Maximize Ethanol Production from Corn Starch, Resistant Starch, Cellulose and Xylan

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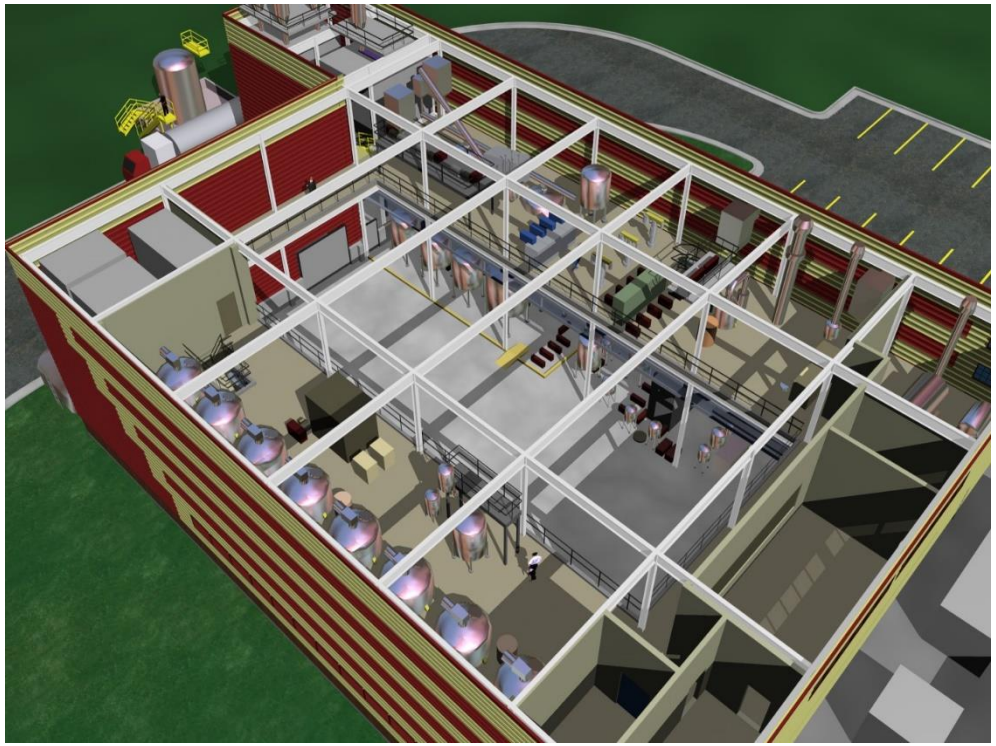

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The Carbohydrates in Corn Flour*
(estimates)

	Starch**	Cellulose	Xylan
dry weight basis (dwb), %	76	1.8	4.0
Range (dwb, %)	*73 - 79*	*1.4 – 2.2*	*3.6 – 4.4*
Standard deviation (±, dwb, %)	2.9	0.36	0.4
Relative standard deviation (RSD)	4%	20%	10%

*All analytical methods are based on published methods with modifications; with QA/QC on each running batch

** total starch includes resistant starch and fermentable starch

What Happened with Industrial 1.5G Processing

Pathway	Carbohydrates used	Pretreatment	Starch hydrolysis	Cellulosic hydrolysis	Fermentation
1G	Starch	No	Yes	No	C6 yeast
1.5 G	Starch and cellulose	No	Yes	Cellulase at 32°C	C6 yeast

Corn to Ethanol Theoretical Mass Change

	starch or cellulose	→	ethanol	+	carbon dioxide
	$(C_6H_{10}O_5)_n$		$2C_2H_5OH$		$2CO_2$
Stoichiometric	162g		2 X 46g		2 X 44g

Theoretical: **ethanol / glucan = 0.57 g/g**

Ethanol / CO2 = 1.05

	xylan	→	ethanol	+	carbon dioxide
	$3(C_5H_8O_4)_n$		$5C_2H_5OH$		$5CO_2$
Stoichiometric	3X132g		5X46g		5X 44g

Theoretical: **ethanol / xylan = 0.58 g/g**

Ethanol / CO2 = 1.05

1G and 1.5 G for Ethanol and Carbon Dioxide

Trial No.	Pathway	Carbohydrates used	Flask No.	Final weight loss (g)	Final ethanol level (g/L)	Final ethanol production (g)
I	1G	Starch	4	13.4	123.0	13.7
	1.5 G	Starch and cellulose	4	13.7	124.8	14.0
	1.5G/1G			102%	101%	102.2%
	p Value			*0.005*	*0.015*	*0.005*
II	1G	Starch	3	13.2	118.6	13.2
	1.5 G	Starch and cellulose	3	13.7	120.4	13.5
	1.5G/1G			103%	102%	102.4%
	p Value			*0.004*	*0.024*	*0.10*

1G and 1.5 G for Ethanol and Carbon Dioxide

Trial No.	Pathway	Carbohydrates used	Flask No.	Final weight loss (g)	Final ethanol level (g/L)	Final ethanol production (g)
III	1G	Starch	6	13.0	116.3	12.9
	1.5 G	Starch and cellulose	6	13.6	117.4	13.1
	1.5G/1G			104%	101%	101.7%
	p Value			*0.001*	*0.039*	*0.036*
IV	1G	Starch	4	13.4	116.7	13.1
	1.5 G	Starch and cellulose	4	13.8	118.4	13.4
	1.5G/1G			102%	102%	102.2%
	p Value			*0.016*	*0.026*	*0.002*

1G and 1.5G Residual Carbohydrates in DDGS (Trial I)

Pathway	Carbohydrates used	Flask No.	Starch in DDGS (%, dwb)	Cellulose in DDGS (%, dwb)
1G	Starch	4	3.0	5.7
1.5 G	Starch and cellulose	4	2.4	5.2
1.5G/1G			80%	91%
			Total starch / ash	Cellulose / ash
1G	Starch	4	0.64	1.2
1.5 G	Starch and cellulose	4	0.47	1.0
1.5G/1G			73%	86%
P value			*0.07*	*0.26*

1G and 1.5G Residual Carbohydrates in DDGS (Trial III)

Pathway	Carbohydrates used	Flask No.	Starch in DDGS (%, dwb)	Cellulose in DDGS (%, dwb)
1G	Starch	6	4.3	9.4
1.5 G	Starch and cellulose	6	3.1	7.1
1.5G/1G			73%	75%
			Total starch / ash	Cellulose / ash
1G	Starch	6	1.1	2.3
1.5 G	Starch and cellulose	6	0.7	1.7
1.5G/1G			69%	71%
P value			*0.000*	*0.026*

1G and 1.5G Residual Carbohydrates in DDGS (Trial IV)

Pathway	Carbohydrates used	Flask No.	Starch in DDGS (%, dwb)	Cellulose in DDGS (%, dwb)
1G	Starch	4	4.1	11.8
1.5 G	Starch and cellulose	4	2.9	9.8
1.5G/1G			71%	83%
			Total starch / ash	Cellulose / ash
1G	Starch	4	1.02	2.9
1.5 G	Starch and cellulose	4	0.73	2.4
1.5G/1G			71%	83%
P value			*0.003*	*0.099*

What Happened to Pure Cellulose and Pure Resistant Starch

Pathway	Pure compound	Starch hydrolysis	Cellulosic hydrolysis and fermentation
1G	Cellulose	Yes	N.D.
	Control corn starch (37% resistant starch)	Yes	N.D.
1.5 G	Pure cellulose	Yes	50% conversion
	Control corn starch (37% resistant starch)	Yes	Barely detected

The Impact of Yeast Biomass on DDGS Cellulose Testing

- Ran pure corn starch based fermentation, with ethanol production similar to the level from corn flour based fermentation
- Harvest the yeast biomass
- Test the yeast biomass for cellulose reading (dwb, %): 10% ± 1%

Summery on Industrial 1.5G Processing Based on Flask Trials

- Compared with 1G processing, we observed about 2% ethanol production increase, verified by the data from carbon dioxide production; both mass changes were statistically sound for the batches we ran
- The ethanol yield increase did vary with individual batches, even though we used the same corn flour, and we suspect the yeast performance was not identical with every batch
- The ethanol increase was due to both cellulose and starch conversions, and likely the cellulosic conversion was responsible for half or more of the 2% ethanol increase
- In order to make about 1% cellulosic ethanol, more than 15% of the cellulose in corn flour is needed to be converted
- We expect the ethanol yield increase with commercial production even higher, due to better mixing of between enzymes and carbohydrates compared with flask trials

Make Cellulosic Ethanol from Cellulose and Xylan (NCERC 1.5GX)

Pathway	Carbohydrates used	Pretreatment	Starch hydrolysis	Cellulosic hydrolysis	Fermentation
1G	Starch	No	Yes	No	C6 yeast
1.5 G	Starch and cellulose	No	Yes	Cellulase at 32°C	C6 yeast
1.5GX	Starch, cellulose and xylan	Yes (mild at 90°C)	Yes	Cellulase & hemicellulase at 32°C	C5C6 yeast

What Happened to Pure Carbohydrates during NCERC 1.5GX

Pathway	Cellulose form	Pretreatment	Starch hydrolysis	Cellulosic hydrolysis at 32°C
1.5 GX	Pure cellulose	Mild at 90°C	Yes	60% conversion
	Pure xylan	Mild at 90°C	Yes	50% conversion
	Pure corn starch	Mild at 90°C	Yes	90 - 100% conversion
	Control corn starch (37% resistant starch)	Mild at 90°C	Yes	50% conversion

What Happened to NCERC 1.5GX

- Cannot find the right C5C6 yeast to consume both glucose and xylose in the hydrolysate to the completion level as we run 1G or 1.5G fermentation

- So far the ethanol yield is not as high as 1.5G processing

- More optimization work is on the way

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