



# C5-6 Technologies<sup>®</sup>

## Simplifying Carbohydrates

David Mead CEO

Phil Brumm CSO

# Simplifying Carbohydrates

Enzymes for the bioconversion of polysaccharides into five and six carbon sugars, high value chemicals, biofuels, fiber and food products, and the degradation of biofilms.

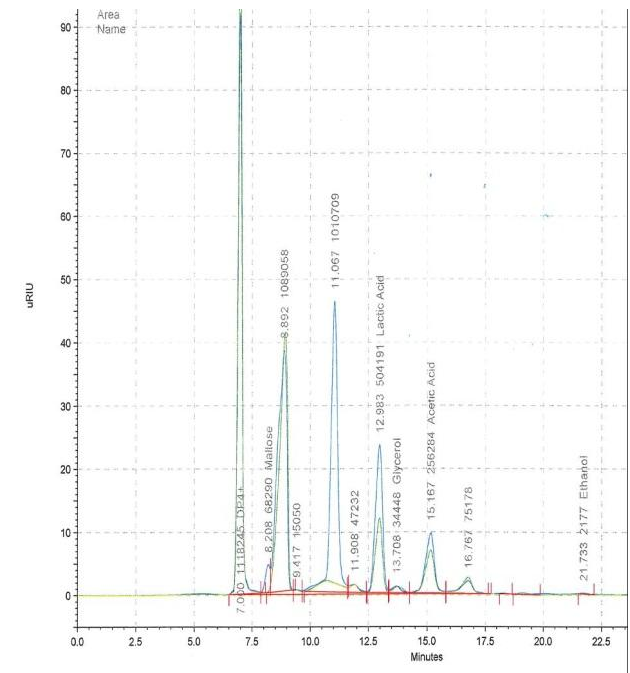


**Oct 2006 spin out  
from Lucigen Corp**

**28,000 SF facility in  
Middleton, WI**

# C5•6 Carbohydrase Expertise

- CAZyme cloning/purification/screening
- Enzyme characterization/evaluation
- Carbohydrate chemistry/analysis
- Gene transfer to Gram +/- hosts
- Fermentation/recovery of enzymes



# C5•6 Management Team

**David Mead, Ph.D.** – CoFounder & CEO

25 yrs biotech R&D & management; 5 patents

**Phil Brumm\***, Ph.D. – CoFounder & Chief Scientific Officer

25 yrs management in industrial enzymes; 7 patents

**Larry Allen\***, Ph.D. – 25 yrs exp. strain development

**Dan Moran\***, Ph.D. – 20 yrs exp. fermentation

**Rick Remeschatis, MBA, CFA, CPA,** – CFO

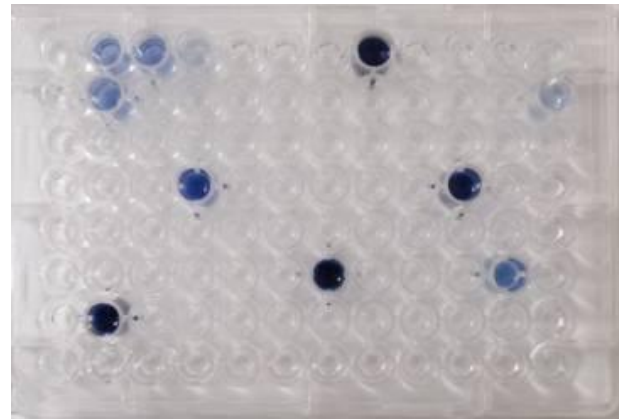
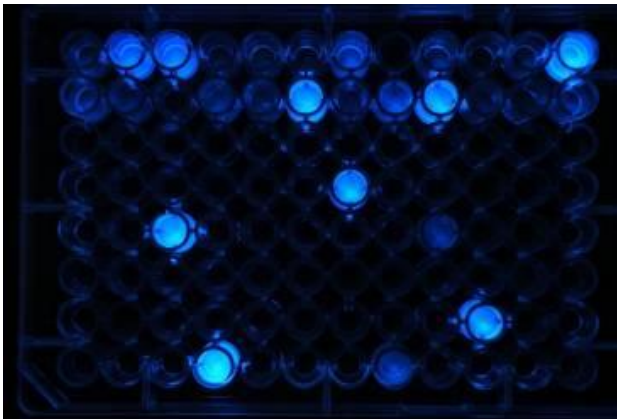
30 yrs private & public companies

\*Industrial enzyme scale up and production experience.

# C5•6 Technical Assets

High throughput multiplex expression screening

- 🔗 150 thermophilic cultures from hot springs
- 🔗 Eight highly cellulolytic microbial genomes sequenced
- 🔗 Genome mining of 100s CAZymes



C5-6 Product	Gene	GH Family
β-Glucosidase 1	Aax_81839	GH42
Xylanase 19	Acel_0180	GH10
Xylanase 20	Acel_0372	GH10
Cellulase 45	Acel_0614	GH5
Cellulase 40	Acel_0615	GH6 GH12
Cellulase 41	Acel_0617	GH48
Cellulase 42	Acel_0619	GH12
Cellulase 46	Acel_0970	GH9
Cellulase 47	Acel_1701	GH9
β-Xylosidase 8	Acel_2050	GH3
β-Glucosidase 3	Bcell_0282	GH1
β-Xylosidase 4	Bcell_0385	GH43
Cellulase 32	Bcell_0437	GH5
Cellulase 33	Bcell_0438	GH5
Bcell_0537	Bcell_0537	GH8
β-Xylosidase 2	Bcell_0538	GH39
Xylanase 17	Bcell_0541	GH10
Xylanase 15	Bcell_0547	GH10
β-Xylosidase 5	Bcell_0554	GH43
Curdianase 1	Bcell_0683	GH16
Curdianase 2	Bcell_0690	GH16
β-Glucosidase 5	Bcell_0705	GH3
β-Xylosidase 6	Bcell_0821	GH30
β-Galactosidase 3	Bcell_1039	GH43
β-Glucosidase 6	Bcell_1042	GH43
Cellulase 38	Bcell_1191	GH5
β-Mannanase 7	Bcell_1368	GH26
Cellulase 34	Bcell_2363	GH16
Galactanase 1	Bcell_2962	GH53
Cellulase 35	Bcell_3371	GH5
Cellulase 36	Bcell_3391	GH9
Cellulohydrolase 3	Bcell_4264	GH3
Expansin	Bsu_18630	CBM63
β-Glucosidase 7	Celf_0140	GH3
Cellulose Phosphorylase 1	Celf_0317	GH94
Xylanase 21	Celf_0374	GH11
Xylanase 22	Celf_0574	GH10 CE4
Xylanase 23	Celf_1271	GH10
Cellulase 50	Celf_1925	GH6
α-Arabinofuranosidase 4	Celf_2726	GH3
β-Xylosidase 9	Celf_2770	GH2
α-Arabinofuranosidase 5	Celf_2983	GH3
Curdianase 3	Celf_3113	GH16

C5-6 Product	Gene	GH Family
β-Mannanase 1 (ManA)	Cthe_0032	GH26
Cellulase 07 (Cel I)	Cthe_0040	GH9
β-Glucosidase 2 (BglA)	Cthe_0212	GH1
Cellulase 03 (Cel A)	Cthe_0269	GH8
Cellulase 48 (Cel L)	Cthe_0405	GH5
Cellulase 10 (Cel K)	Cthe_0412	GH9
Cellulohydrolase 1	Cthe_0413	GH9
Cellulase 06 (Cel R)	Cthe_0578	GH9
Cellulase 04 (Cel E)	Cthe_0797	GH5
Cellulase 11 (Cel D)	Cthe_0825	GH9
Xylanase 05 (Xyn Y)	Cthe_0912	GH10
Cellulase 09 (Cel H)	Cthe_1472	GH5 GH26
Xylanase 03 (XynZ)	Cthe_1963	GH10
Cellulase 08 (Cel O)	Cthe_2147	GH5
Cellulase 02 (Cel C)	Cthe_2807	GH5
Cellulase 05 (Cel G)	Cthe_2872	GH5
Xylanase 04 (XynA)	Cthe_2972	GH11
β-Glucosidase 8	Dhur_0462	GH1
α-amylase 1	Dhur_0675	GH57
Cellulase 01	Dhur_0670	GH5
Xylanase 06	Dhur_1647	GH10
α-Glucuronidase 1	Dhur_1714	GH67
β-Galactosidase 1	Dhur_1799	GH1
α-Fucosidase 1	FG11254.1	GH29
Cellulase 20	Fisuc_0057	GH9
Xylanase 12	Fisuc_0207	GH8
Cellulase 21	Fisuc_0241	GH8
Xylanase 11	Fisuc_0362	GH11
Cellulase 12	Fisuc_0471	GH8
Cellulase 13	Fisuc_0678	PL10
β-Mannanase 2	Fisuc_0727	GH26
β-Mannanase 5	Fisuc_0728	GH5
β-Mannanase 3	Fisuc_0730	GH26
Cellulase 22	Fisuc_0786	GH5
Cellulase 28	Fisuc_0860	GH77
Cellulase 14	Fisuc_0897	GH5
Cellulase 26	Fisuc_1224	GH5
Xylanase 13	Fisuc_1426	GH45
Cellulase 23	Fisuc_1523	GH5
Cellulohydrolase 2	Fisuc_1584	GH5
Esterase 1	Fisuc_1641	CE2
β-Mannanase 6	Fisuc_1688	GH26
Cellulase 29	Fisuc_1751	GH3
Xylanase 14	Fisuc_1765	GH30

C5-6 Product	Gene	GH Family
Xylanase 24	Fisuc_1769	GH43
Esterase 2	Fisuc_1771	CE1
α-Galactosidase 1	Fisuc_1773	GH27
ChBP1	Fisuc_1789	GH95
Xylanase 09	Fisuc_1793	GH10
Xylanase 07	Fisuc_1794	GH10
Cellulase 30	Fisuc_1802	GH8
Cellulase 15	Fisuc_1859	GH9
Cellulase 24	Fisuc_2033	GH9
β-Glucanase 1	Fisuc_2065	GH3
Cellulase 16	Fisuc_2081	GH51
Xylanase 10	Fisuc_2201	GH11
Cellulase 17	Fisuc_2230	GH5
Xyloglucanase 1	Fisuc_2317	GH74
Cellulase 27	Fisuc_2362	GH9
Xylanase 08	Fisuc_2442	GH11
ChBP2	Fisuc_2485	CBM6
Esterase 3	Fisuc_2534	CE6
Cellulase 18	Fisuc_2579	GH8
Cellulase 31	Fisuc_2686	GH43
β-Mannanase 4	Fisuc_2933	GH5
Lichenase 1	Fisuc_2961	GH16
β-Galactosidase 2	Fisuc_3049	GH2
Cellulase 25	Fisuc_3081	GH5
Cellulase 19	Fisuc_3111	GH51
α-Arabinofuranosidase 1	Prop.	GH51
Xylanase 01	Prop.	GH10
Xylanase 02	Prop.	GH10
β-Xylosidase 1	Prop.	GH43
Xyloglucanase 2	Sfa_0771	GH12
Xylanase 16 (Xylanase EX3)	Tre_120229	GH10
β-Xylosidase 7	Tre_121127	GH3
Cellulase 37 (Cellulase EG1)	Tre_122081	GH7
Xylanase 18	Tre_123818	GH10
α-Glucuronidase 2	Tre_72526	GH67
Cellulohydrolase 4	Tre_72567	GH6
Cellulase 39	Tre_73643	GH61
α-Arabinofuranosidase 2	Tre_76210	GH62 GH43
β-Glucosidase 4	Tre_76672	GH3

# Hundreds of Recombinant CAZymes

# CAZyme Research Products

## CAZyme™ Carbohydrases.

All CAZyme Carbohydrases are active at 50-70°C. See lucigen.com for supporting data.

CAZyme	Gene Locus	Product Name	Cat. No.
<i>Clostridium thermocellum</i> enzymes			
<b>β-glucosidase activity</b>			
BglA	Cthe_0212	CAZyme CthBglA	30572-1
<b>endo-cellulase activity</b>			
CelE	Cthe_0797	CAZyme CthCelE	30553-1
CelD	Cthe_0825	CAZyme CthCelD	30554-1
CelH	Cthe_1472	CAZyme CthCelH	30555-1
CelC*	Cthe_2807	CAZyme CthCelC	30552-1
CelG	Cthe_2872	CAZyme CthCelG	30556-1
<b>endo-mannanase activity</b>			
ManA	Cthe_0032	CAZyme CthManA	30610-1
<b>exo-cellulase activity</b>			
Cell	Cthe_0040	CAZyme CthCell	30557-1
CelA	Cthe_0269	CAZyme CthCelA	30558-1
CelO	Cthe_2147	CAZyme CthCelO	30559-1
CelK (reducing activity)	Cthe_0412	CAZyme CthCelK	30560-1
CelR (nonreducing activity)	Cthe_0578	CAZyme CthCelR	30561-1
<b>xylanase activity</b>			
XynY	Cthe_0912	CAZyme CthXynY	30534-1
XynZ	Cthe_1963	CAZyme CthXynZ	30535-1
XynA	Cthe_2972	CAZyme CthXynA	30533-1

CAZyme	Gene Locus	Product Name	Cat. No.
<i>Bacillus</i> enzymes			
<b>xylanase activity</b>			
Xylanase 1	Proprietary	CAZyme Xylanase 1	30531-1
Xylanase 2	Proprietary	CAZyme Xylanase 2	30532-1
<b>β-xylosidase activity</b>			
Xylosidase 1	Proprietary	CAZyme Xylosidase 1	30511-1
<b>β-glucanase activity</b>			
β-Glucanase 1	Proprietary	CAZyme β-Glucanase 1	30591-1
β-Glucanase 2	Proprietary	CAZyme β-Glucanase 2	30592-1
<b>β-glucosidase activity</b>			
β-Glucosidase 1	Proprietary	CAZyme β-glucosidase 1	30571-1
<b>arabinofuranosidase activity</b>			
Ara 1	Proprietary	CAZyme Ara 1	30501-1
<b>Other activities</b>			
Expansin	Proprietary	CAZyme Expansin	30502-1
Only commercial source of recombinant expansin!			
<i>Dictyoglomus turgidum</i> enzymes			
<b>endo-cellulase activity</b>			
CelA**	Dtur_0670	CAZyme DturCelA	30551-1

\*Formerly called CAZyme Cellulase 2

\*\*Formerly called CAZyme Cellulase 1

# Ethanol Bio-Refineries

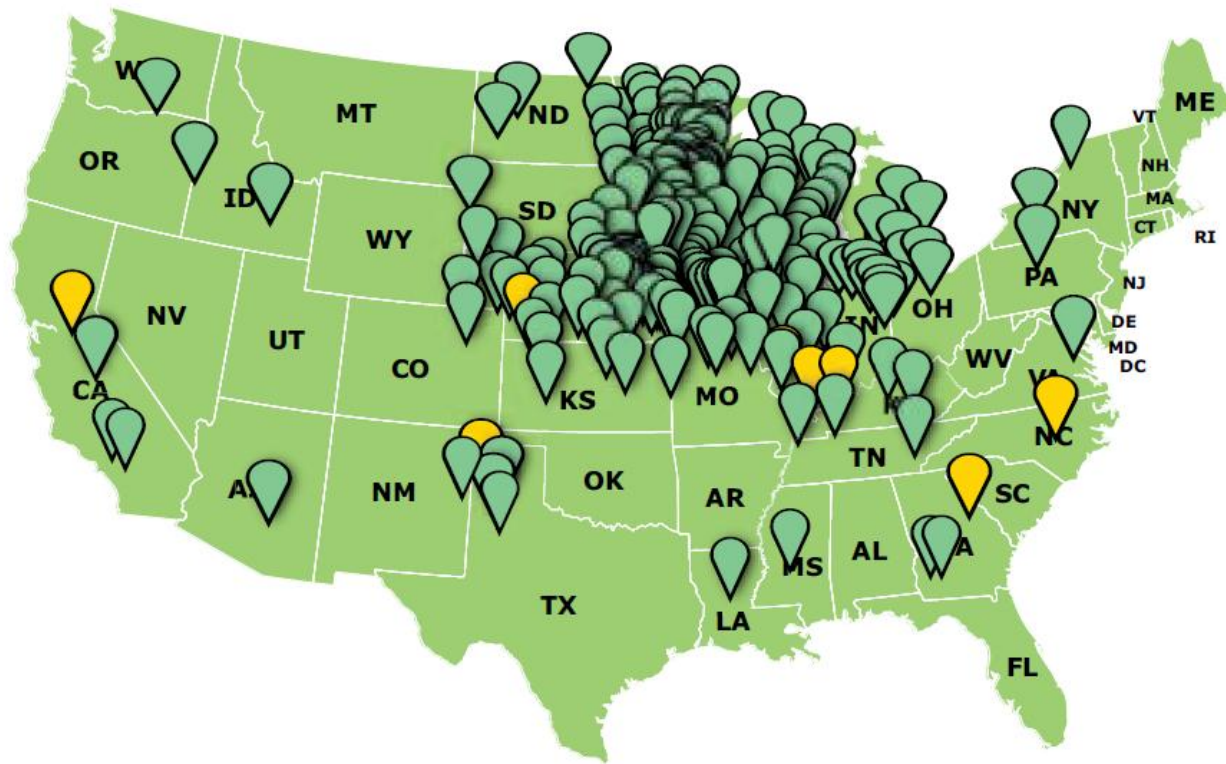
1,400+ conventional bio-refineries worldwide

## 2011 Top Global Ethanol Producers (in millions of gallons)

U.S	13,900
Brazil	5,573
E.U.	1,199
China	554
Canada	462
Total	21,688



# US Ethanol Bio-Refineries



**209 Nameplate Refineries in N. America**  
**14744.9 MGY Total (13B or 88% Dry Mill)**  
**\$38B Industry @ \$2.34/GAL ETHANOL**

# Corn Ethanol Plant Efficiencies

Bushel of Corn Costs	\$7.42	
Conventional Ethanol Plant Makes	\$8.54	13%
<b>C5•6 Process Improvements</b>	<b>\$9.89</b>	<b>25%</b>

**15% fiber + starch carbohydrate is not converted**

# Conventional Corn Processing



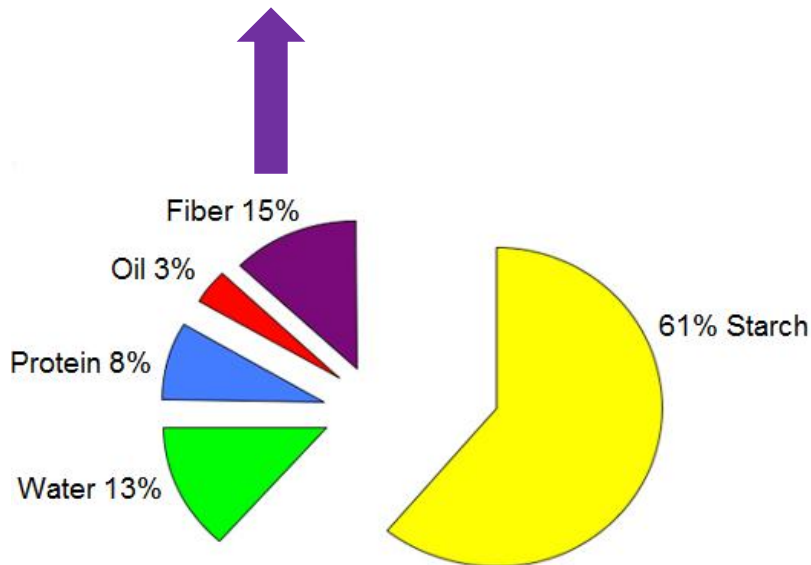
5B bushels/yr

## Animal Feed

42M tons/yr

## Ethanol

14.8B gal/yr



# CornBuster™ Enzyme Technology

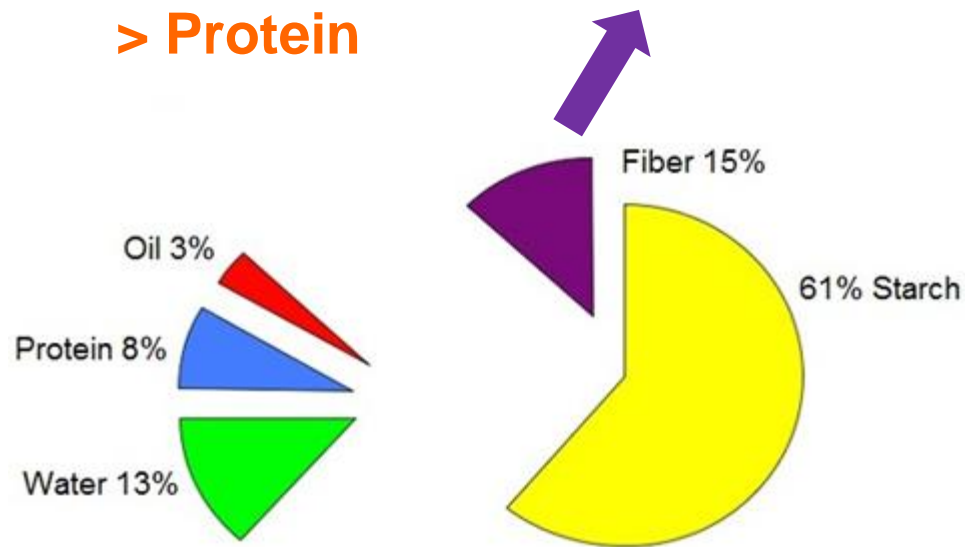


## Animal Feed

< Fiber  
> Protein

## Ethanol

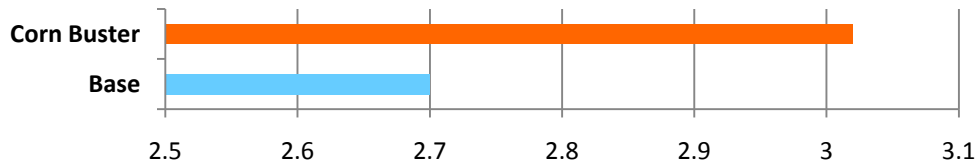
10% > Yield



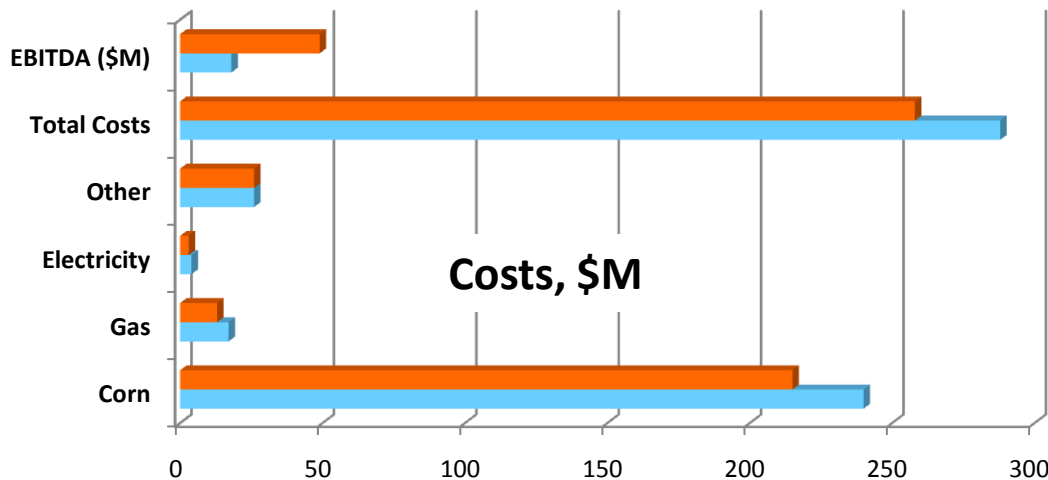
# CornBuster™ Value Proposition

for 100 MGY Plant

### Yield, gal/bushel



**10% Ethanol  
Yield Increase**



**266% EBITDA Increase**

**10% Less Electricity  
22% Less Gas  
10% Corn Decrease**

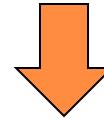
**CornBuster™ Gain vs Base Case**

**\$31,057,000 EBITDA**



# Dry Mill Corn Ethanol Process

**Grind corn**



**Cook with amylase**



**Cool, add second amylase and yeast, and ferment**



**ETHANOL**

**DDGS – ANIMAL FEED**



# Dry Mill Corn Ethanol Products

## **CornBuster™ I**

High temp enzymes increase starch conversion yield.

## **CornBuster™ II**

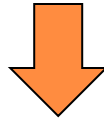
Low temp enzymes convert corn fiber to C6 fermentable sugars and improves feed value.

## **BranBuster™**

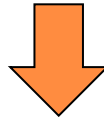
Converts bran from fractionation processes to C6 fermentable sugars.

# CornBuster™ | Enzyme Technology

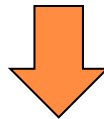
**Grind corn**



**Cook with amylase and  
CornBuster™ |**

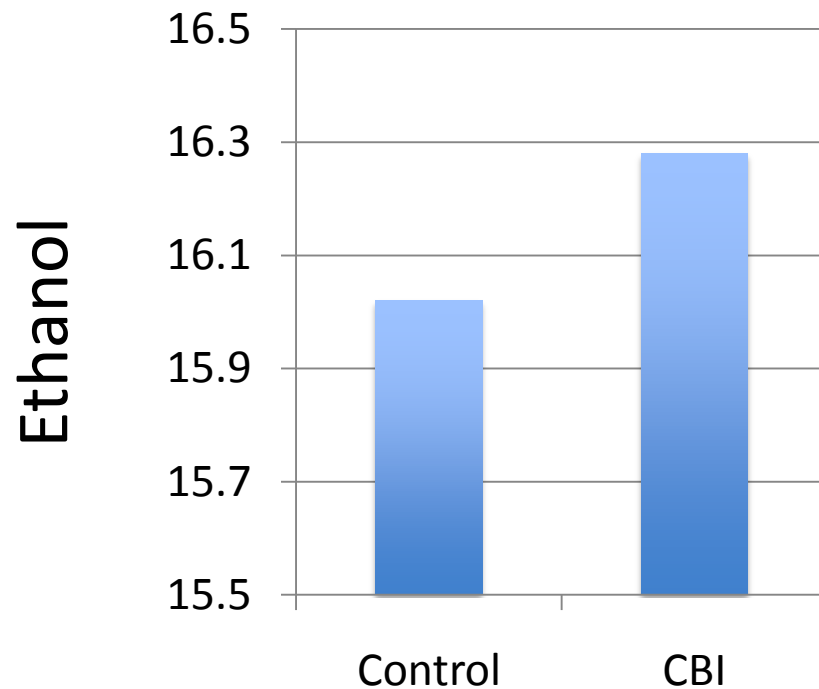


**Cool, add second amylase and  
yeast, and ferment**



**MORE ETHANOL  
LESS DDGS**

# CornBuster™ I Enzyme Technology



Corn Buster I Ethanol Plant Field Trial run in plant laboratory. Average of three replicates.

**2-3% More Ethanol with first gen product.**  
Second gen version going out for evaluation!

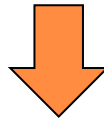


# CornBuster™ II Enzyme Technology

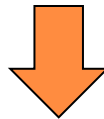
**Grind corn**



**Cook with amylase**



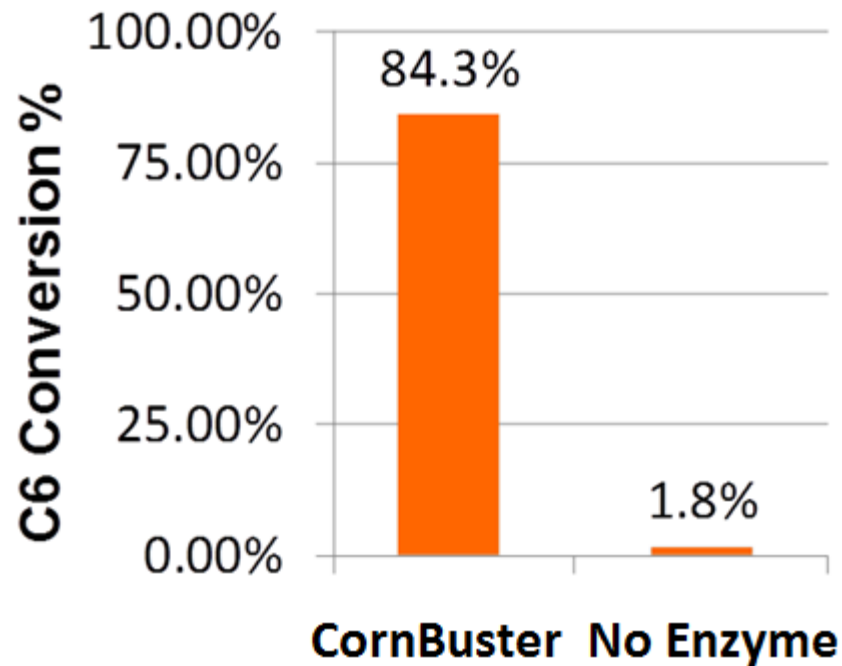
**Cool, add second amylase and  
CornBuster II, yeast, and ferment**



**MORE ETHANOL  
LESS DDGS**

# CornBuster™ II Enzyme Technology

Drop in Enzyme to Convert Fiber to More Sugar  
**10% More Ethanol**



# Competitive Advantages

Drop in solution with Minimal CAPEX

Current cellulosic enzymes require large CAPEX

Wrong temp & pH optimum for existing ethanol plants

CleanTech Benefits

**Reduces energy, water use, manure, methane, GHG!**

Corn Buster Adds Value Today

Higher protein content DDGS feed

Corn Buster Adds Value Tomorrow

Technology compatible with cellulosic biofuels

# Competitive Products

- 1) Fungal Enzymes – Wrong temp & pH
- 2) Mechanical 1  
Pursuit Dynamics Ethanol Reactor System  
Gas disruption, does not convert fiber
- 3) Mechanical 2  
Elusieve process from MSU  
Separates fiber from feed, no conversion

# Dry Mill Corn Fractionation Process

**Grind corn**



**Fractionate**

**Bran**

**Endosperm**

**Germ**



**Saccharify and ferment**

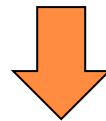


**ETHANOL**

**ANIMAL FEED**

# BranBuster™ Enzyme Technology

Grind corn

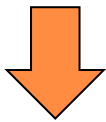


Fractionate

Bran

Endosperm

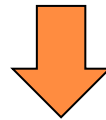
Germ



**BranBuster™**



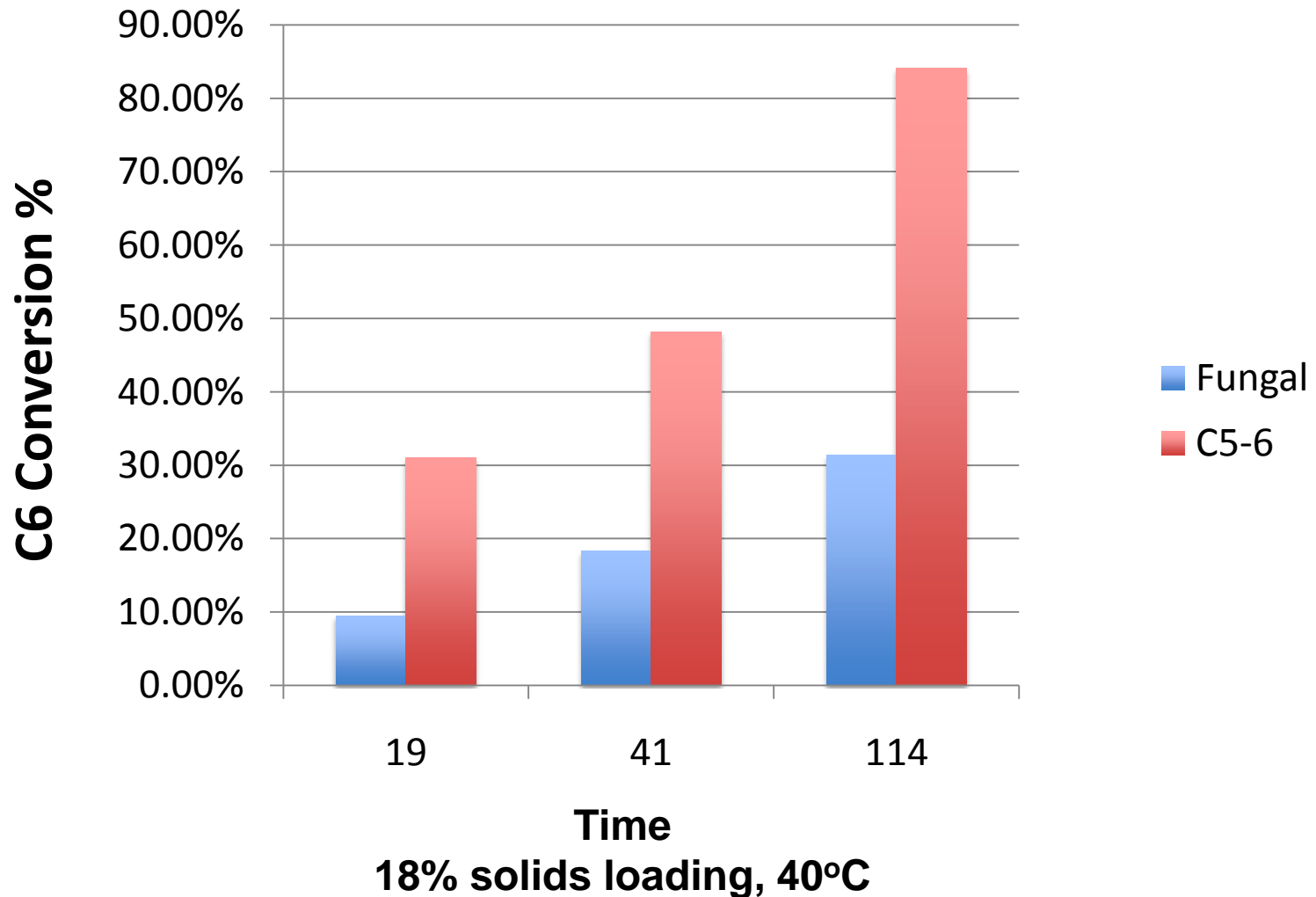
Saccharify and ferment



**MORE ETHANOL**

**HIGHER PROTEIN ANIMAL FEED  
(like wet mill corn gluten feed )**

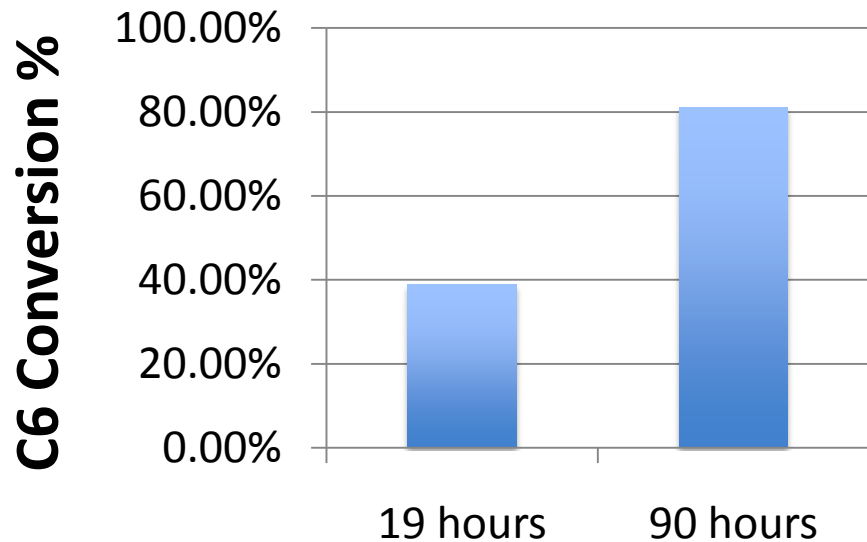
# BranBuster™ Results



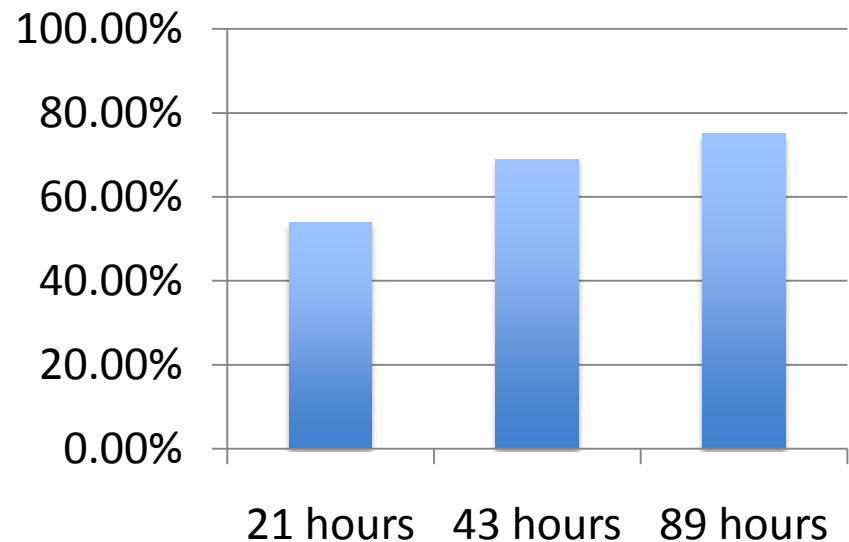
# Cellulose Buster

Based on CornBuster and BranBuster Technologies

Robust System for Converting Biomass into C5 & C6 Sugars



**Filter Paper**  
40°C, pH5.8



**Pretreated Corn Stover**  
40°C, pH5.8



# Milestone Achievements

## Project Funding \$7M

Multinational Joint Venture Cellulosic Ethanol \$1M

Great Lakes Bioenergy Research Center Enzyme Research ~\$700k/yr (Yr 4)

DOE Soybean Enzyme Grant \$1.3M

NSF SBIR Biomass Degradation Phase I Grant \$150K

DOD SBIR Navy Biofuels Grant \$100K

NIH SBIR Biofilms SBIR Phase I \$230K

WI DOC Corn Ethanol Grants \$100K & \$350K

## Corn Buster IP

100% C5•6 Technologies – Developed by Phil Brumm, PhD, CSO

2 Patents Issued

Multiple patent applications underway

## 7 Peer Reviewed Publications

# Have a C5 & C6 Sweet Day!

**608.444.9518**

**Visit our Booth**



**David Mead**  
CEO

**C5•6 Technologies Inc.**  
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Middleton WI 53562

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