

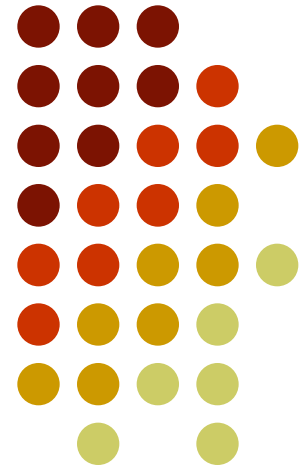


Dyadic International



The C1 technology platform

*Facilitating the economic production of
lignocellulose based biofuels and chemicals*



Mark A. Emalfarb

President and CEO



Dyadic International



- ❖ A global biotechnology company
- ❖ Founded in 1979 by Mark A. Emalfarb
- ❖ Provider of licensed patented and proprietary technologies for on-site manufacturing of enzymes
- ❖ Applications in bioenergy, biopharmaceutical and industrial enzyme markets
- ❖ Manufacturing/selling enzymes since 1994
- ❖ Publicly traded since 2004 (DYAI)
- ❖ Headquartered in Jupiter, Florida, USA
- ❖ R&D arm located in the Netherlands





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



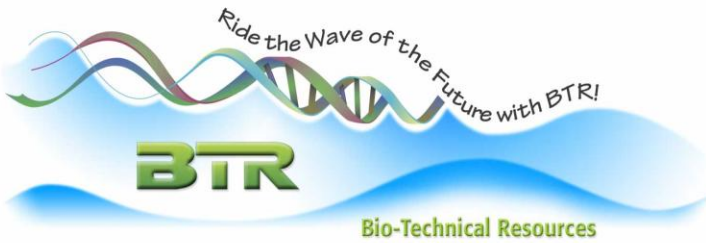
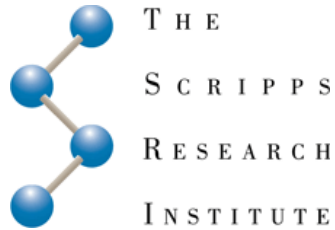
Animal Feed



Pulp & Paper



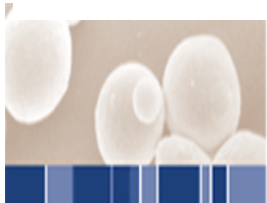
Scientific Collaborations



Moscow State University



Savannah River National Laboratory



Kluyver | CENTRE | Kluyver Centre for Genomics of Industrial Fermentation



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)

❖ Core competencies

- ❖ Genome Mining
- ❖ Fungal Molecular Biology
- ❖ Fermentation technology
- ❖ Enzymology

❖ Located in Wageningen, the Netherlands

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





Dyadic's International Initiative

The Abraham Group LLC

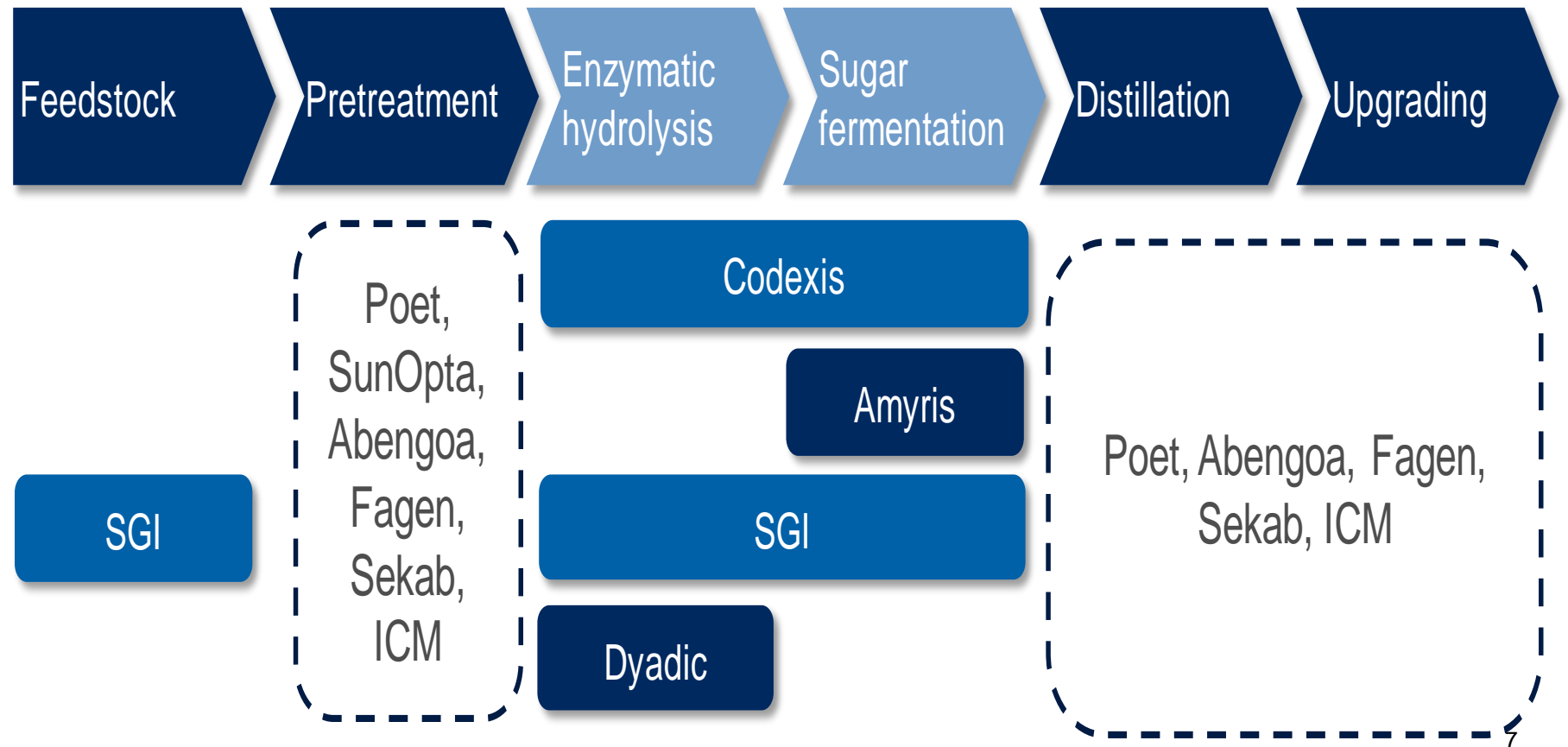
- ❖ Influential consulting firm led by former U.S. Secretary of Energy, Spencer Abraham
- ❖ Secretary Abraham also serves as non-executive Chairman of AREVA, Inc. and as a member of the Board of Directors of Occidental Petroleum
- ❖ Pursuing a global strategy to communicate advantages of Dyadic's C1 platform technology and its R&D capabilities to major international energy groups committed to cellulosic ethanol and other forms of sustainable energy





2nd Generation Biofuels

Value Chain





Dyadic's Biofuels Partners

ABENGOA

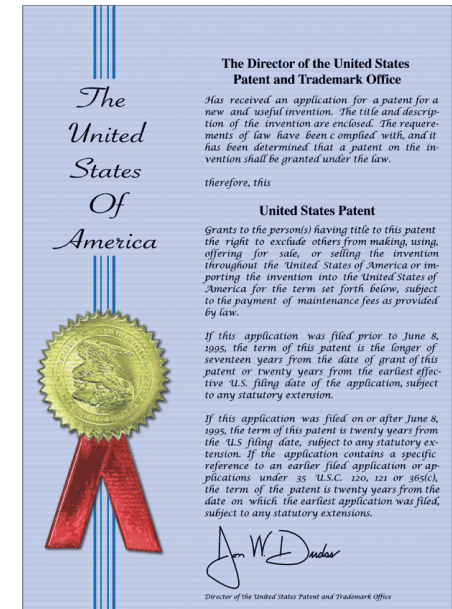


❖ Non-exclusive licensees



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



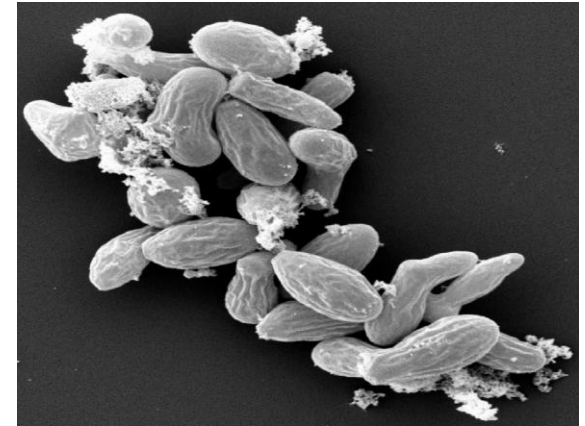


The C1 Technology Platform

*Chryso sporium 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 *Chryso sporium luckowense* 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



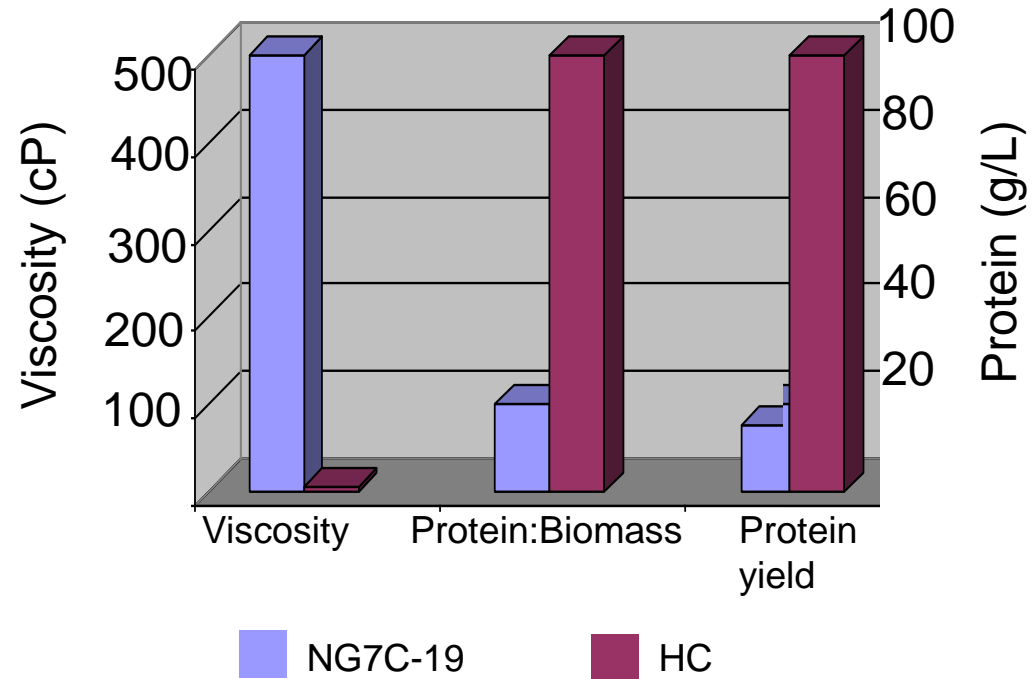
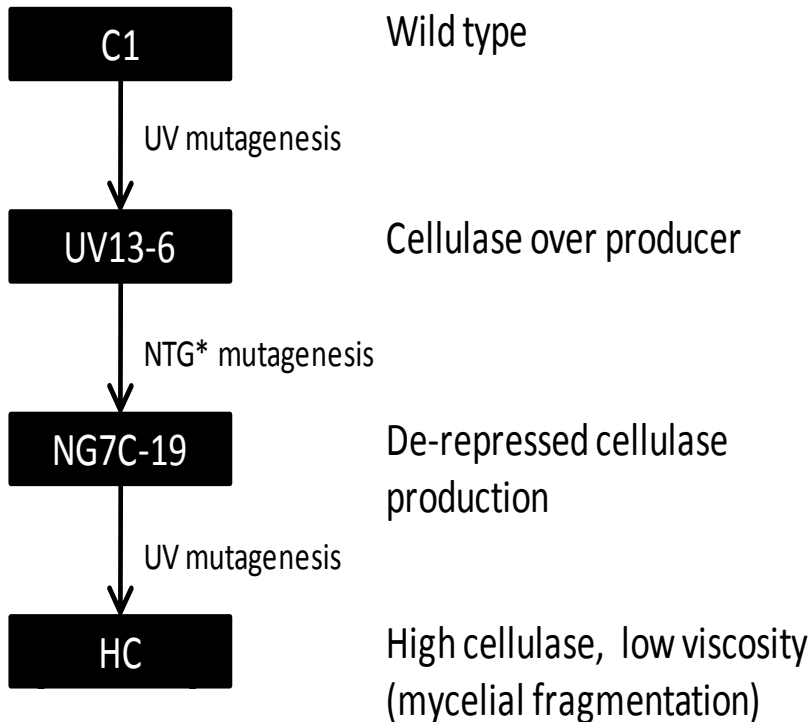
On-Site vs. Off-Site Production

<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





The C1 technology Platform

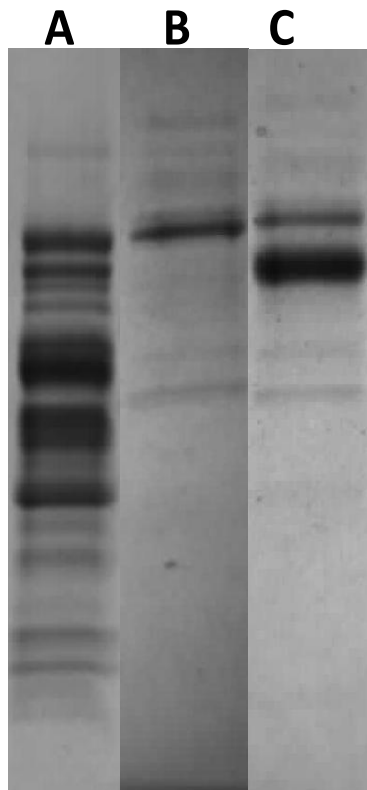
Construction of tailored strains

Result of constructing more than 1000 modified C1 strains:

- 1. Construction of “clean” background strains (LC-strains)**
 - Suitable for pure single enzyme expression
- 2. Strains with high level expression of specific enzymes (HC-strains)**
 - Successfully have over expressed 7 different genes simultaneously
- 3. Strains exhibiting low protease profile**
 - Suitable for heterologous enzyme expression



Construction of “clean” background strains (LC-strains).



← Individual target enzyme

(A) Baseline C1-strain:
High cellulolytic activities
Diverse enzyme mixture
Up to 100 g/L total protein

(B) Low cellulase background strain (LC):
Almost no cellulolytic activities
Very few endogenous secreted
Suited for enzyme characterization

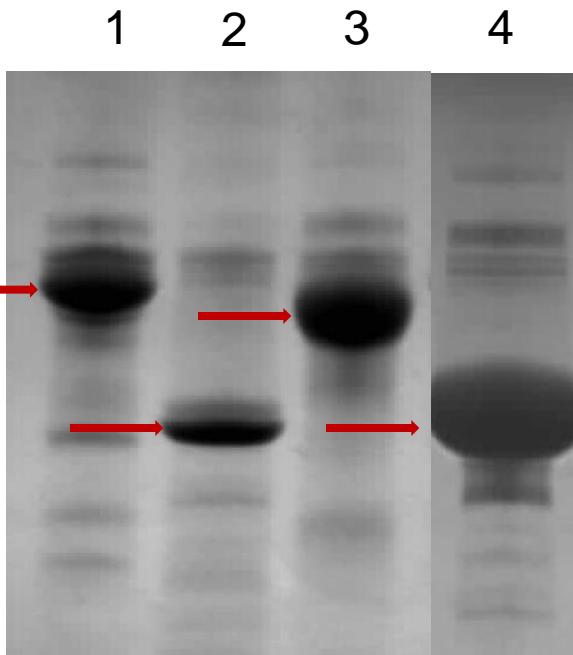
(C) ~ Up to 80% target protein



A library of LC-strains producing single enzymes

- ❖ 70 functional single (hemi-) cellulase expressing strains obtained
- ❖ Production levels: up to 40 g protein/l
- ❖ Approx. 30g/l of relatively pure target enzyme has been produced

➡ Important for both research and commercial purposes



SDS-Page analysis of end of fermentation broth of single enzyme producing LC-strains



The C1 Technology Platform

Development of versatile genetic tools

- ❖ Transformation system: High efficiency, stable integration
- ❖ Several genetic markers available: Auxotrophic and dominant
 - ❖ **Allows for multiple rounds of transformation**
- ❖ Gene expression: Variety of expression signals.
 - ❖ Constitutive, inducible at various strengths
- ❖ Protein production: Efficient secretion signals
- ❖ Targeted gene disruption: Efficiency up to 90%
- ❖ Variety of optimized C1-hosts
- ❖ Based on self cloning: **No foreign DNA needed**



The C1 Technology Platform

Production of Heterologous Proteins

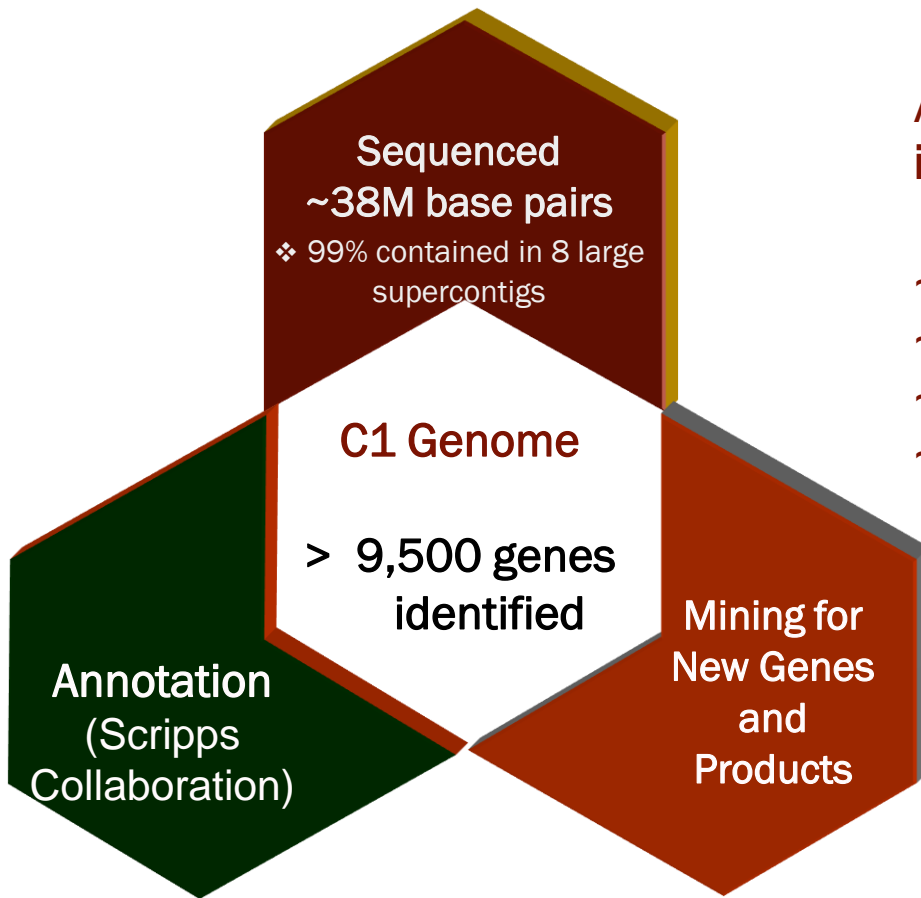
Functional **heterologous** enzymes/proteins from a variety of organisms have been produced in the g/L scale:

Source	Enzyme/protein	Yield (range)
Fungal	Xylanases, amylase, cellulase, endo-polygalacturonase, oxidase, phytase	Up to 15 g/L
Bacterial-directed evolution	Confidential	Up to 6 g/L No extensive yield optimization done
Human	Immunoglobulin IgG1	IgG 1 – 2 g/L No extensive yield optimization done



The C1 Technology platform

Exploration Enzymatic Potential: Genome Mining



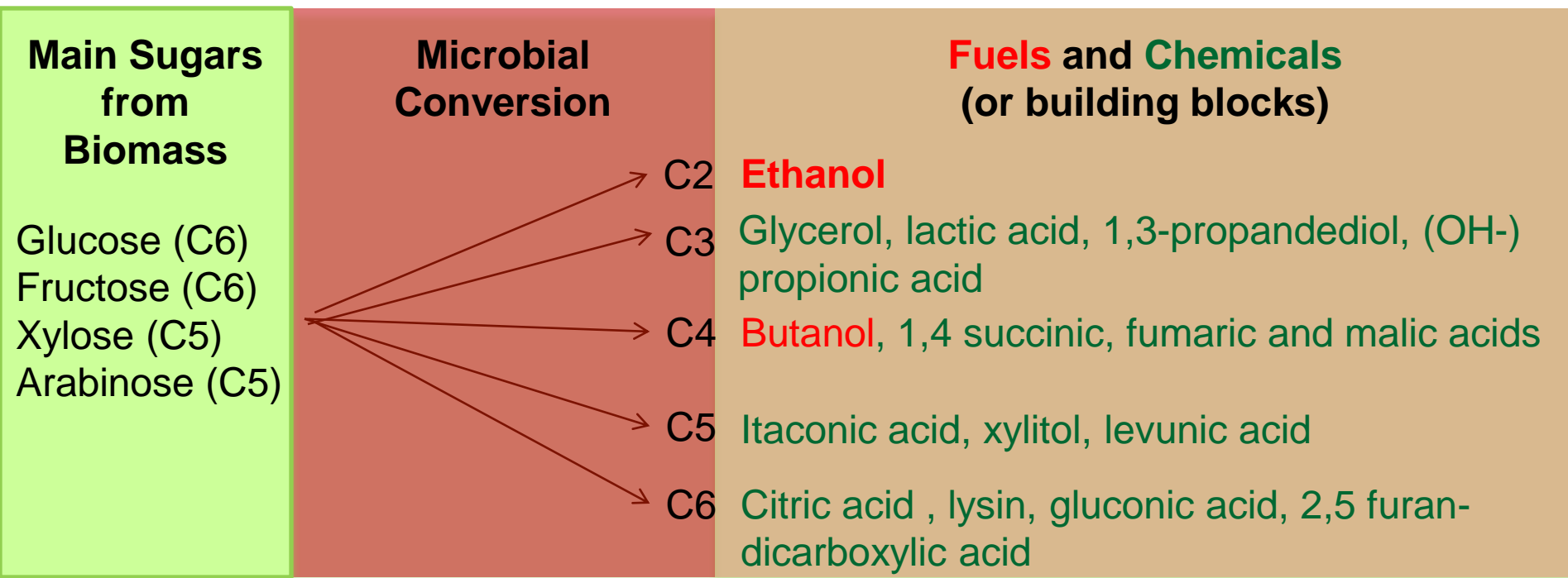
A large number of genes putatively encoding **industrially important** enzymes discovered:

- ~250 Carbohydrate-active Enzymes (CAZy)
- ~150 proteases
- ~700 oxido-reductases
- ~75 lipases / esterases.



Sugar-based Fuels and Chemicals

Replacing chemicals now derived from fossil oil with **sugar-based** fuels and chemicals





C1 vs. *Trichoderma*

Lignocellulolytic Potential of C1 vs. *Trichoderma reesei*
 (the 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#	

Based on literature and JGI database searches

C1 is a Rich Source of Lignocellulolytic Enzymes!



Biomass Degrading Potential of C1

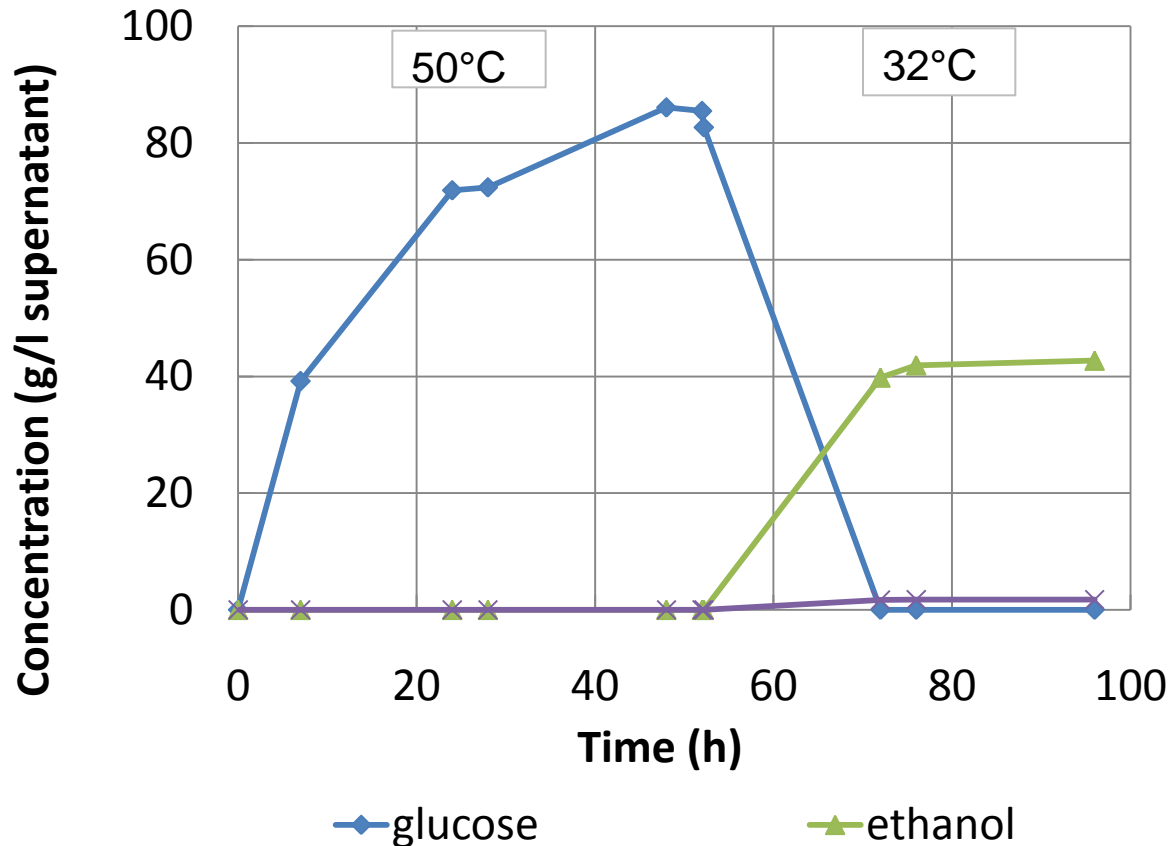
Carbohydrate active enzymes (CAZy) as revealed from automated annotation

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



Hybrid Saccharification and Fermentation by G3

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



Conclusions:

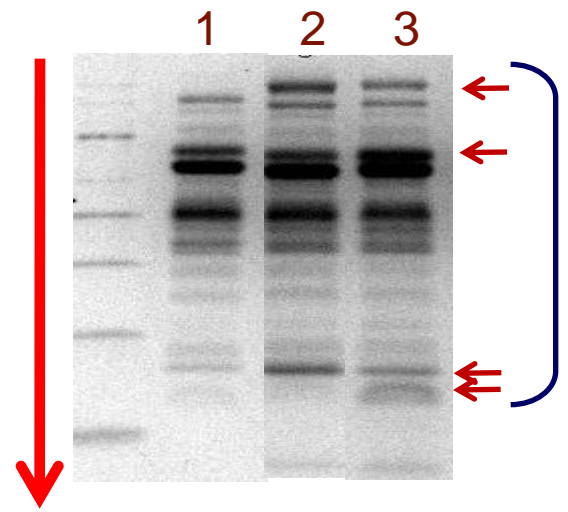
- ❖ Efficient conversion of glucan to ethanol enabled by G3 (80%) in 3 days
- ❖ Both clarified enzyme and **crude fungal broth** lead to efficient ethanol production



Construction of Strain to Produce Cost Effective Enzyme Mixtures

Enzyme Loading to Achieve >70% Saccharification

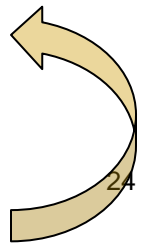
C1 Strain: Baseline Enzyme Mix (G1) 100%



Example:
Specifically overexpressed
cellulases

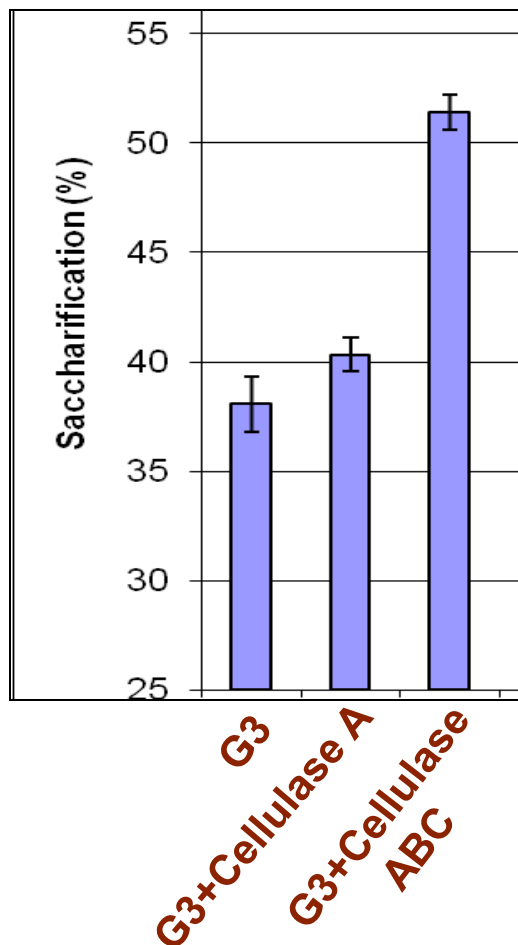
Next Generation Optimized Strain: Improved Enzyme Mix (GX) 20%

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

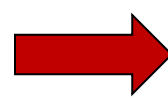




Eliminate Limitations by Mixing with Single Enzymes



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



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

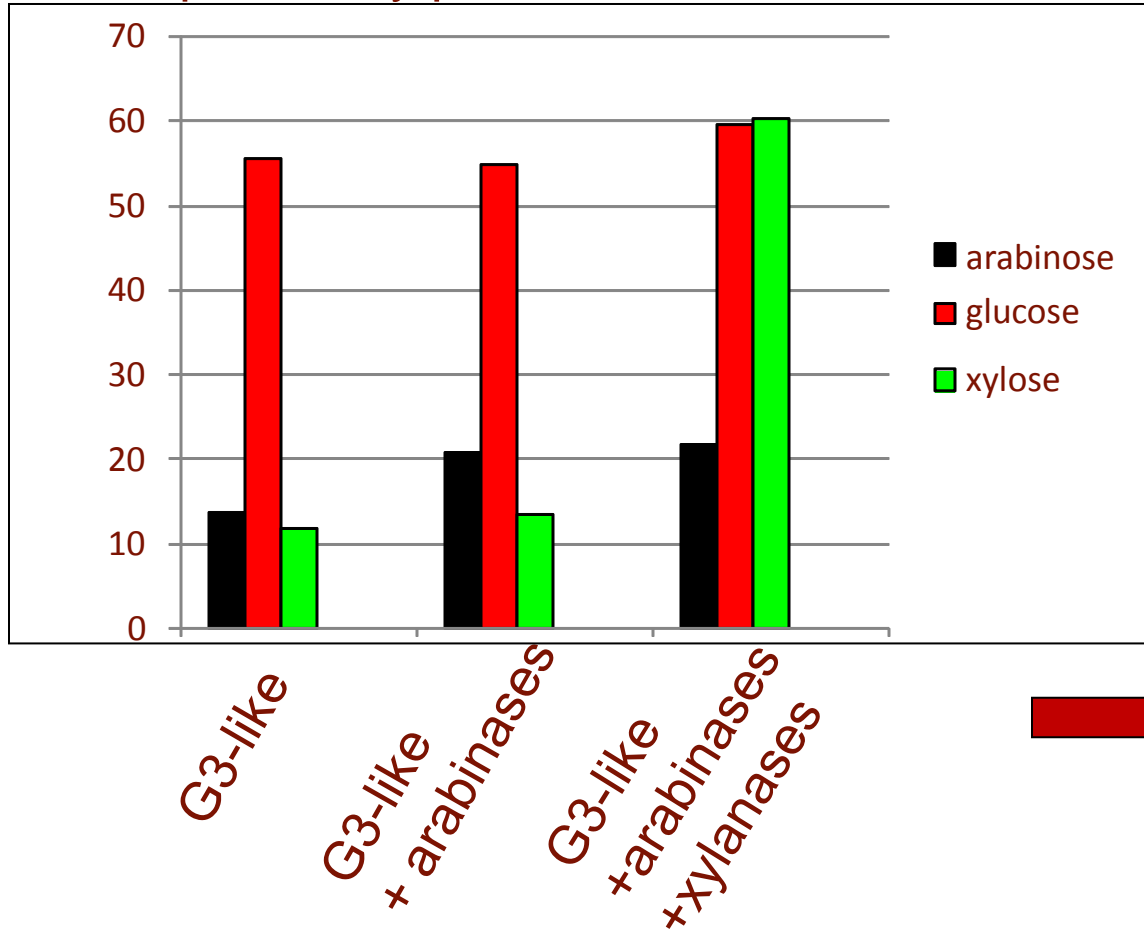


New C1-strains developed: G5 and G7



Eliminate Limitations by Mixing with Single Enzymes

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

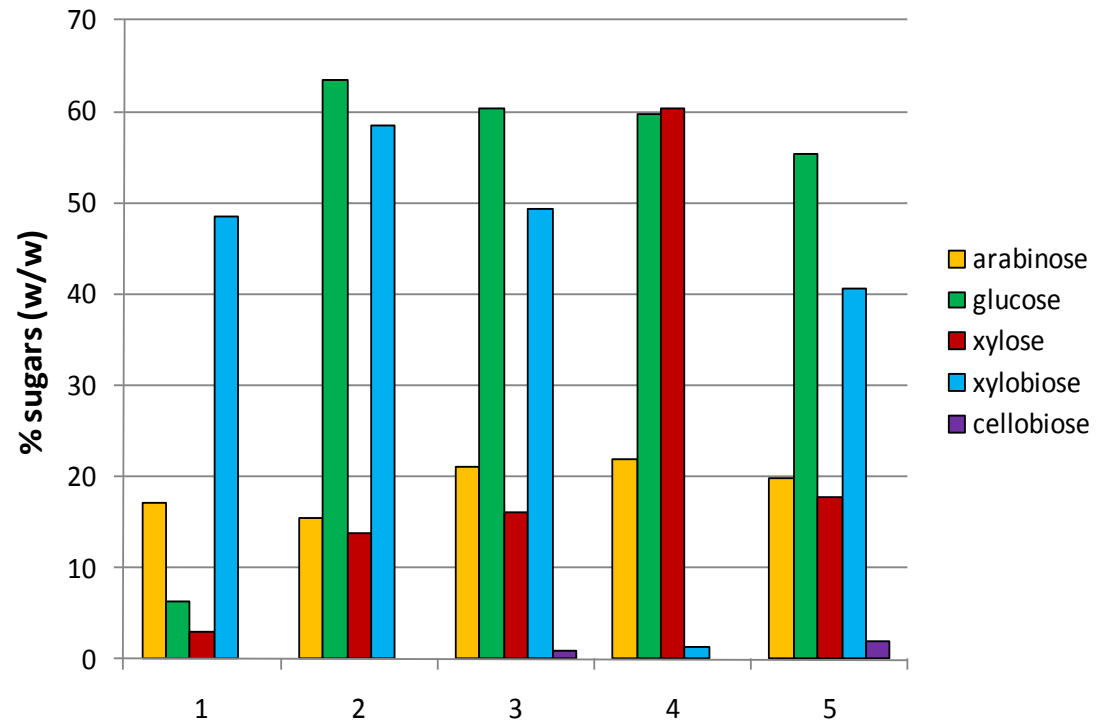


Eliminate Limitations by Mixing with Single Enzymes

Pretreated wheat bran (insoluble), 10% (g DW/L), 1% enzyme loading, pH 5, 50°C, 24h

Glucose, xylose and arabinose determined by HPAEC analysis

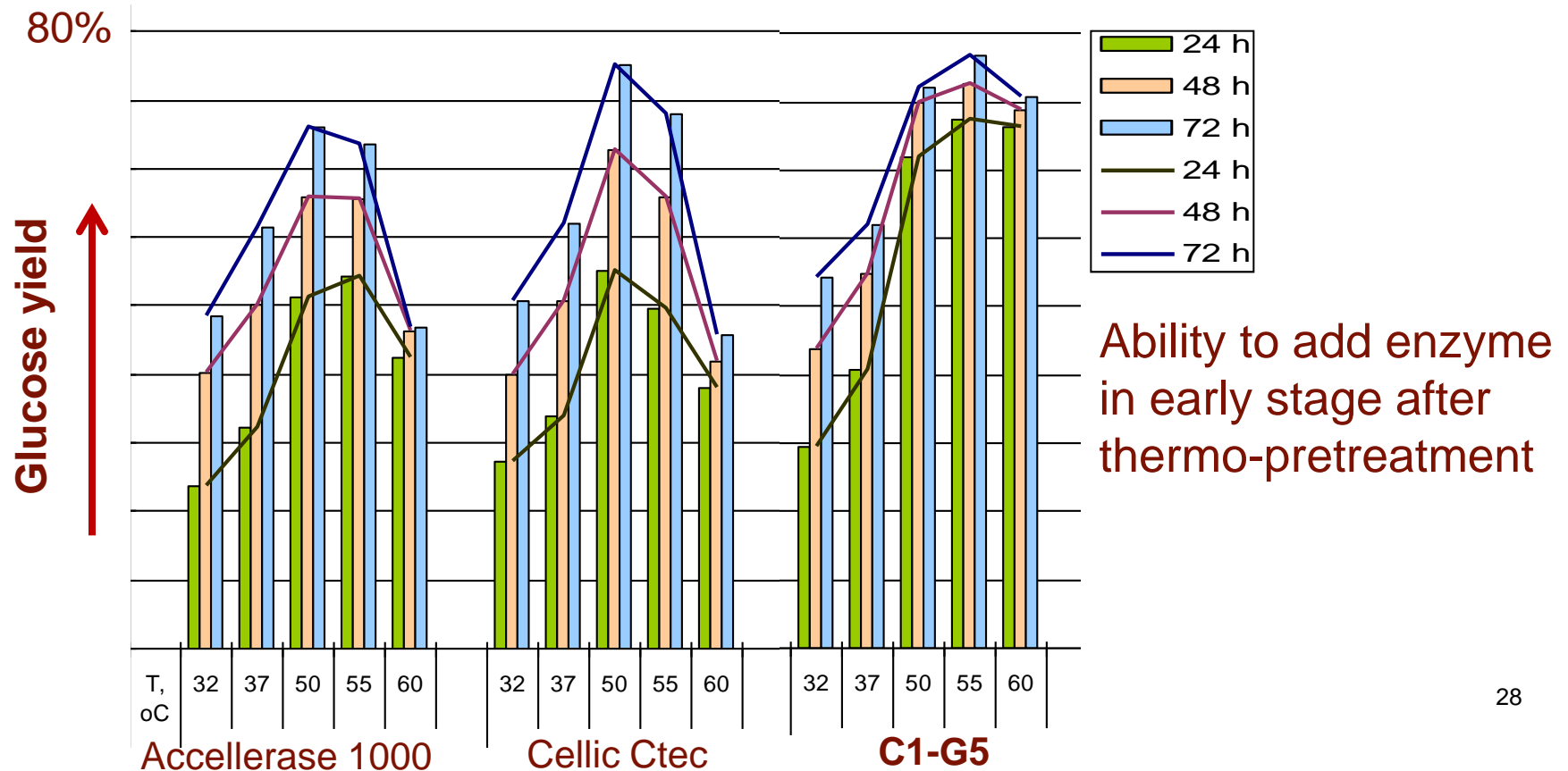
1. C1 Host strain
2. (Hemi-)cellulase Mix1
3. Mix1 + 2 AXH's
4. Mix1 + 2 AXH's + β -xylosidase
5. Mix1 + 2 AXH's + β -glucosidase





C1 Enzymes: Broad Temperature Range

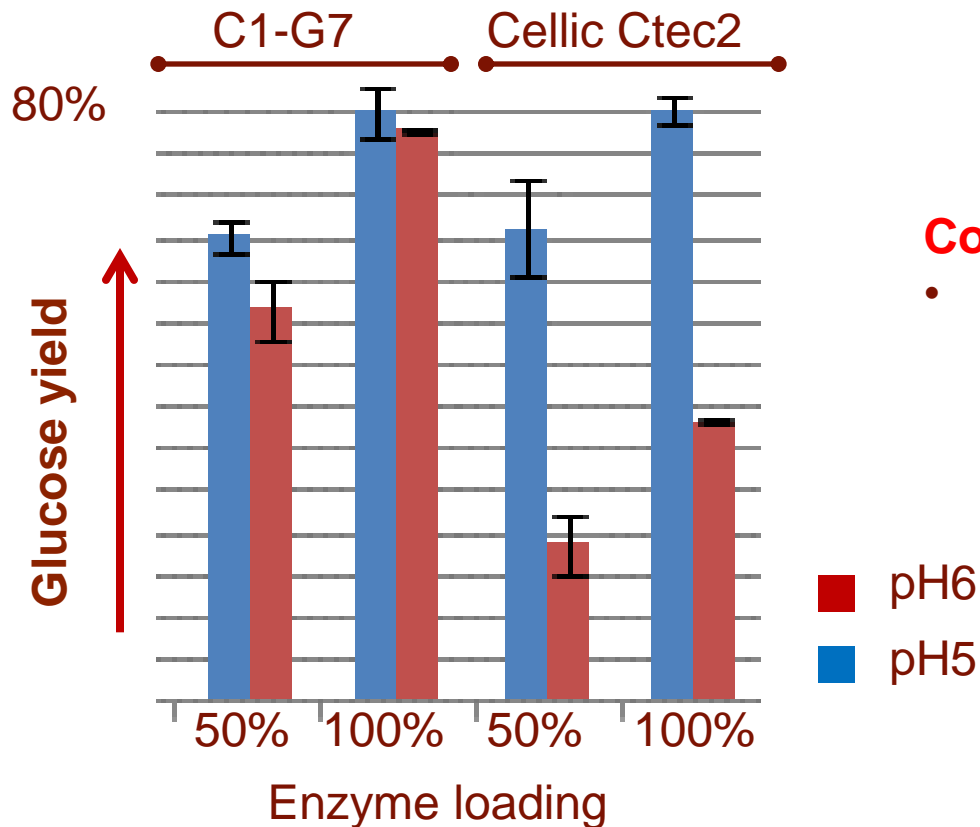
Dilute acid pre-treated corn stover, 10% DM





C1 Biofuel Enzymes: Broad Active pH Range

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



Conclusion:

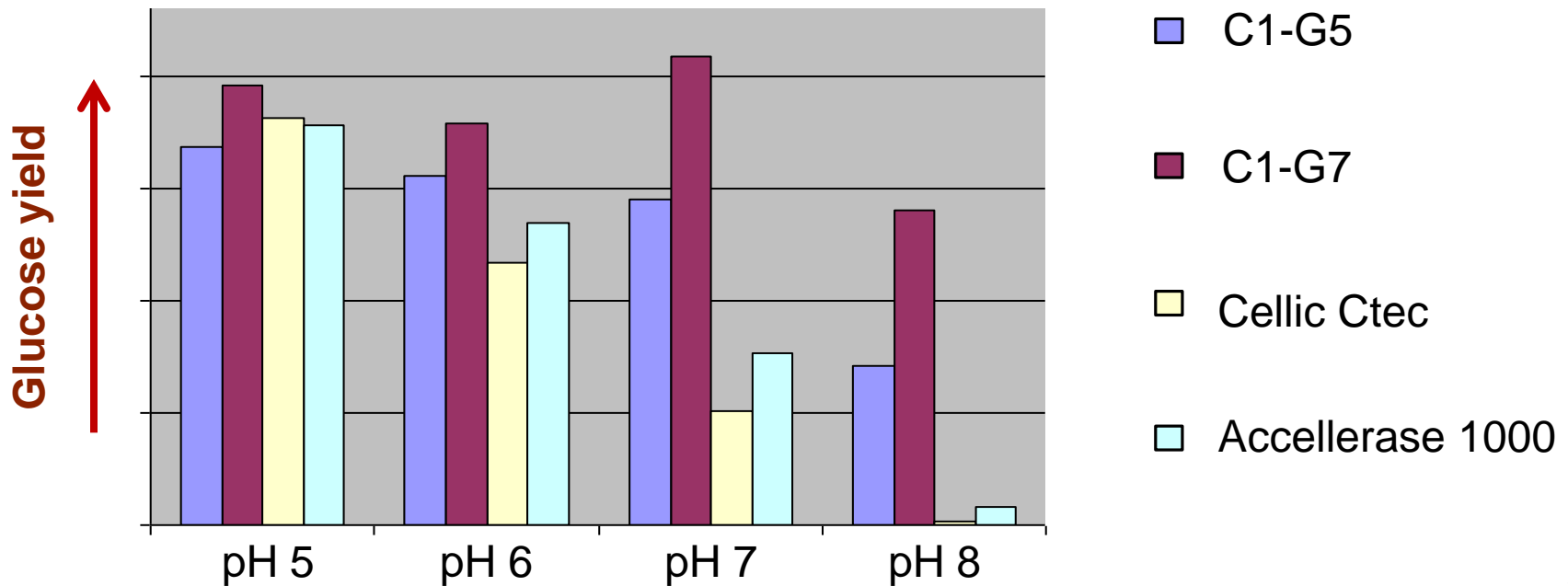
- G7 makes an excellent enzyme mixture for **BOTH** acidic and more neutral processes



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





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



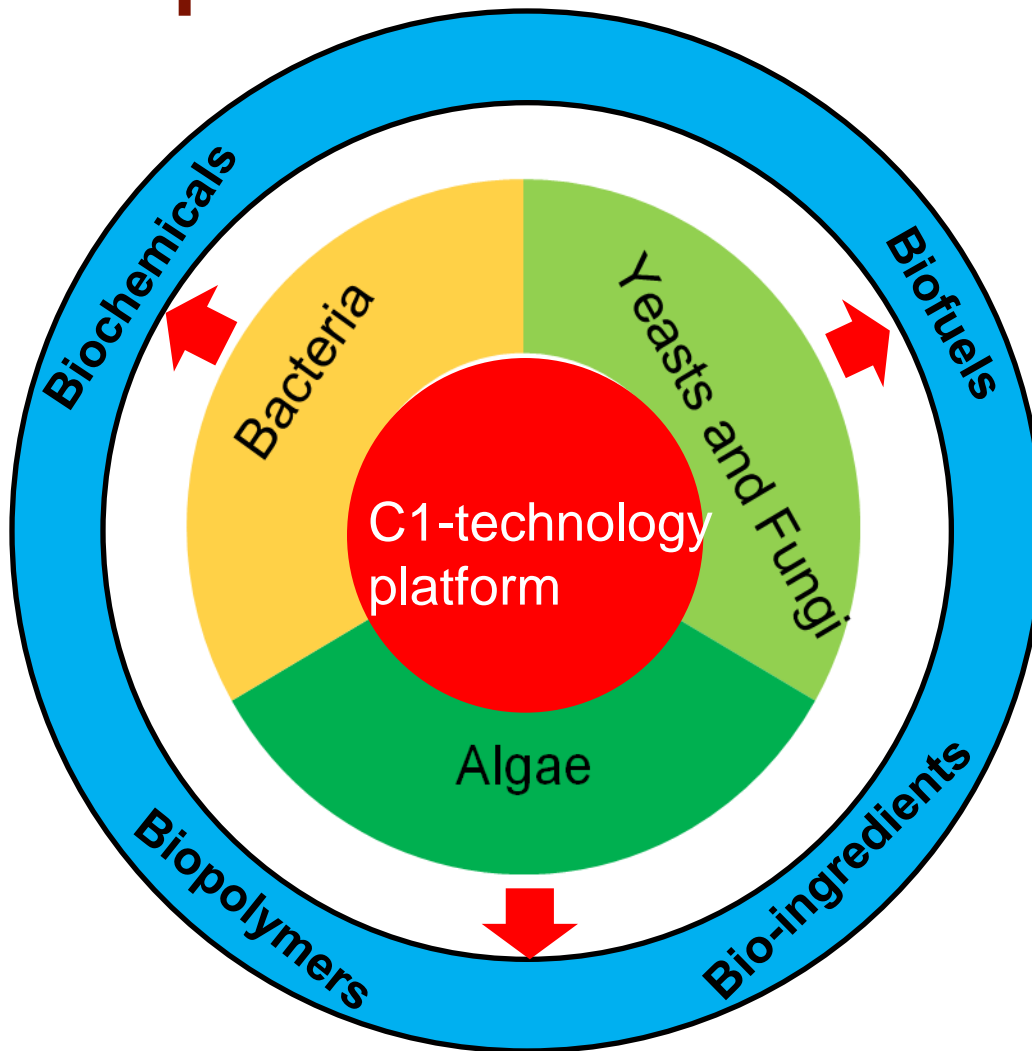
C1--G Series Improvements

Versatile (hemi)-cellulosic Enzyme Mixtures from a Single C1 Strain

- ❖ **Fast Saccharification:** Ethanol process completed in 72 hrs
- ❖
- ❖ **Fast reduction of viscosity**
- ❖ **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,
you are invited to visit our stand**

