Dyadic®

Inside®

The Power of Nature
The Speed of Technology

Mark Emalfarb, Founder and CEO
Dyadic International, Inc.

Safe Harbor Statement

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DYADIC®

Dyadic Profile

- Headquartered in Jupiter, Florida, USA
- R&D lab in Wageningen, The Netherlands
- Experienced management team
- Solid financial position

Producer of Enzymes Since 1994

- **Dyadic has manufactured commercial enzymes in 150,000, 50,000 and 30,000 liter fermenters since 1994**
- **Over 50 products sold to customers in over 35 countries**
- **Sales exceeded 1,300 metric tons through December 2009**

Food, Brewing Animal Feed

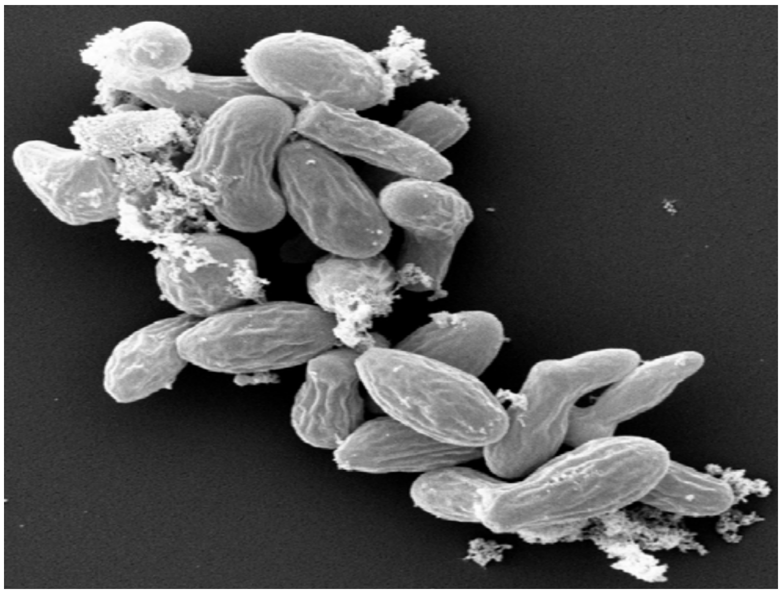
Pulp & paper

Bioethanol

Textiles

C1 Patented Technology Platform

An integrated system for gene discovery, expression and protein production



- Based on fungus isolated from alkaline soil in Eastern Russia
- Platform for enzyme and protein production
  -favorable fermentation characteristics
  -high yield
- 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. thermophilia based on genetic tests.*

Biofuels

Energy Independence and Security Act of 2007

Requirement to increase the volume of renewable fuel
9 billion gallons in 2008 → 36 billion gallons by 2022

Biomass
Corn stalks
Wheat straw
Pulpwood
Switch Grass

→

Cellulosic Sugars

→

Ethanol
Butanol
Plastics
Polymers
Chemicals

Renewable
Environmentally friendly
Reduces dependence on imported oil

Challenges in Biofuel Production

ECONOMICS

- **Producing ethanol at a competitive price**
- **Reduction of pretreatment and saccharification costs**
- **Reduction of enzyme production costs**

>>>>> DYADIC C1 APPROACH

The Dyadic C1 Approach

- Supply an optimized STRAIN and an efficient PROCESS for on-site production of enzymes for the efficient saccharification of raw materials
- The fermentation medium can be used to hydrolyze raw materials
- Avoiding down stream processing, formulation, packaging and transportation = Substantial reduction in the cost of a unit enzyme activity

>>> LOWER COST per GALLON of ETHANOL

The Dyadic C1 Approach

Generate C1 strains that produce a mix of enzymes that efficiently hydrolyze the Lignocellulosic Biomass into C5 and C6 sugars.

- **Exploration of (hemi-) cellulolytic potential of C1:**
- Development of strains that produce mainly one carbohydrase
- Test artificial mixes of enzymes on biomass
- **Increase level of limiting enzyme in production strain**
- **Increase amount of enzyme produced in industrial fermenters**

>>> More efficient enzyme mix; lower dosage; higher productivity

>>>> LOWER COST per GALLON of ETHANOL

C1 Production Platform

1. Efficient protein production system

- Production and secretion: ~100 g/L protein
- Robust controlled fermentation
- Low viscosity yields low energy input in fermentations

2. Versatile genetic tools and hosts developed

- Transformation system: high efficiency, stable integration
- Gene expression: strong expression signals
- Protein production: efficient secretion signals
- Targeted gene disruption: efficiency >1% up to 90%
- Variety of optimized C1-hosts.

3. Genome sequenced and annotated

- A wealth of new exploitable information

C1 Safety Profile

GRAS status acknowledged by the FDA

C1 strain

- Pathogenicity and toxicogenicity data: strain is non-infectious/no known toxins are produced
- Peer-reviewed scientific literature:
  No known pathogenicity
- No mycotoxins found

C1-Enzyme preparation

- *In* vivo feeding trials:
  14 day dose study in rats
  13 week subchronic rat study
- Genotoxicity testing:
  AMES bacterial mutagenesis
  Chromosomal aberration test
  Genetic mutation test
- No adverse effects observed

C1 Commercial Highlights

- **Patented C1 Protein Production Platform**
  - C1 fungus-based expression system offers significant advantages over other microbial systems

- **World Class Non-Exclusive Licensees**
  - Platform capabilities have been validated in industrial enzymes and biofuels through non-exclusive license agreements
    - 2008—Codexis, Inc.
    - 2009--Abengoa Bioenergy New Technologies, Inc.

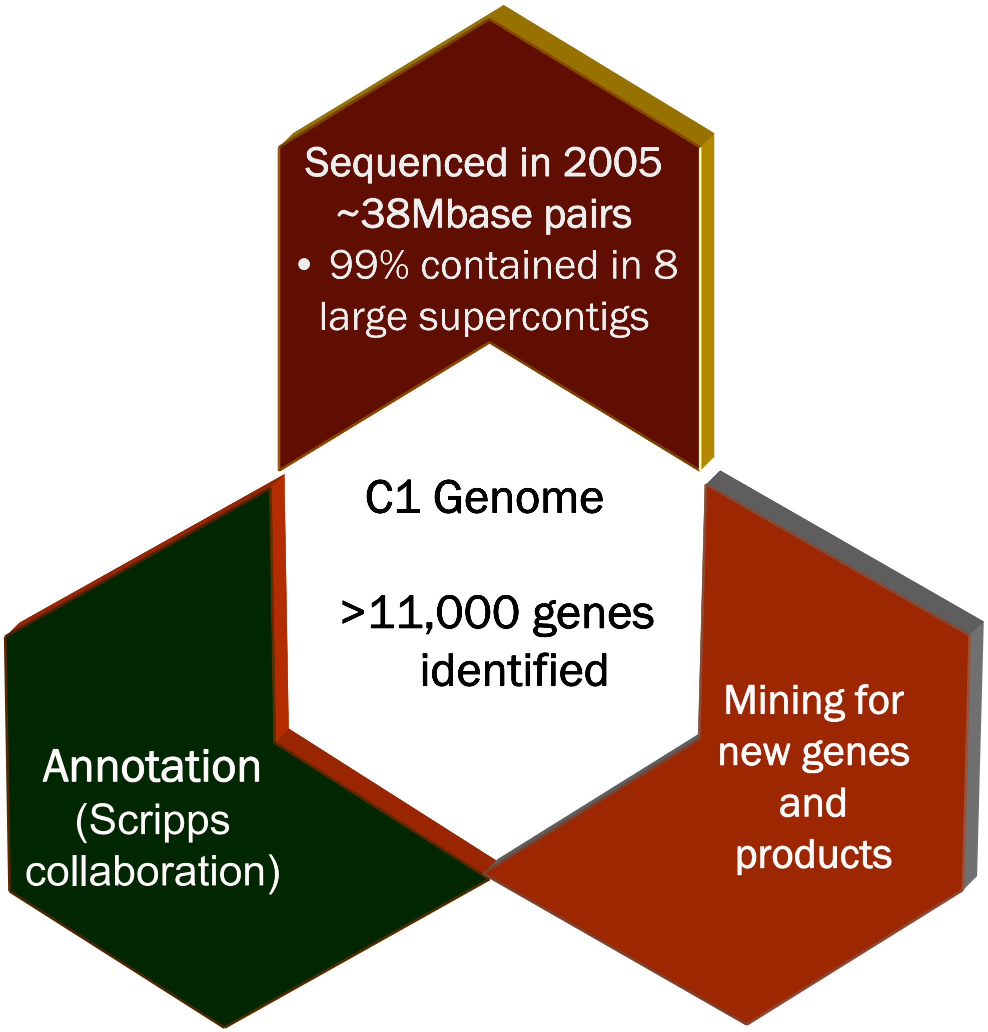
CODEXIS®

ABENGOA BIOENERGIA
The Global Ethanol Company

Scripps Collaboration

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

C1 Gene Discovery



Over 200 genes encoding putative carbohydrate-active enzymes:

•cellobiohydrolases
•endo-/exo-β-glucanases
•endo-/exo-xylanases
•xyloglucanases, mannanases, arabinases, galactanases
•pectinases (pectin-/pectate lyases, polygalacturonases, etc.)
•α-amylases, glucoamylases glycosidases (α-/β-glucosidases, α-/β-xylosidases, α-/β-galactosidases, α-L-arabinofuranosidases, α-/β-mannosidases, etc.)
•ferulic acid esterases, cutinases, esterases, polyesterases

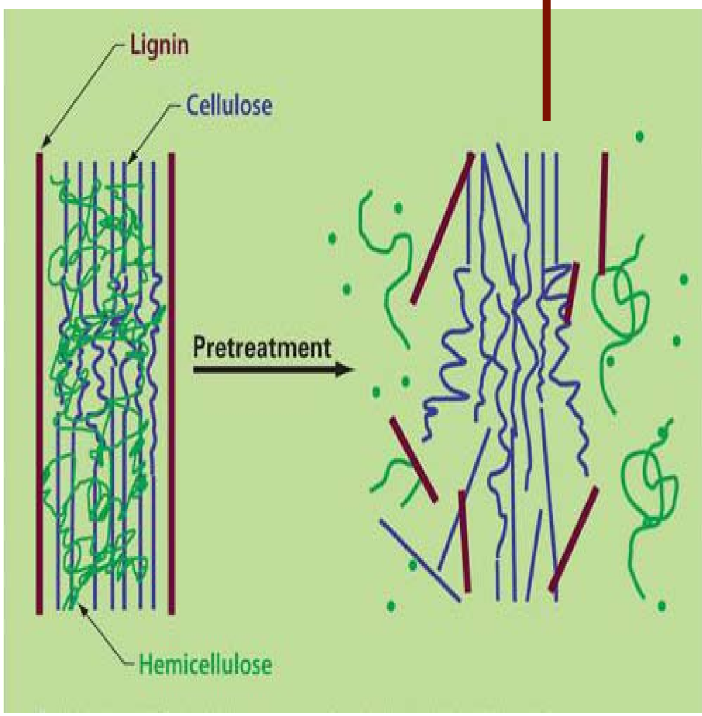
A Rich Source of Hydrolytic Enzymes

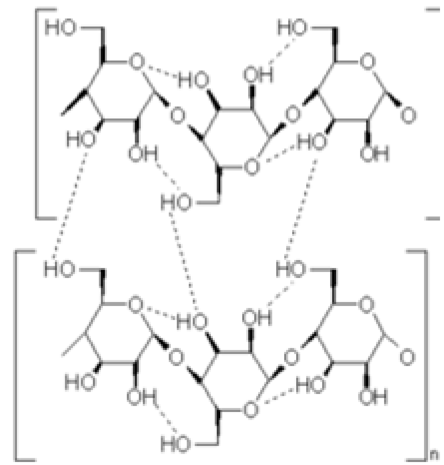
70 genes have been expressed and partially characterized to date

Annotated enzyme	Number of enzymes in C1	Number of enzymes in *T.reesei* *(JGI data base)*
β-Galactosidases/1,4-galactanases	6	2
β-Glucosidases/β-xylosidases	11	12
Endoglucanases/cellobiohydrolases	18	7
Xylanases	11	5
Endoglucanases	26	10
α-Galactosidases	2	8
Polygalacturonase	2	4
Arabinases/arabinofuranosidases/β-xylosidases	10	2
Arabinofuranosidases	4	1
α-Glucuronidase	2	1
Xyloglucanase	1	1
Exo-arabinases	2	-
Acetyl xylan esterases/ferulic acid esterases	13	2
Acetyl esterases/ pectin methyl esterase	3	-
Lyases	7	-
Total	115	55

Enzymes for Biomass Saccharification

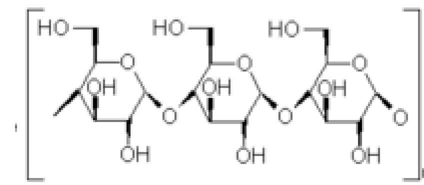
Cellulases





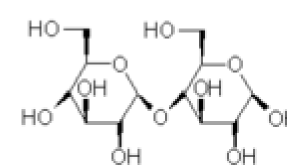
Crystalline cellulose

Endo-glucanases
Accessory enzymes



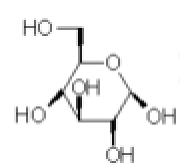
oligomers

Exo-glucanases/
cellobiohydrolases



cellobiose

B-glucosidase

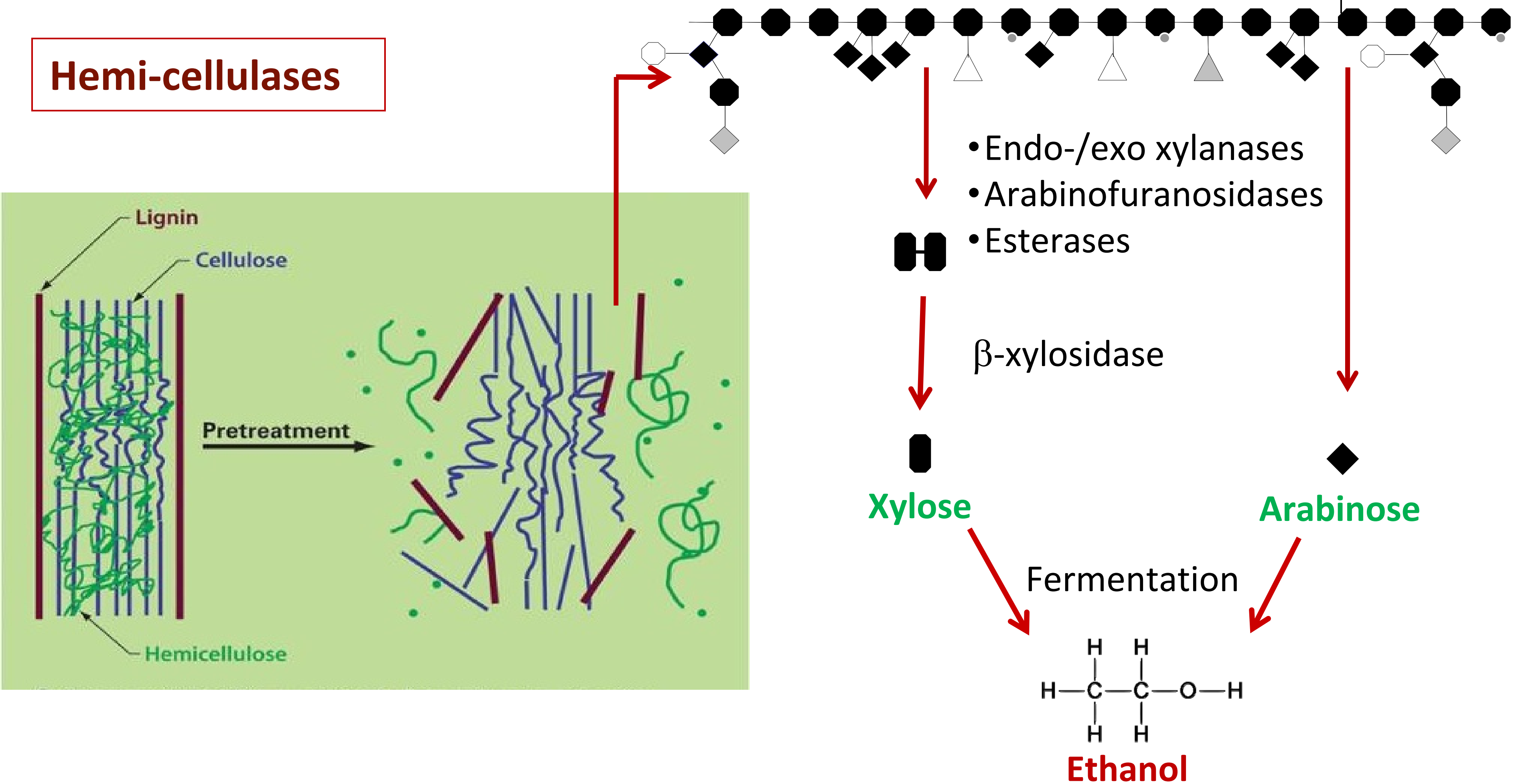


glucose

Fermentation

ETHANOL

Enzymes for Biomass Saccharification



C1 vs. *Trichoderma*

C1 is a rich source of lignocellulolytic enzymes!

Comparison of the lignocellulolytic potential of C1 and *Trichoderma reesei* (the main industrial source for biofuel enzymes (e.g. Accellerase™)

	C1	***T.reesei*****
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

The Dyadic C1 Approach

ADDITIONAL ADVANTAGES

- **C1 enzymes are more robust and effective at a wider range of applications**
  - **Wider ph range than enzymes from *Trichoderma***
  - **Higher temperature optimum**

>>> LOWER COST per GALLON of ETHANOL

C-1 Technology Platform

From Promise to Product in 5 Steps:

From Gene to Product in a Single Host Strain

1. Gene discovery
2. Gene expression
3. Characterization
4. Optimization
5. Commercial Manufacturing
   - Demonstrated scale-up to 150,000L

BioSugars: The Vision

- **Development of proprietary enzymes for cellulosic sugar production**
  - **Discovery and optimization of new enzymes**
  - **Improve performance**
  - **Lower costs**

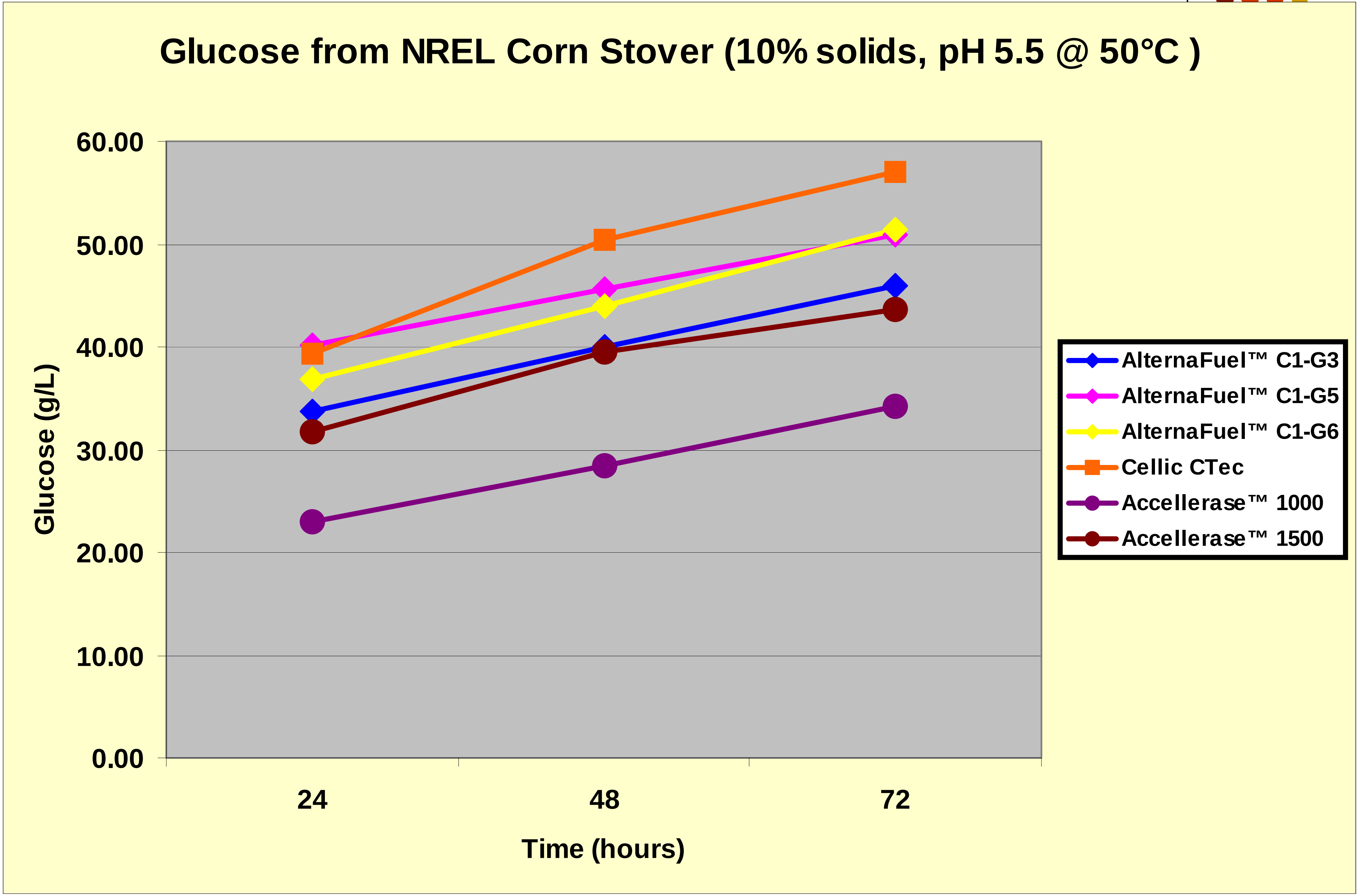
Performance of Dyadic's C1 Enzyme vs. Genencor's AccelleraseTM 1000*

Enzyme Preparation	**Protein dose** (mg protein/g total solids) **	**Temp** (^{0}C)	**pH**	**Total Solids** (% wt)	**Glucose productivity** (g/Lh^{-1})***	**Relative Glucose productivity** (%)
Dyadic C1 (Chrysosporium sp)	20	50	5.0	10	0.86	97
	20	50	6.0	10	0.89	100
Accellerase™ 1000 (Trichoderma reesei)	20	50	5.0	10	0.73	82
	20	50	6.0	10	0.46	52

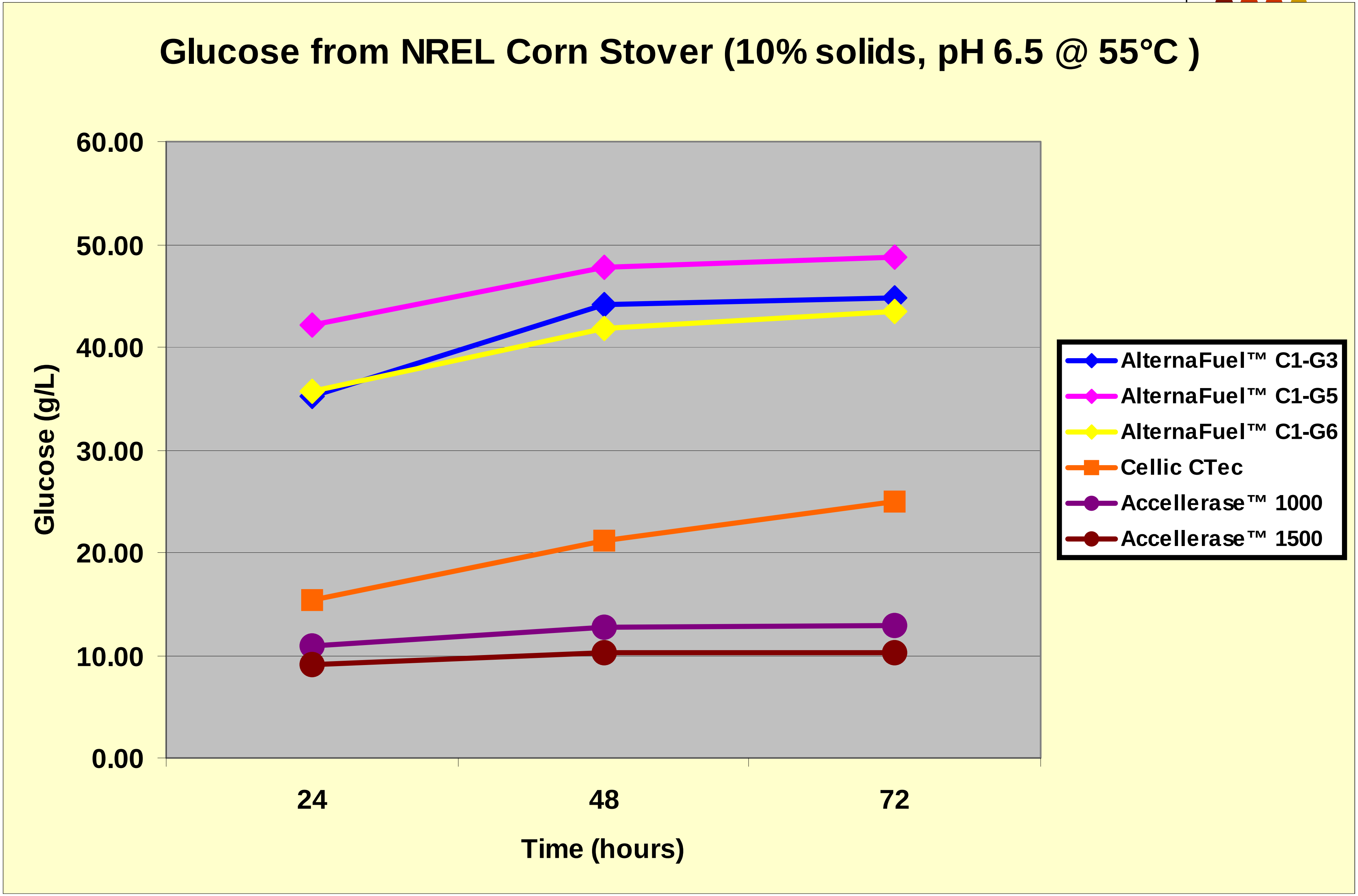
*Substrate: pretreated hardwood, **protein measured by BCA, *** 96h reaction

Source: Lignol Innovations Ltd

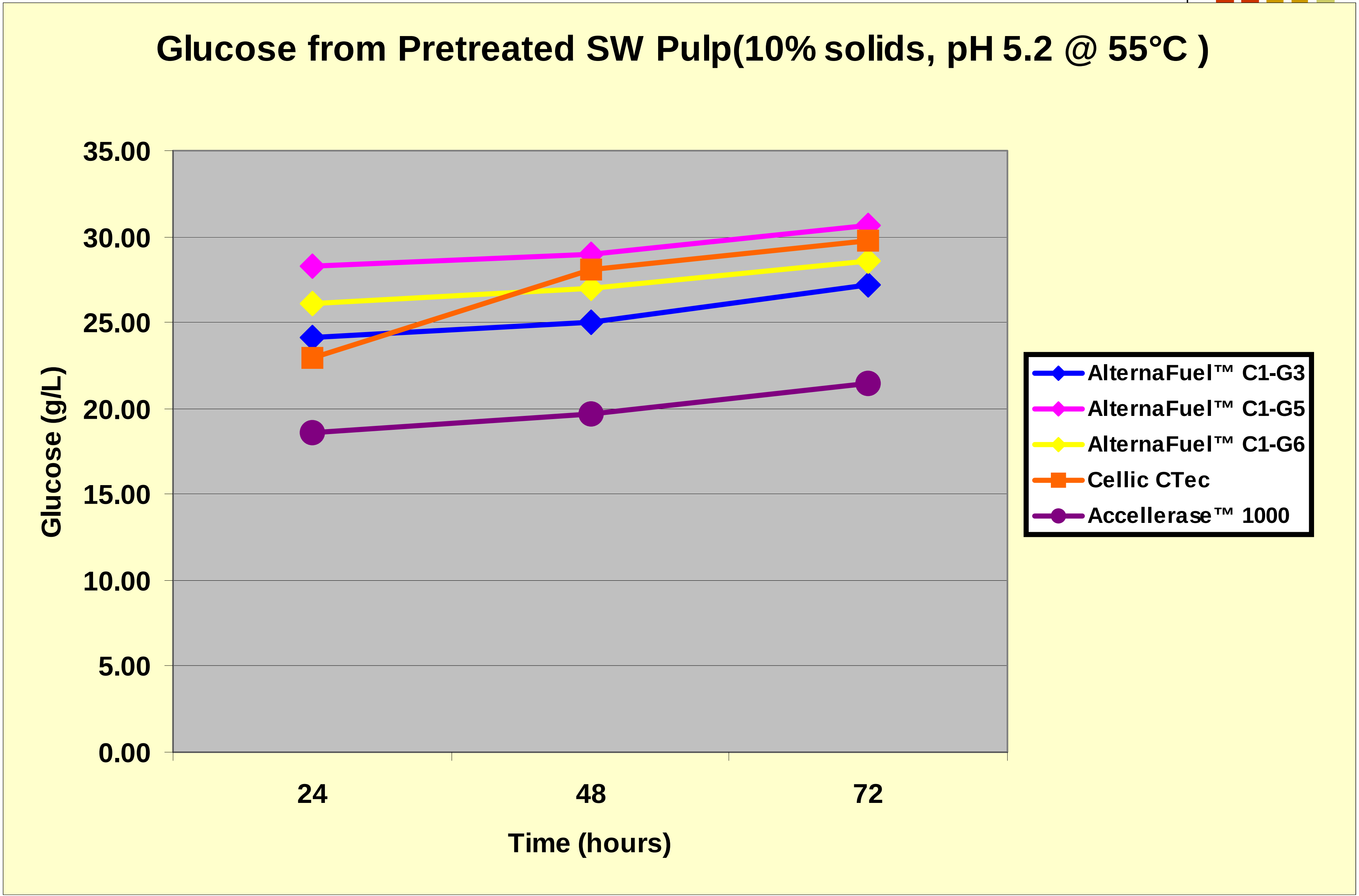
NREL Corn Stover



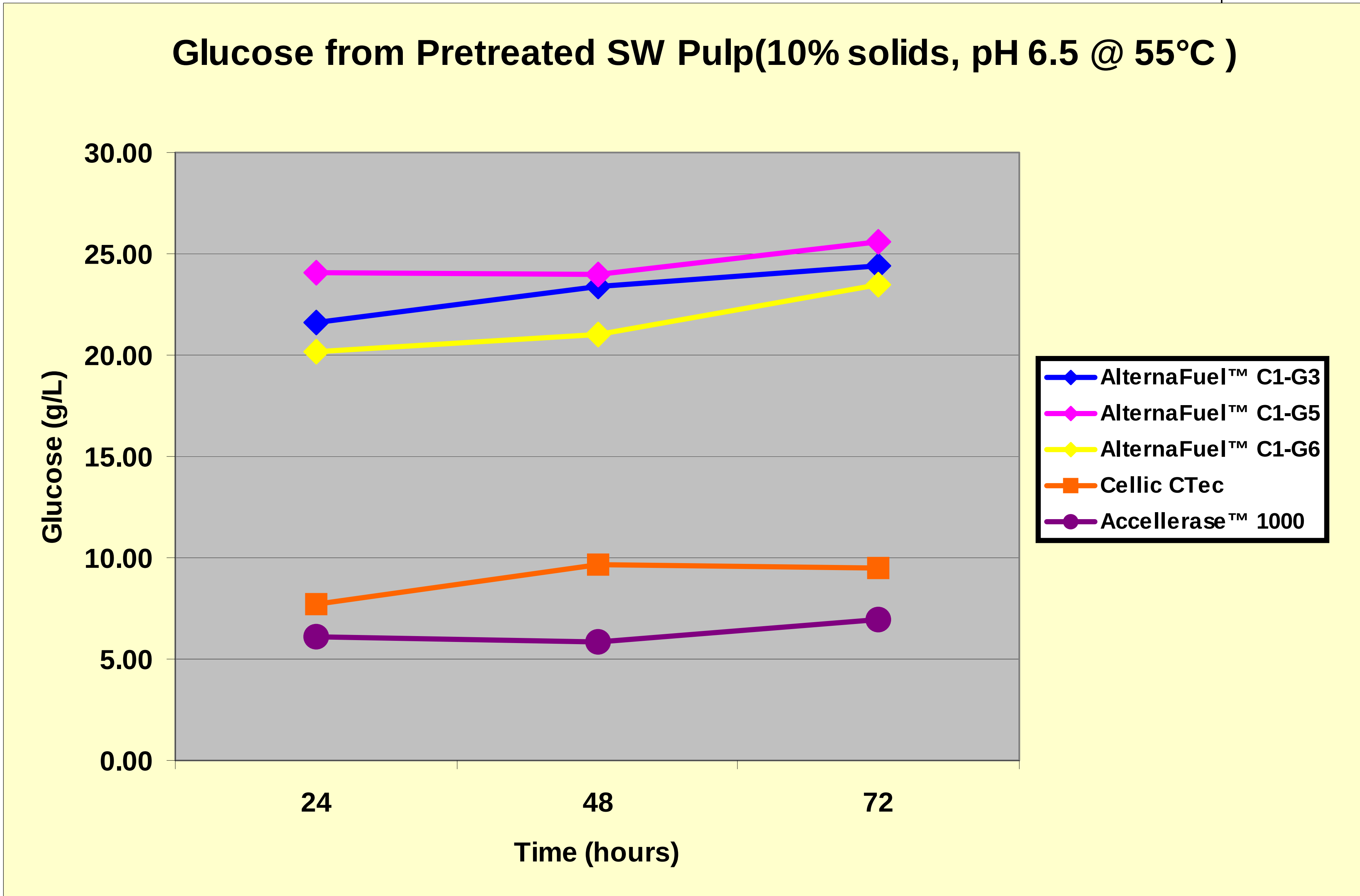
NREL Corn Stover



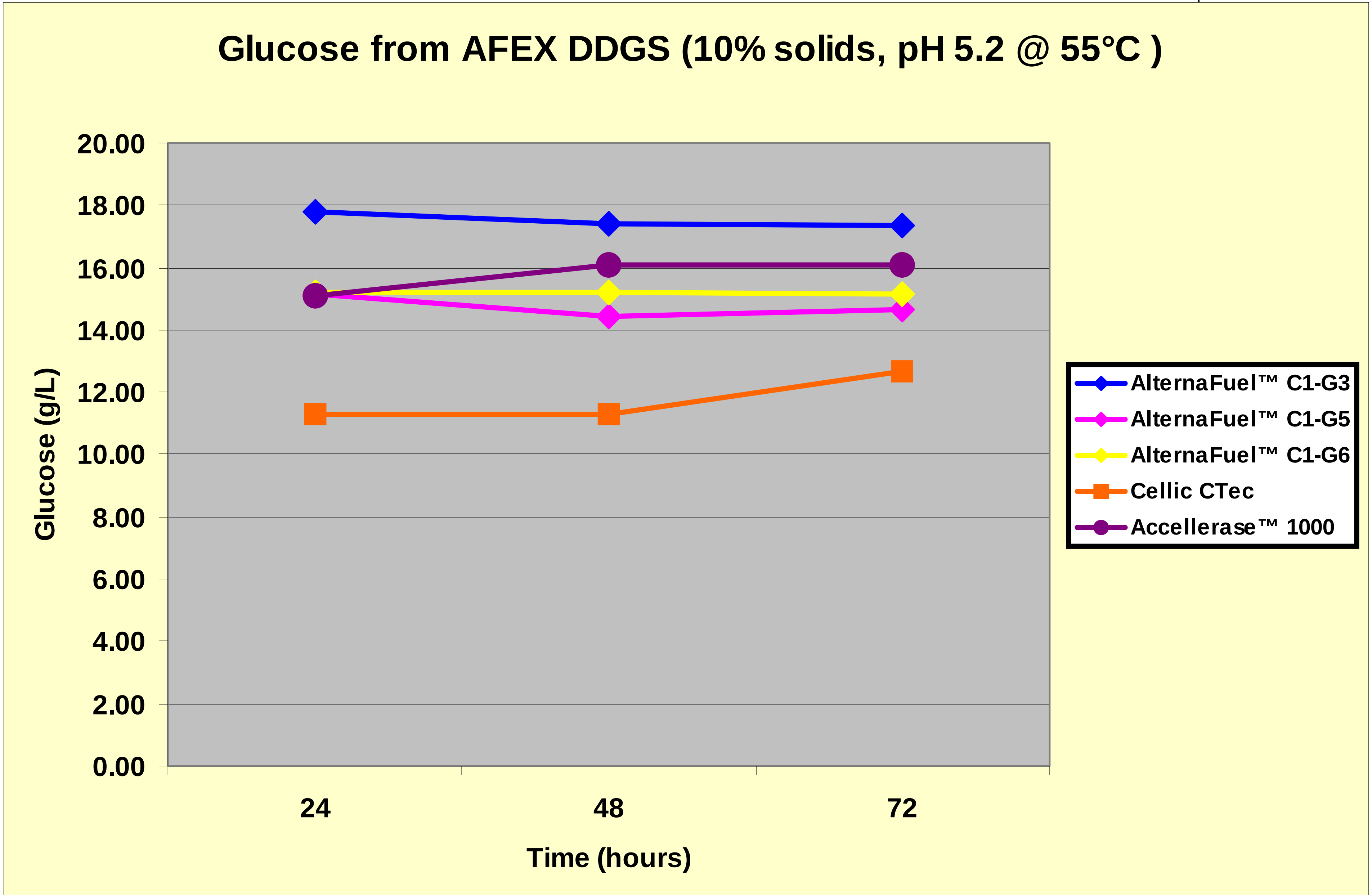
Soft Wood Pulp



Soft Wood Pulp



AFEX Dry Distiller Grains



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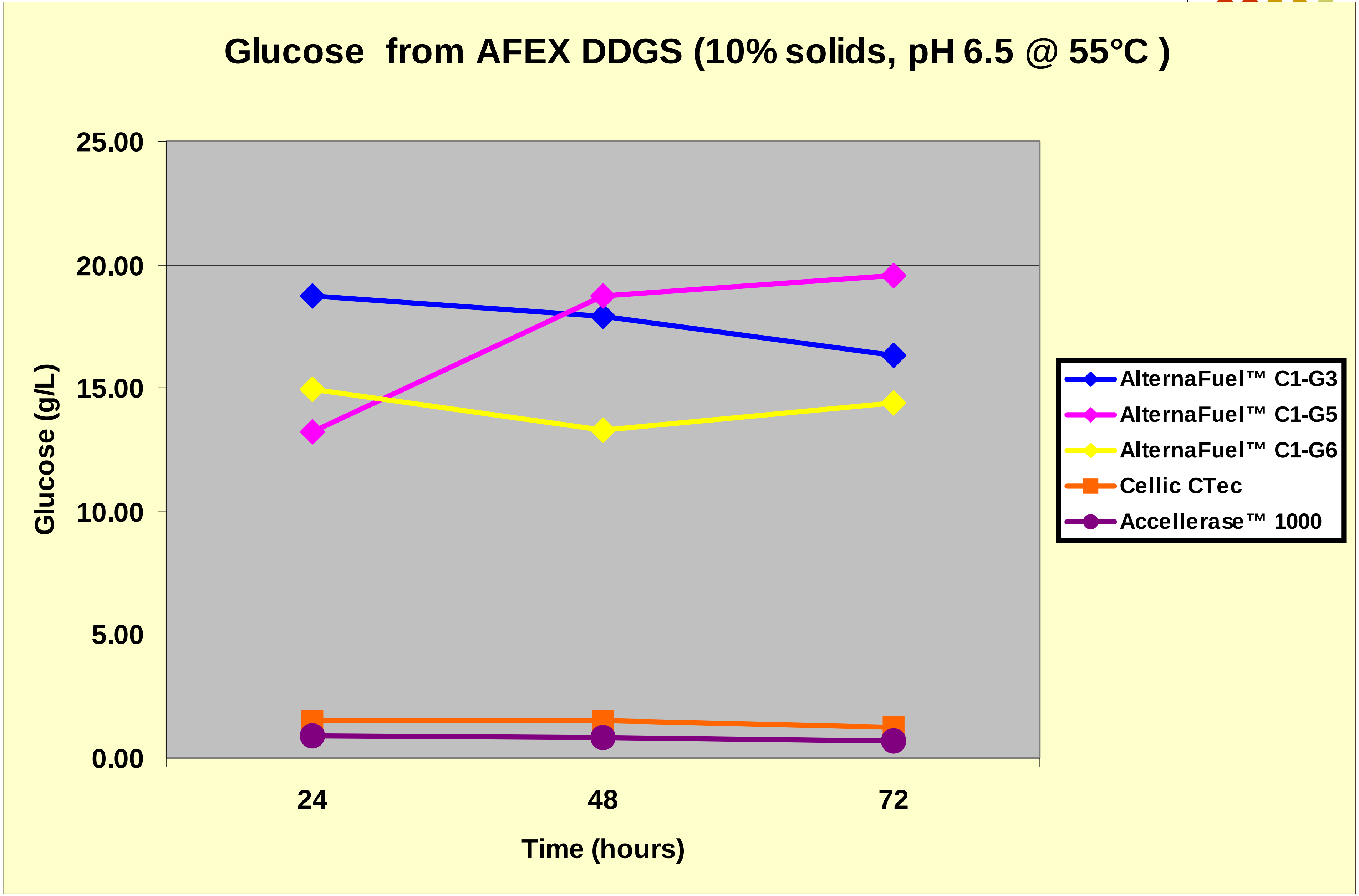
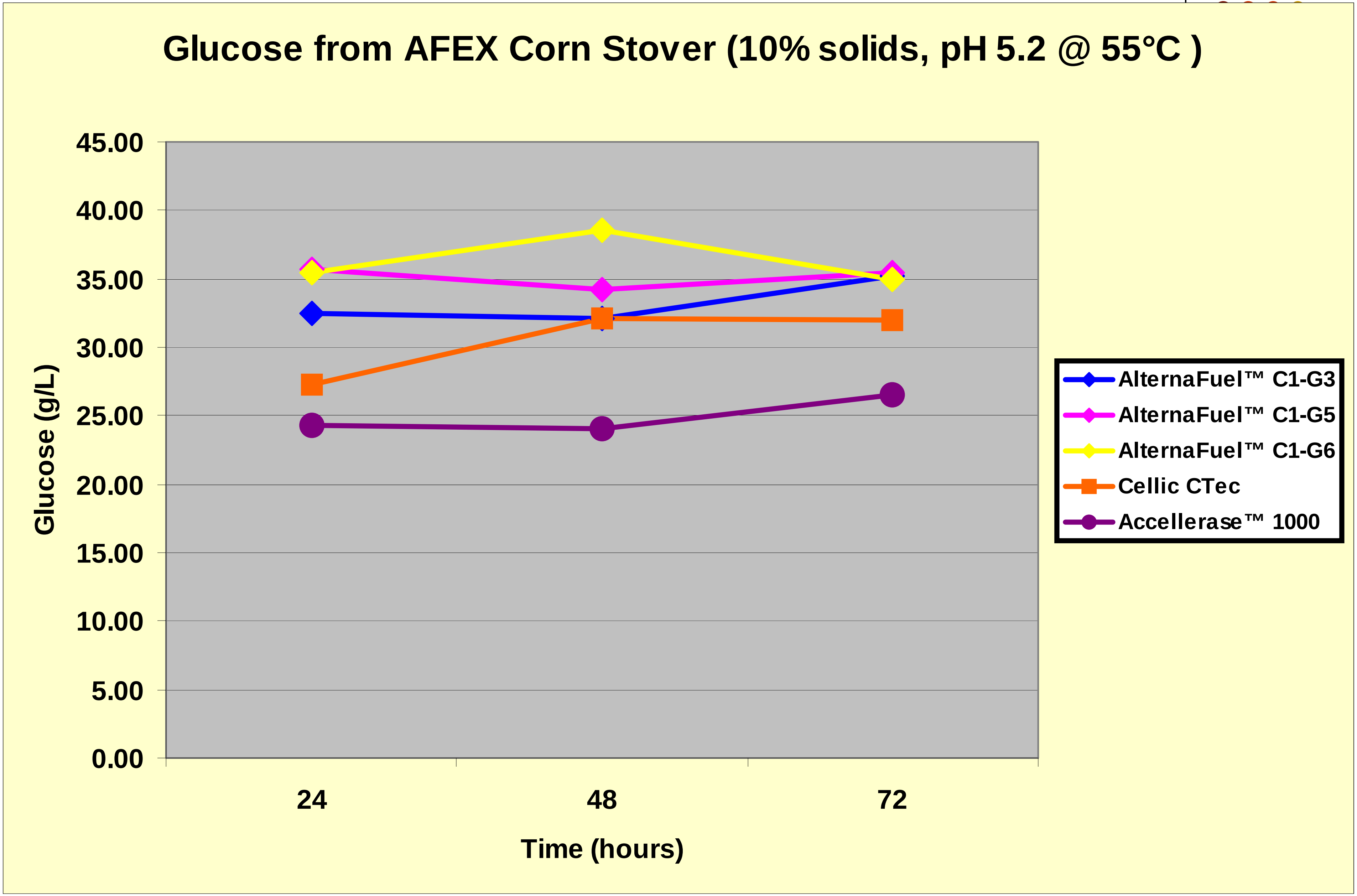


AFEX Corn Stover



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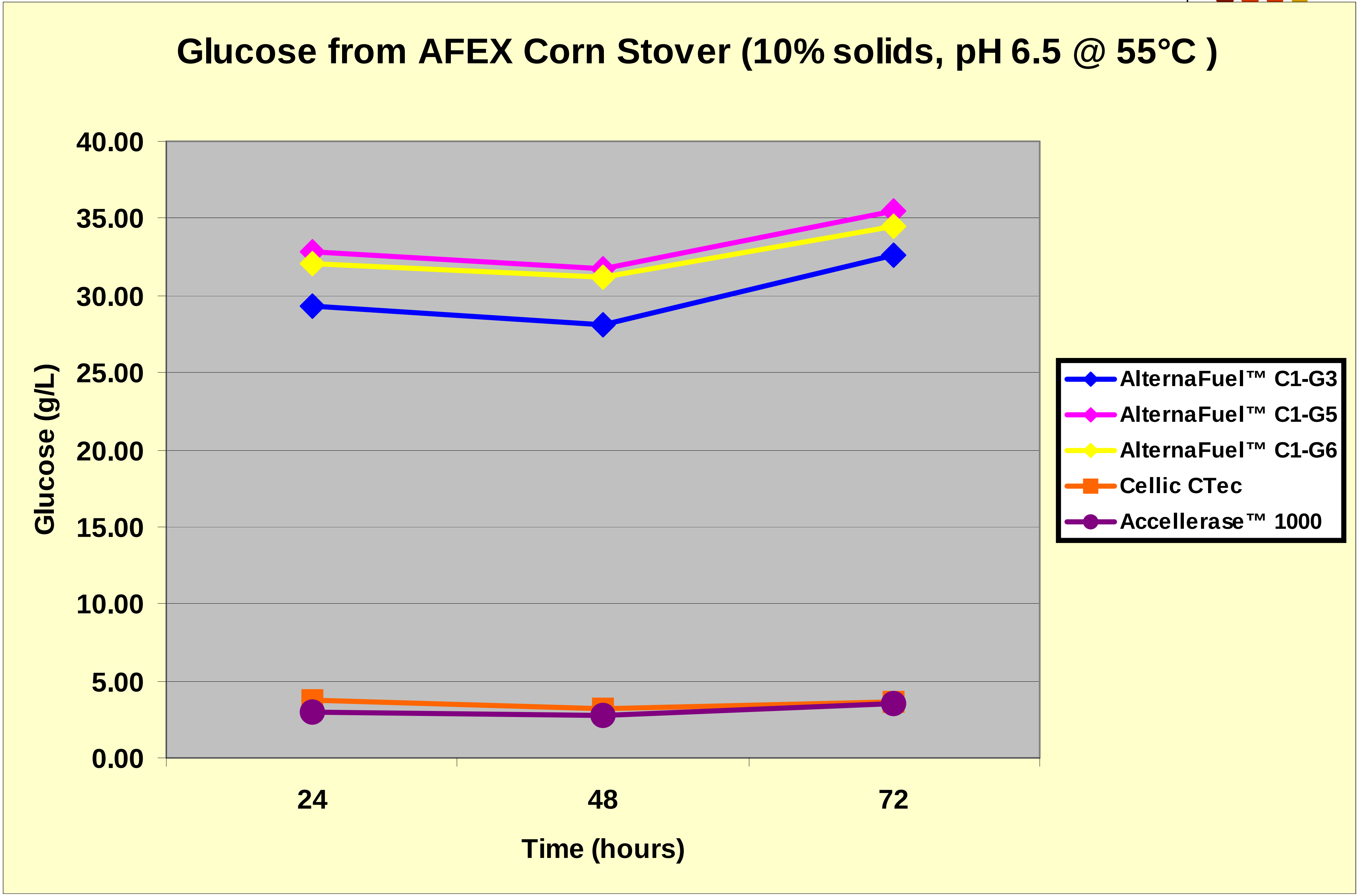
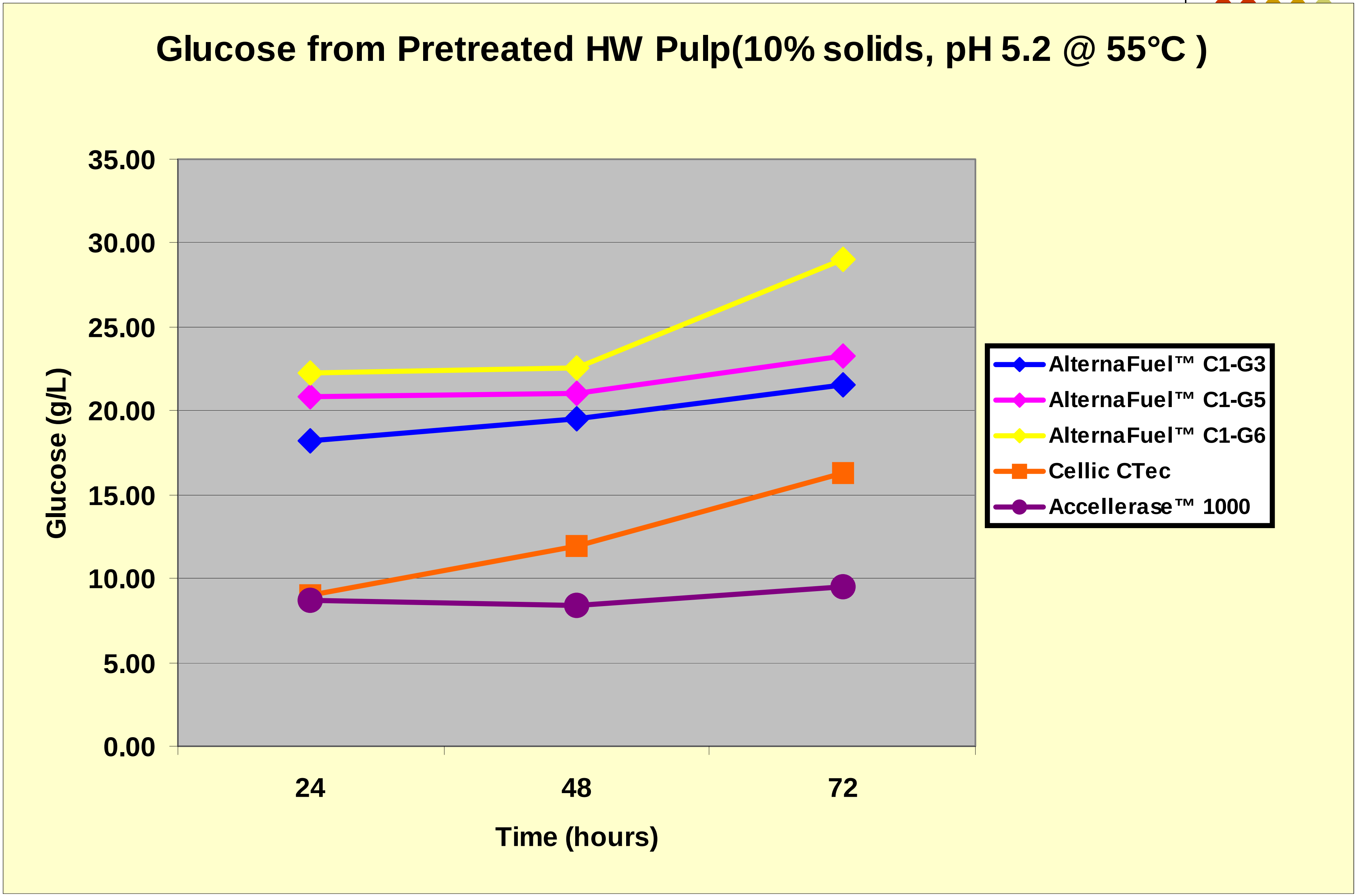
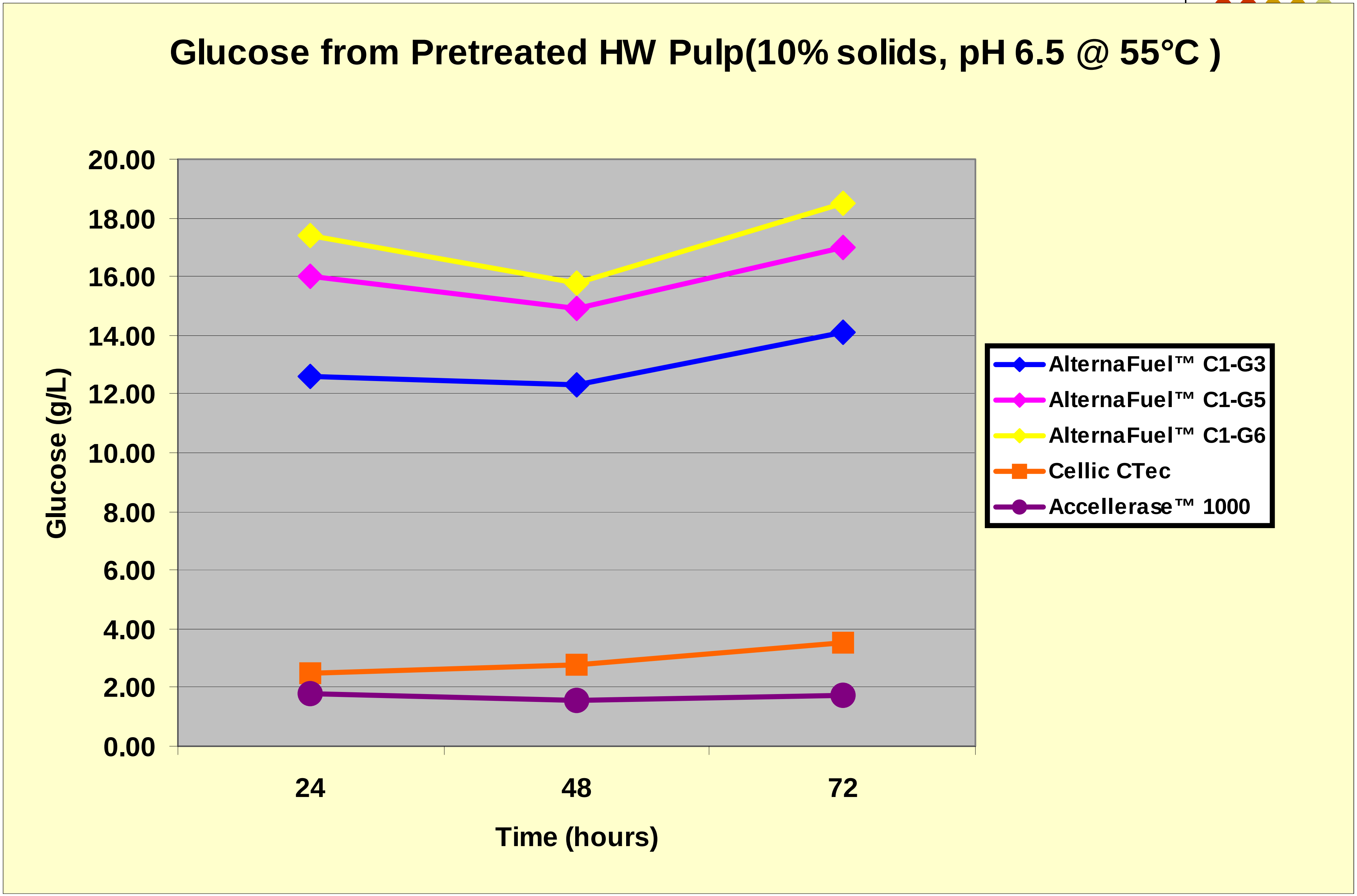


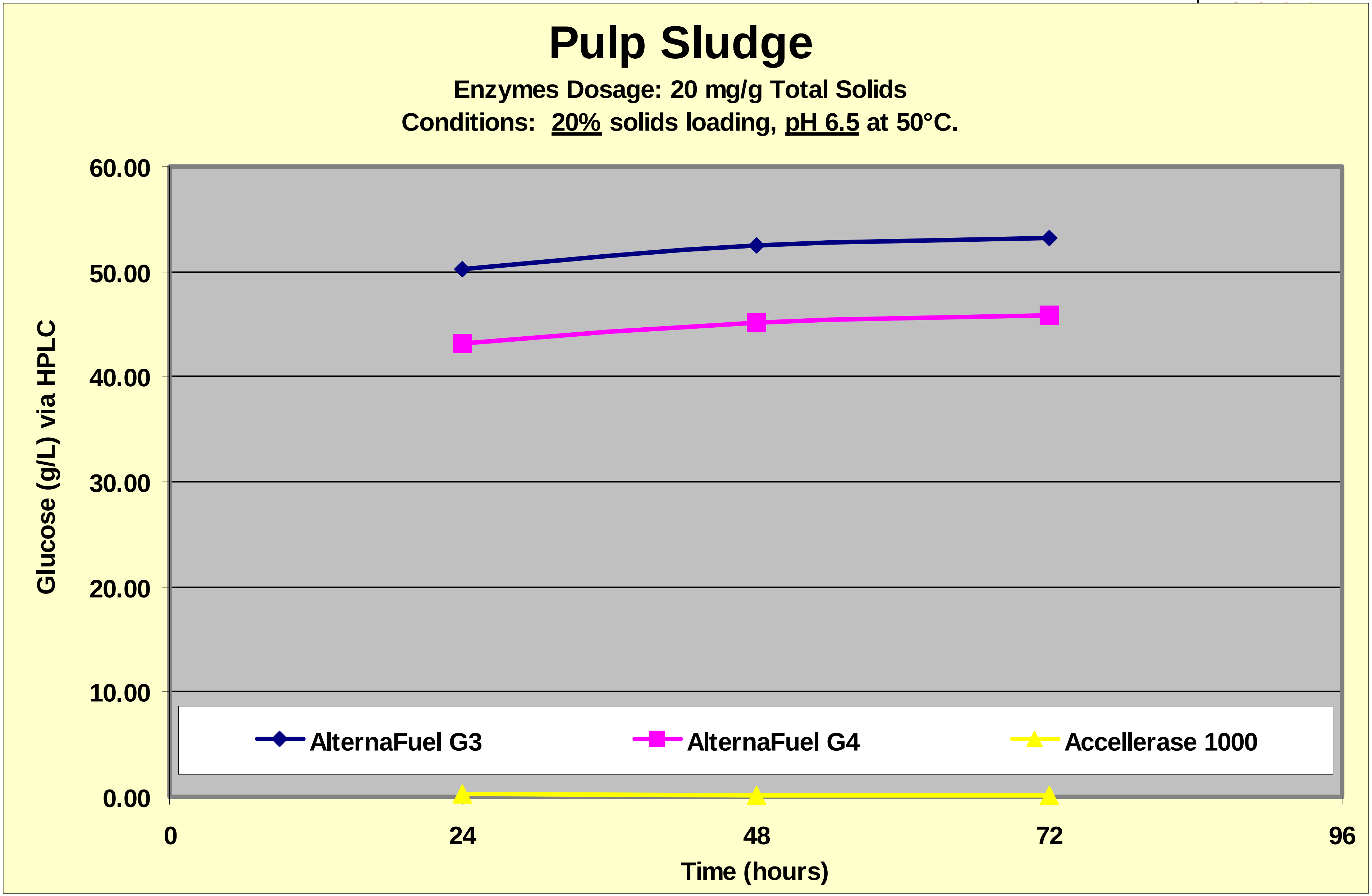
Hard Wood Pulp



Hard Wood Pulp



Pulp Sludge



Pulp Sludge
Enzymes Dosage: 20 mg/g Total Solids
Conditions: 20% solids loading, pH 6.5 at 50°C.
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Glucose (g/L) via HPLC
AlternaFuel G3
AlternaFuel G4
Accellerase 1000
0
24
48
72
96
Time (hours)

Recent Publications

- “Hemicellulase production in *Chrysosporium lucknowense* C1” - *Journal of Cereal Science* 1–6, July, 2009

- *Chrysosporium lucknowense* arabinohydrolases effectively degrade sugar beet arabinan *

- Branched arabino-oligosaccharides isolated from sugar beet arabinan *

* Publications Submitted

Strong Intellectual Property

- **5 issued U.S. patents**
  - Broad claims blocking use of C1
- **9 pending U.S. patent applications**
- **Large number of issued foreign patents and applications**

Invitation

- To learn more about C1, please meet us at stand B1

- Let us show you why companies like Codexis, Abengoa and others have chosen to work with the Dyadic C1 Approach