

Evaluating Corn-to-Ethanol Production with the Addition of Sorghum Grain

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- Section I Ethanol from 100% Sorghum and Mixed with Corn, **1G**
- Section II Ethanol from 100% Sorghum, *in situ* **1.5G and 1G**
- Section III Use NCERC's Starch and Cellulose **Testing Methods to Build Mass Balance** for Flask Fermentation
- Section IV **Hemicellulose** in Feedstocks (corn, sorghum), and in DDGS
- Section V Cellulose methods: NCERC method and NREL method



1G Ethanol Production

100% Sorghum vs 100% Corn

Sorghum	Ethanol level (g/L) in final beer compared with 100% Corn 1G	Ethanol production (g) per flask* compared with 100% Corn 1G
Total of 6 varieties [‡] , plus samples from three states [#] in the U.S.	88% to 102%	88% to 102%

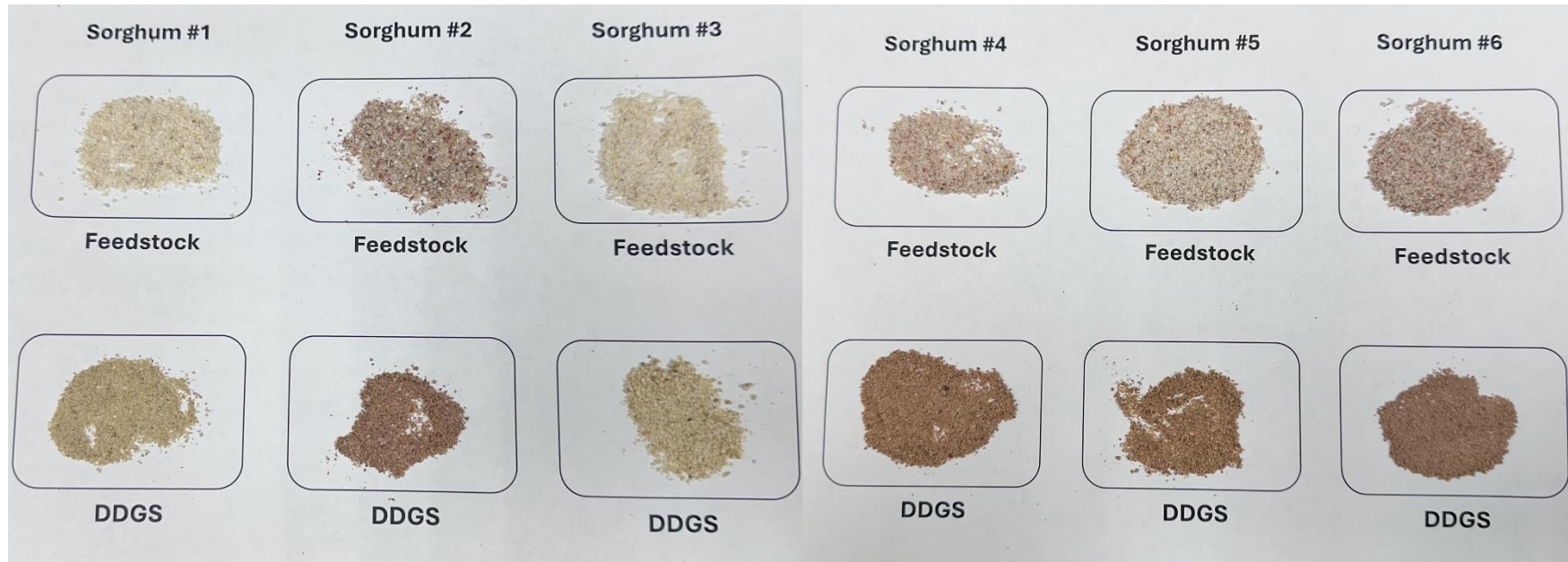
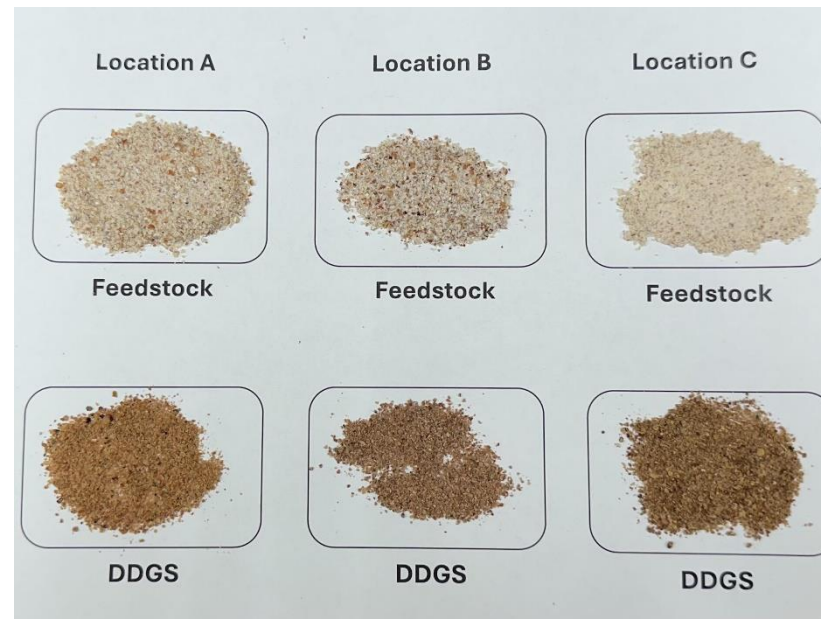
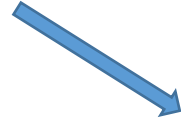
* Ethanol production per flask data were consistent with **carbon dioxide production data**

‡ Sorghum varieties received from **Nuseed**

Location A, B, C

100% Sorghum Flour VS 100% Sorghum DDGS

Nuseed Variety 3 Best Performer



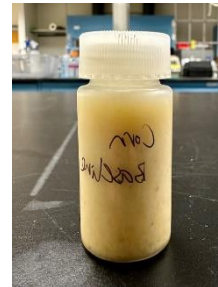
Flask Fermentation to Make Ethanol from **Corn Mixed with Sorghum**

Corn; Sorghum

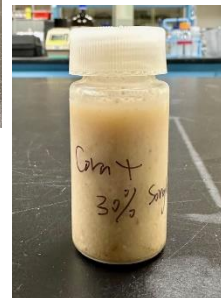


Final Beer from

100% Corn 10% Sorghum
in Corn



30% Sorghum
in Corn



100% Sorghum



DDGS from
corn,
10% sorghum in corn
30% sorghum in corn
100% sorghum



Section I Summary

- The 1G ethanol production from sorghum can range between **88% and 102%** of that from corn, depending on the sorghum variety
- When mixing sorghum with corn, the ethanol production level could be reduced, if the sorghum variety has **lower starch content and lower conversion capability of the starch** in sorghum under 1G conditions



Industrial *in situ* 1.5G Ethanol Production Comparison

Feedstocks	<i>In situ</i> 1.5G ethanol production per flask compared with Corn 1G	Ethanol production per flask <i>in situ</i> 1.5G / 1G sorghum
Location A	98%	104%
Location B	94%	106%
Sorghum V1	96%	102%
Sorghum V4	101%	102%
Corn	101%	



NCERC in situ 1.5G Ethanol Production Comparison

Feedstocks	<i>NCERC In situ 1.5G</i> ethanol production per flask compared with Corn 1G	Ethanol production per flask <i>NCERC in situ 1.5G</i> / 1G sorghum
Location B	96%	108%
Sorghum V3	105%	
Corn	104%	



Section II Summary

- **1G to industrial *in situ* 1.5G,**
 - 1% ethanol production increase** **corn**
 - 2 - 6% ethanol production increase** **sorghum**

- **1G to **NCERC** *in situ* 1.5G,**
 - 4% ethanol production increase** **corn,**
 - 8% ethanol production increase** **sorghum**



Mass Balance

1G Ethanol, Starch in Feedstocks and in DDGS

Feedstock	Ethanol production per flask (g)	Total Starch in feedstock (% DWB)	Total starch in DDGS (% DWB)	Starch/ash in DDGS / starch/ash in feedstock (%)
Sorghum V1	10.5	69.9	9.0	4
Sorghum V2	10.4	61.5	15.0	8
Sorghum V3	11.4	70.7	6.0	2
Sorghum V4	11.1	65.1	8.7	5
Corn Baseline	11.2	70.6	3.9	2



Mass Balance: Industrial *in situ* 1.5G / 1G

Feedstock	Ethanol production per flask	Residual starch in DDGS	Residual cellulose in DDGS
	g	%, dwb	%, dwb
Corn 1G*	13.1	2.6	9.4
Corn <i>in situ</i> 1.5G	13.4	1.8	7.1
1.5 G/1G	102%	71%	75%
Sorghum Location A 1G	12.4	15.7	10.7
Sorghum Location A <i>in situ</i> 1.5G	12.9	13.0	4.4
1.5 G/1G	104%	83%	41%
Calculated ethanol increase from the reduction of starch or cellulose (g)		0.16**	0.37
Ethanol increase (g)	0.50		0.53 g

*Corn DDGS data were from the publication of *Industrial Biotechnology* 19 (3), 163-167

**Estimated by 1. Carbohydrate mass in DDGS: DDGS mass X residual carbohydrate level

2. Reduction of carbohydrates: carbohydrate mass in 1G DDGS – mass in 1.5G DDGS

3. Ethanol (g): results from #2 X 0.57 (assuming 100% carbohydrate conversion to ethanol and CO₂)



Mass Balance: *in situ* 1.5G / 1G

Feedstock	Residual starch in DDGS	Residual cellulose in DDGS
	%, dwb	%, dwb
Sorghum V1 1G	9.0	11.0
Sorghum V1 1.5G	4.3	7.6
1.5 G/1G	48%	70%
Sorghum V3 1G	6.0	10.6
Sorghum V3 NCERC Boost 1.5G	5.4	6.6
1.5 G/1G	90%	62%



Section III Summary

- Under **1G**, higher starch in sorghum leads to higher ethanol production; the residual starch level in sorghum DDGS was likely higher than that in corn DDGS
- Under *in situ* **1.5G**, sorghum likely has more potential to make cellulosic ethanol production than corn
- **Nuseed Variety 3** can make more total ethanol and cellulosic ethanol than corn under various conditions



Hemicellulose in Grains

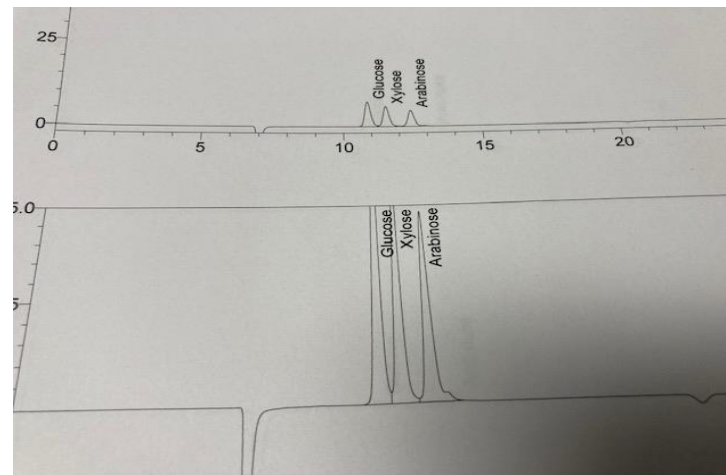
Definition: Xylan, dominant, β -1,4-linked xylose

Arabinan, minor, alpha-1, 5 linked arabinose

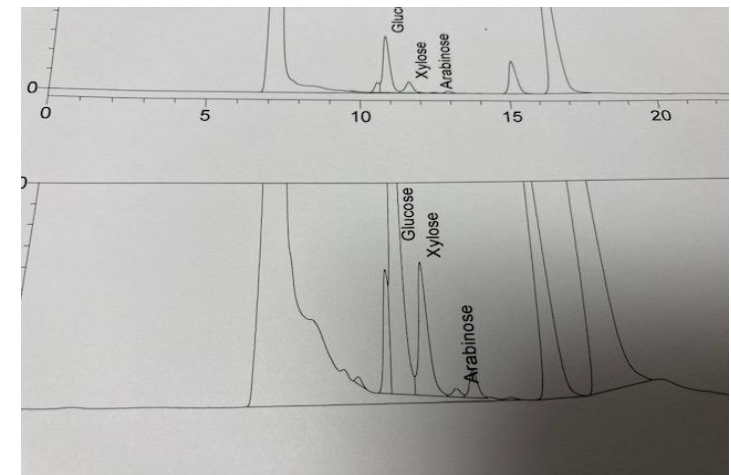
Testing method: NREL Technical Report NREL/TP-510-42618

(can be validated with the help of LC/MS/MS, to verify the selectivity of the xylose peak identified using LC/RID)

Typical HPLC Chromatograms



HPLC Check Standard



Sorghum flour



Carbohydrates in Flour and DDGS*

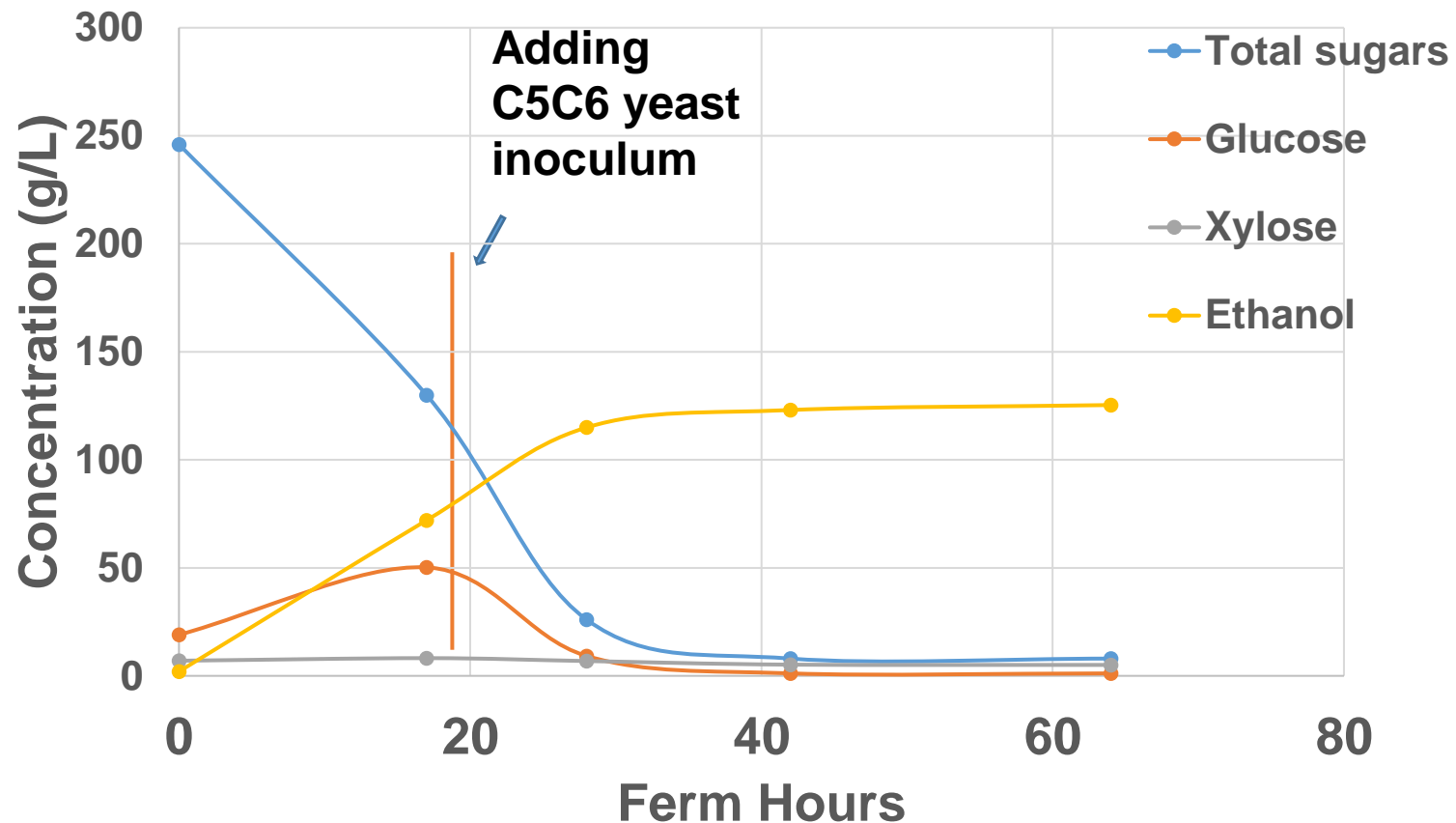
Samples	Starch	Cellulose	Xylan
	%, dwb	%, dwb	%, dwb
Corn flour	76	1.8	4.0
Corn 1G DDGS	3.9	9.4	N.A.
Sorghum flour V3	71	3.9	3.4
Sorghum V1 1G DDGS	9.0	11.0	9.5
Sorghum V3 1G DDGS	6.0	10.6	10.1

* The error bar for data points from 10% to 20%; also consider the range for each data category



HPLC for 150 L Fermentation

Corn Starch, Cellulose and Xylose Conversion



NCERC Cellulose Method for Corn Matrix

- A VCSB Method - Published in *Industrial Biotechnology 19 (3), 163-167*
- Used to test the following samples:
 - ❑ Two NIST biomass samples
 - ❑ A pair of before conversion (BC) and after conversion (AC) samples from three dry grind ethanol plants in the U.S.
 - ❑ NCERC in-house synthetic standard run with every batch

When using the testing method on sorghum-based samples, the data on cellulose and starch change from 1G to 1.5G can explain the ethanol increase with accurate mass balance



Results - NIST Samples (corn matrix)

NIST BC Biomass			NIST AC Biomass		
NREL method	NCERC method	NREL/NCERC	NREL method	NCERC method	NREL/NCERC
2.00%	2.80%	0.7	5.20%	8.60%	0.6



Results - Dry Grind Ethanol Plant Samples (corn matrix)

	Plant BC Sample			Plant AC Sample		
	NREL Method	NCERC Method	NREL/NCERC	NREL Method	NCERC Method	NREL/NCERC
Plant I	1.9	3	0.6	6.5	9.1	0.7
Plant II	2.2	2.2	1	6.4	8.9	0.7
Plant III	1.8	2.7	0.7	5.8	8.5	0.7



Summary

- **The NCERC proprietary analytical methods for testing starch and cellulose in both corn and sorghum matrix have delivered scientifically sound results**
- **Those methods are strong to be used to support D3RINs analytical testing package for ethanol plants**
- **In general, the NCERC cellulose method delivered about 30% higher value than the NREL method when testing the same corn matrix samples**



Acknowledgement

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