, 20% DM



Conclusions:

- ❖ Efficient conversion of glucan to ethanol enabled by G3 (80%) in **3** days
- ❖ Both clarified enzyme and **crude** fungal broth lead to efficient ethanol production

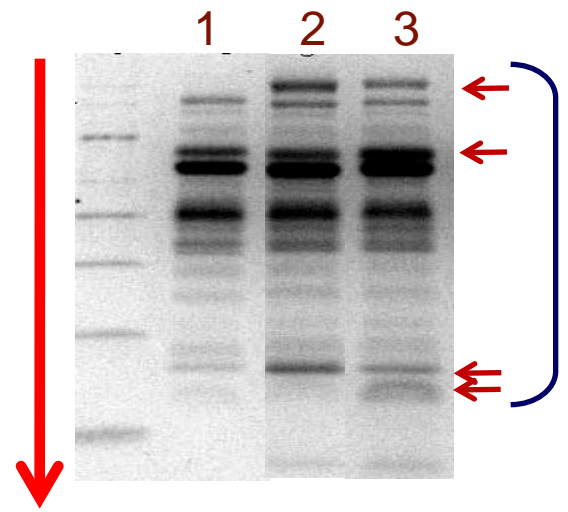


Dyadic Approach:

Single Strain to Produce Cost Effective Enzyme Mixtures

Enzyme Loading to Achieve >70% Saccharification

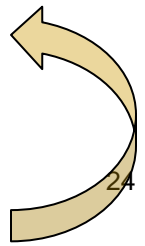
C1 Strain: Baseline Enzyme Mix (G1) 100%



Example:
Specifically overexpressed
cellulases

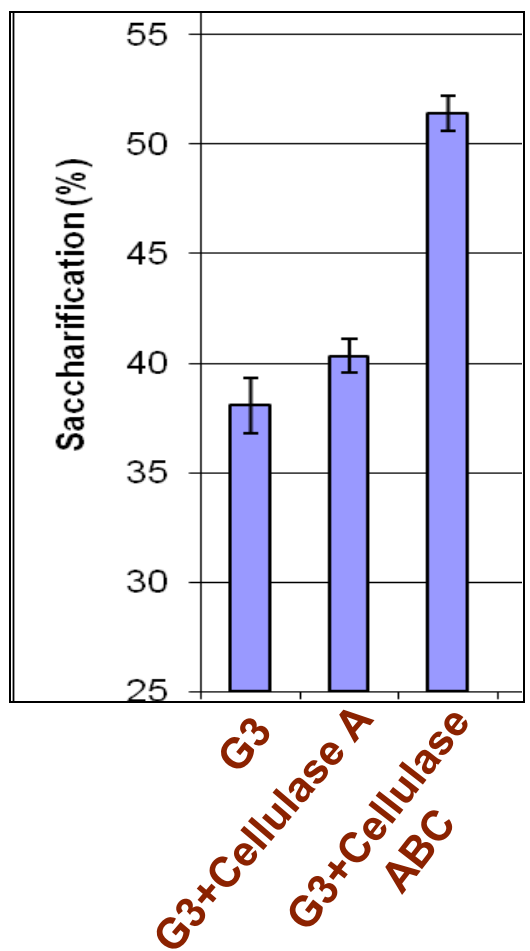
Next Generation Optimized Strain: Improved Enzyme Mix (GX) 20%

Addition of Specific Single (hemi-) Cellulases 20-X %

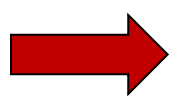




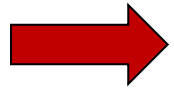
Eliminate G3 Limitations by Mixing with Single Enzymes



- ❖ Substrate: Acid pretreated corn stover
- ❖ Low enzyme loading G3 used



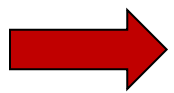
Addition of distinct single cellulases to G3 yielded a tremendous increase in efficiency



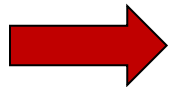
New C1-strains developed: G5 and G7



Eliminate G3 Limitations By Deleting Genes Encoding Adverse Activities



Deletion of Cdh and other oxidase genes that cause gluconate and other acid formation

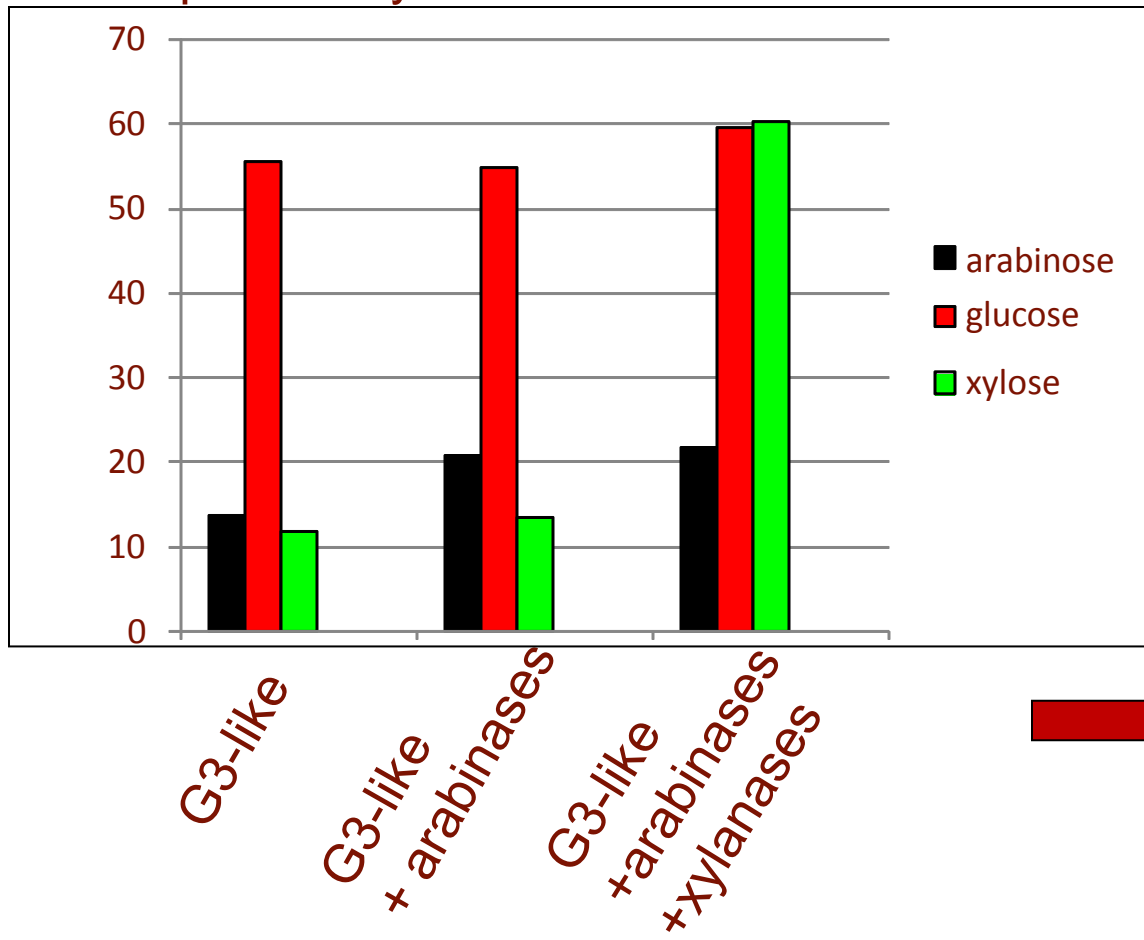


New C1-strains developed lead to higher glucose yields and reduced acidification



Enzyme Development for Hemicellulosic Substrates

Example: Mildly Pretreated Wheat Bran



- ❖ High hemicellulose content (20%)
- ❖ Low enzyme loading G3 used
- ❖ Addition of distinct single hemicellulases to G3 yielded a tremendous increase in efficiency

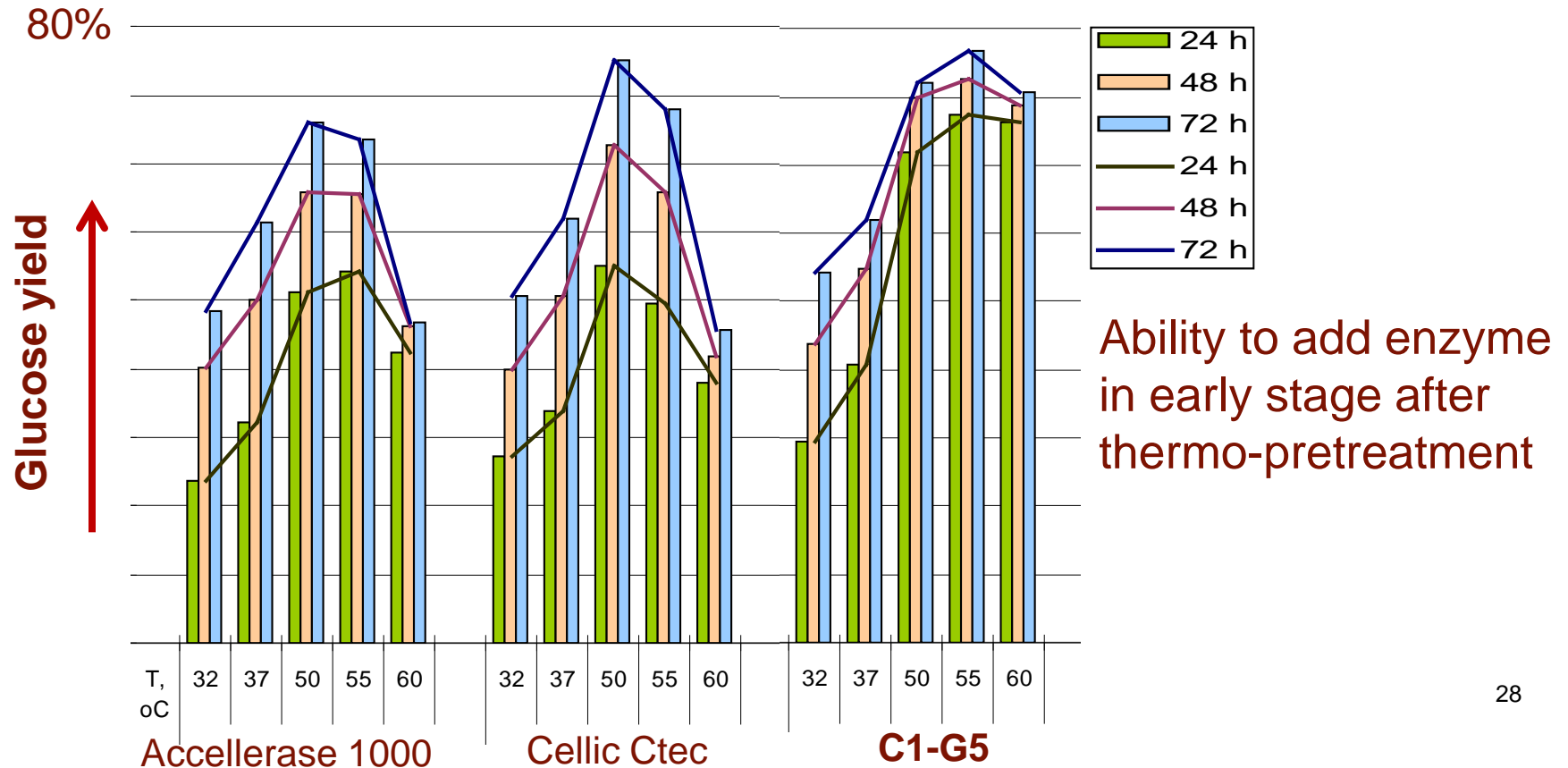


New C1-strains developed for ligno-hemi-cellulosic biomass: G4, G6, G8



C1 Biofuel Enzymes: Broad Active Temperature Range

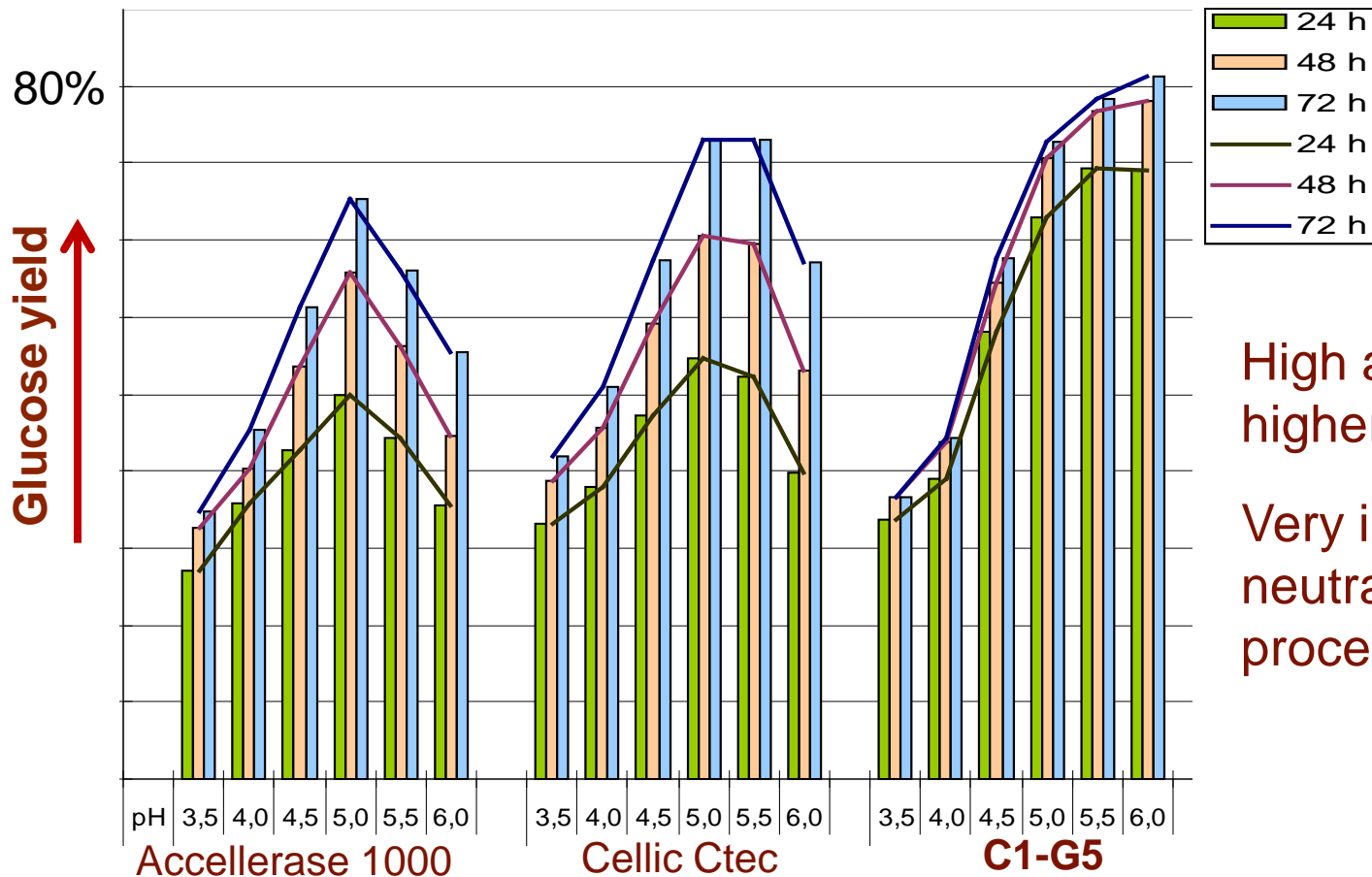
Dilute acid pre-treated corn stover, 10% DM





C1 Biofuel Enzymes: Broad Active pH Range

Dilute acid pre-treated corn stover, 10% DM



High activity at higher pH's:

Very important for neutral (bacterial) processes



Matching Enzyme Activity to Microbial Conversion (SSF conditions)

Fuel/chemical	Micro-organism	T (°C)	pH-range	Selection of Companies
Ethanol	Yeast	32-37	4-5	Nedalco, DSM, Mascoma (CBP)
Ethanol	<i>Z. mobilis</i>	30	7	Dupont/Genencor
Ethanol	<i>E. coli</i>	37	6-7	
Ethanol	<i>T. saccharolyticum</i>	50-60	5-6	Mascoma
Ethanol	<i>T. mathranii</i>	50-80	6.5-7.5	Biogasol
Ethanol	<i>C. phytofermentans</i>	37	6 - 9	Qteros
Butanol	<i>C. acetobutylicum, E.coli, yeast</i>	30-37	4-7	BP/Dupont, Butalco, Gevo, Tetravitae
1,3-Propanediol	<i>E. coli</i>	37	6-7	Dupont/Genencor
Succinic acid	<i>E. coli</i> and other	37	6-7	DSM/Roquette, Myriant
Fatty acids (diesel)	<i>E. coli</i>	37	6-7	LS9/JBEI (DOE)
Farnesene (building blocks, biodiesel)	Yeast	32-37	4-5	Amyris
Isoprene (building block chemical)	<i>E. coli</i>	37	6-7	Genencor/Goodyear
Lactic acid	Bacteria and Fungi	30-60	6-6.5	Purac, Myriant

Critical process variables: pH and T



Algae: A Route to Numerous Biobased Compounds

Deployed by e.g. Martek Biosciences Corp, Solazyme Inc.

Cellulosic Feedstock



Heterotrophic Algae



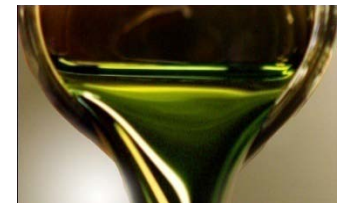
pH 6-9
T=25-30°C

C1 Enzymes

Products



Health supplements

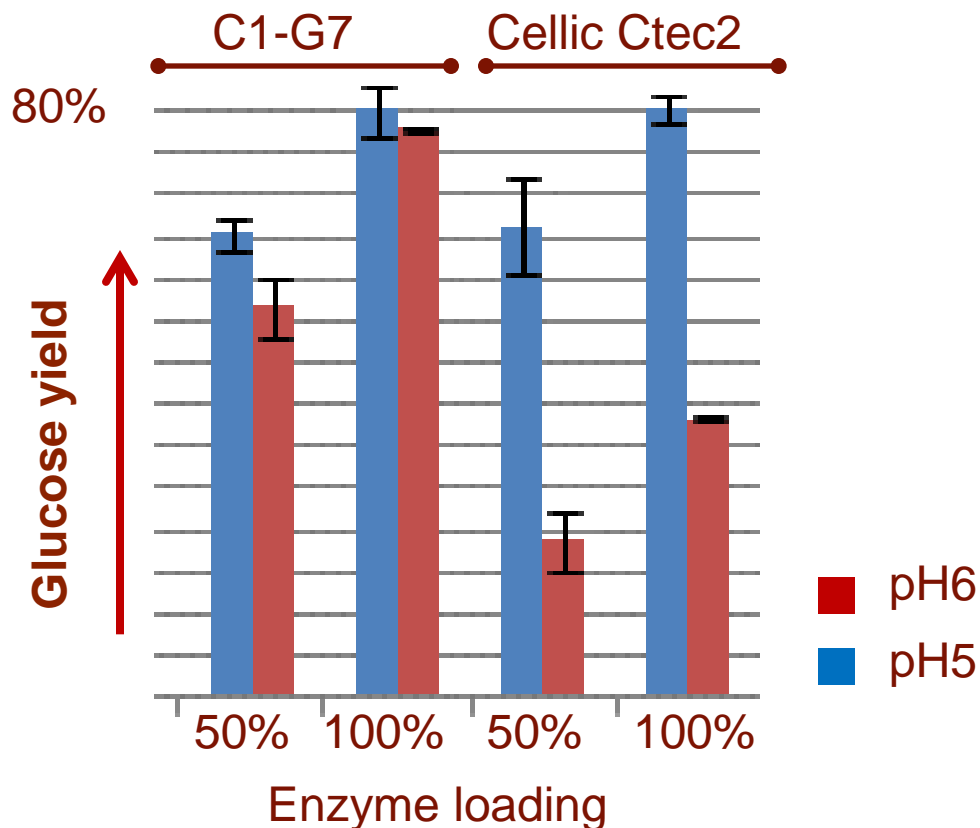


Food ingredients
Transport fuel
Lubricants, polymers



C1 Biofuel Enzymes: Broad Active pH Range

Dilute acid pre-treated corn stover, 10% DM, **24h** saccharification time



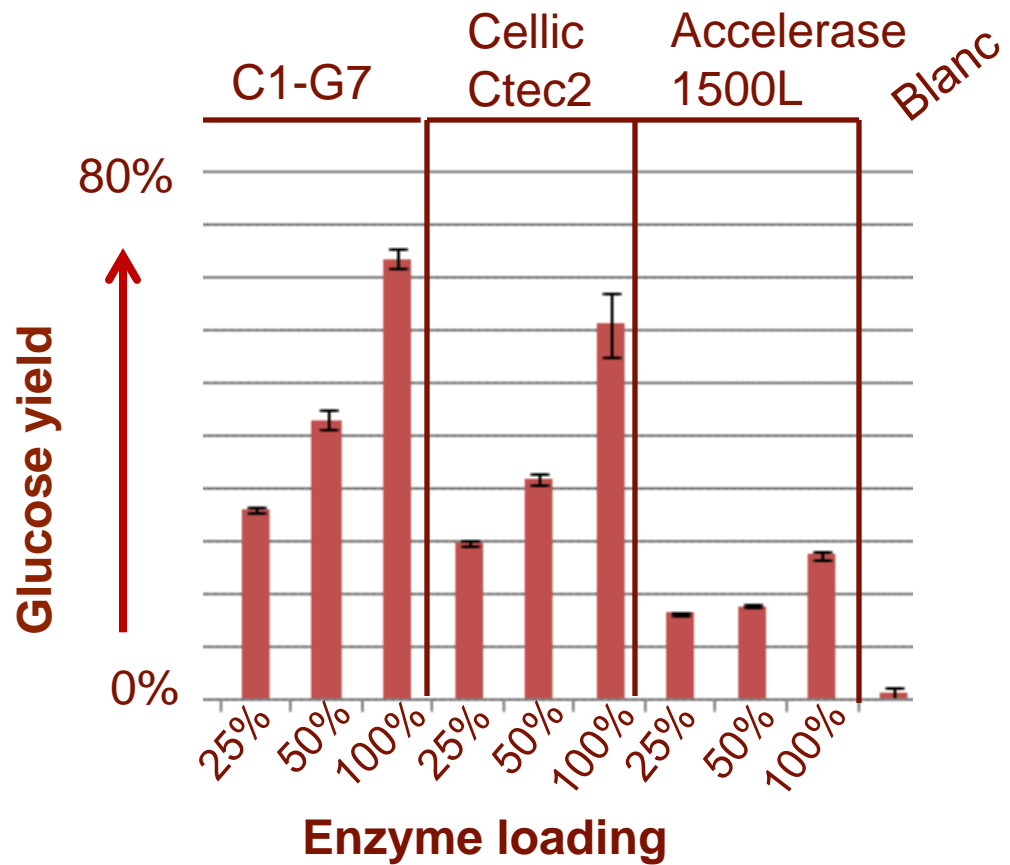
Conclusion:

- G7 makes an excellent enzyme mixture for **BOTH** acidic and more neutral processes



C1 Biofuel Enzymes: Broad Active pH Range

Pre-treated Empty Fruit Bunch, 10% DM, **pH6**

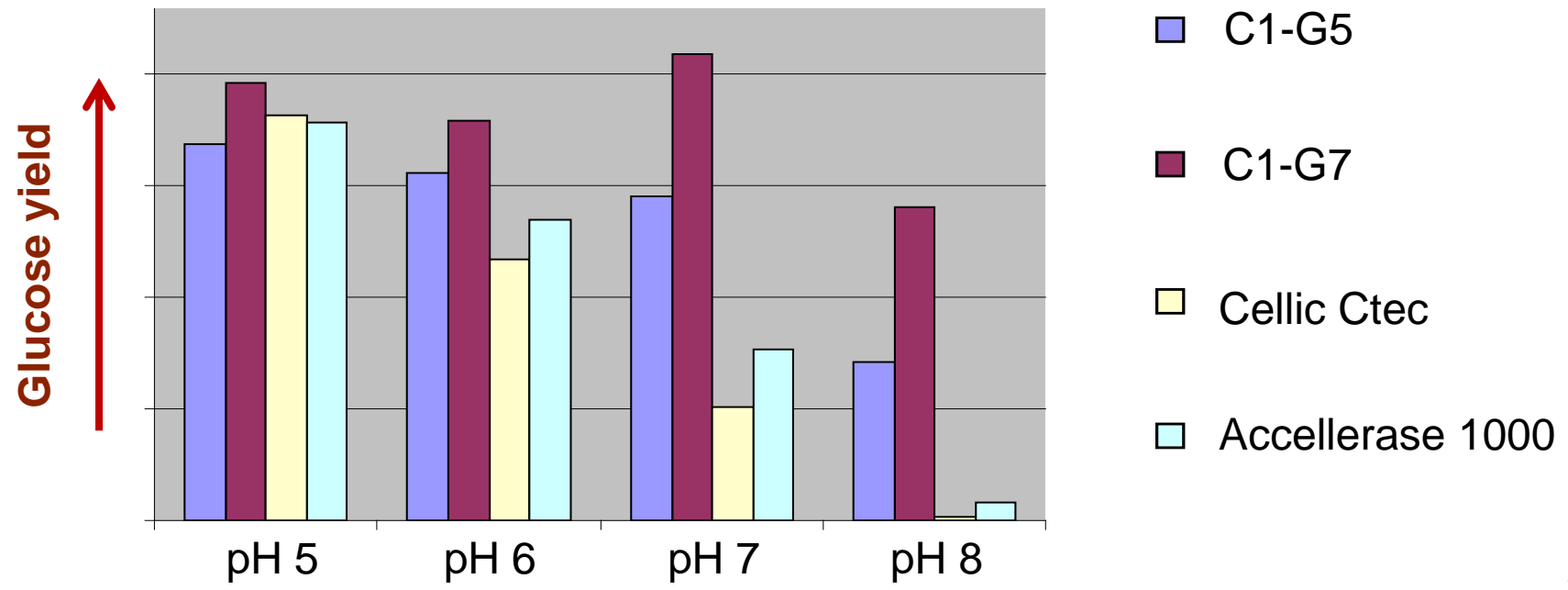




C1 Biofuel Enzymes: Broad Active pH Range

Testing the upper limits of relevant SSF pH's:
pH5, pH6, pH7 and pH8

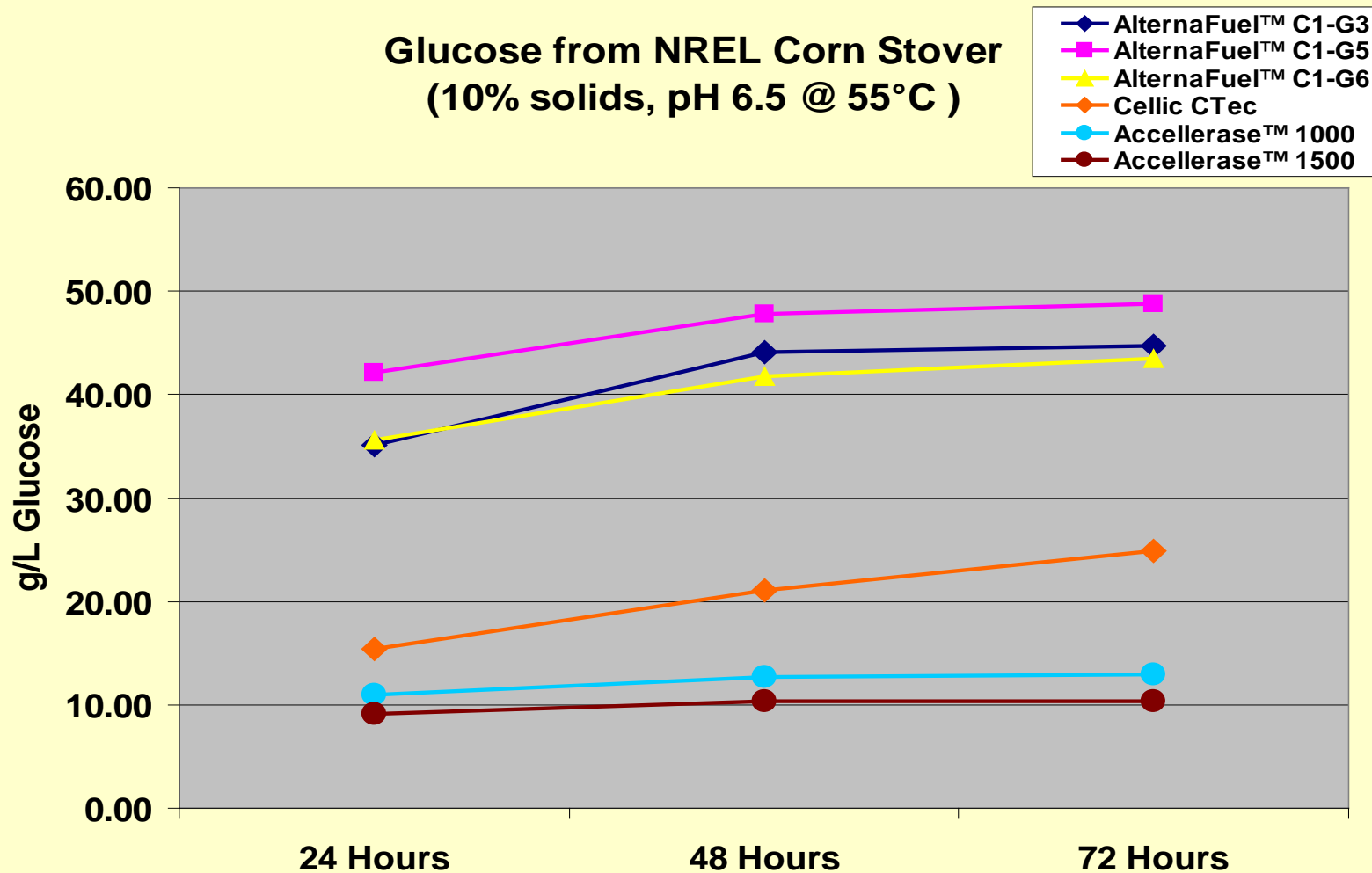
Dilute acid pre-treated corn stover, 10% DM





C1 vs. Genencor/Novozymes at pH 6.5

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





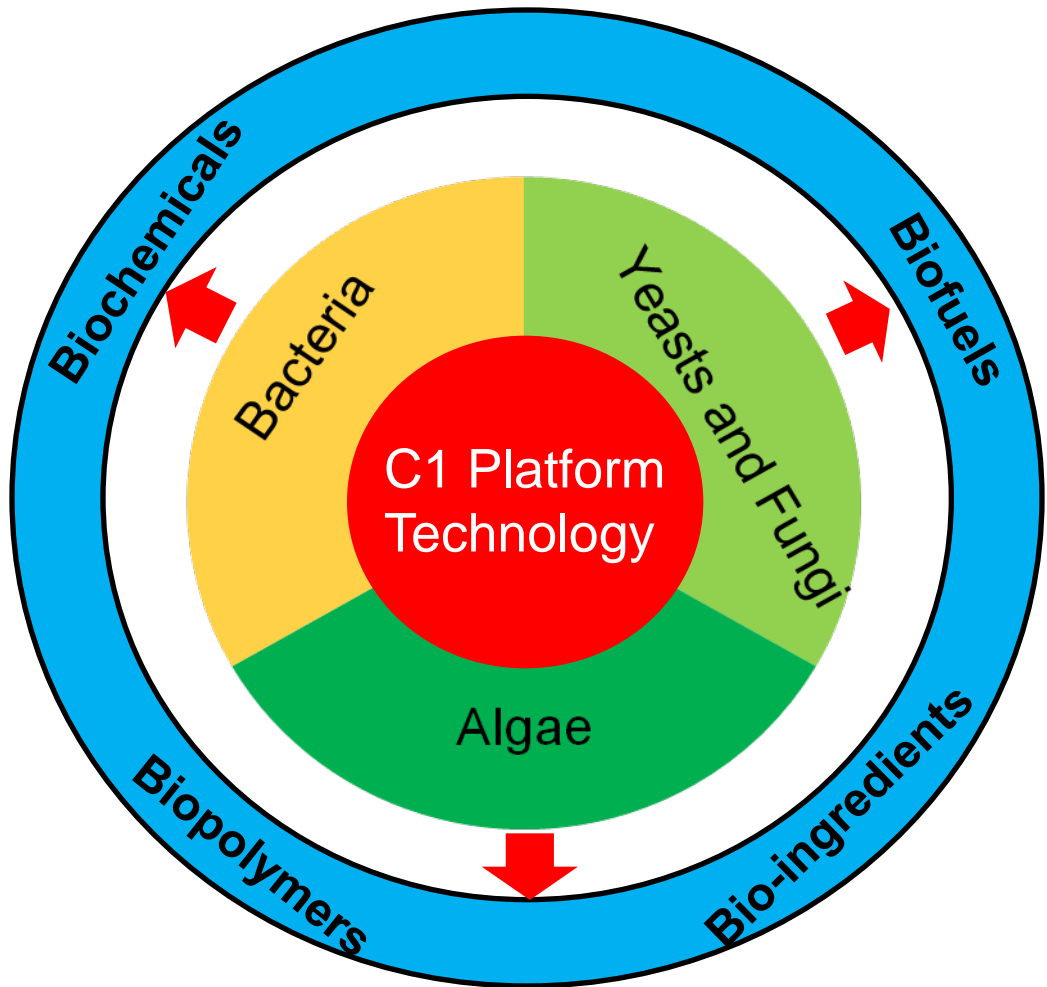
C1--G Series Improvements

Versatile Cellulosic Enzyme Mixtures from a Single C1 Strain

- ❖ **Fast Saccharification:** Ethanol process completed in 72 hours
- ❖ **Broad Temperature Range:** Active between 32°C and 60°C
- ❖ **Broad pH Range:** High activity between pH 4 and pH 8
- ❖ **Active on a Variety of Biomass Substrates:** e.g., Corn Stover, Wheat Straw, Wheat bran, Sugar Cane Bagasse, Switch Grass, Sorghum, Wood



C1 Technology: High Compatibility with Biobased Processes





Summary—Key Points

- ❖ **Broad variety of single (hemi-) cellulase enzymes in C1 is much higher than in the traditional *Trichoderma* host**
 - ❖ Better positioned to develop tailor-made enzyme mixtures for a variety of second generation feedstocks
- ❖ **Broad operating conditions (pH, temperature) allow applications in different process set-ups**
- ❖ **C1 strains have been developed that already produce very efficient and versatile (hemi-) cellulosic enzyme mixtures**
- ❖ **Further immediate optimization of C1 is ongoing**
- ❖ **Excellent outlook for additional significant enzyme cost reductions in the short term**



Thank You

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

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