The Impact of Yeast Biomass on AC Sample Testing for RINs Application

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Vision, Values, and Results

NCERC Services Include:

- Engineering Design
- Method development
- Process control and automation
- Strong client confidentiality and intellectual property protection
- Commercialization services: Project Design, Concept, Validation, Scale-Up





From Concept to Commercialization

NCERC's team of nationally renowned experts provides services that our clients value through these key facilities:

- Fermentation Lab Shaker flask system (250 mL), 5 L Fermenter
- Fermentation Suite 30, 150, and 1,500L Fermenters
- Pilot Plant (4) 22,000L Fermenters
- Analytical Lab Supports research areas and all client projects





Conveniently Located in the Heart of the "Biobelt"

- 50% of US agriculture is produced within a 500 mile radius of St. Louis.
- 30 minute drive from Lambert International Airport (STL)
- Member of the growing STL agtech community





NCERC's Efforts related to in situ D3RINs Testing

Based on flask fermentation (NCERC), compared with 1G, *in situ* 1.5G delivered

- about 2% ethanol production increase due to both cellulose and resistant starch conversions
- the cellulosic conversion led to about 1% ethanol increase
- more than 15% of the cellulose in corn flour was converted

Our GOAL To assist ethanol plants to get approved by US EPA for D3 RINs Application



Work with Ethanol Plants



Assumptions in General

- 1 L composite sample can represent 1 million gallon (MG) size fermenter;
- The 1 MG slurry material is 100% homogeneous
- Only one corn feedstock (no other grains or sugars), no salt addition during fermentation; addition of cellulase at T0;

so based on NCERC's lab study, we predict

BCACTC/Ash> TC (corn) / ash, with at least relatively 10% - 20% reduction



Assumptions in General

How to calculate TC (corn) from TC (total, corn + yeast) of the AC sample:

- Assuming yeast biomass in AC ranges from 10% to 20% (dry weight basis), then choose 15%
- Make yeast biomass in corn starch flask fermentation using yeast from the ethanol plant, then test TC (yeast) of the yeast biomass, and then use the following equation to calculate TC (corn)

TC (total, corn + yeast) = 0.15*TC (yeast) + 0.85 *TC (corn)



Modeling Yeast Impact on TC (corn) / Ash

| %, DWB | TC (total) | Yeast% | TC (yeast) | TC (corn) | Ash | TC (total) /Ash | TC (corn) /Ash | Ratio changed considering yeast impact |
|---------------------|---------------|----------|---------------|------------|----------|--------------------|-------------------|--|
| | Lab test | assuming | Lab test | calculated | Lab test | Calculated | Calculated | |
| Currently reporting | 9.1 | 10% | 11.6 | 8.8 | 5.7 | 1.60 | 1.55 | - 3% |
| What if | 0 1 | 10% | 20.0 | 7 9 | 57 | 1 60 | 1 2 2 | _1/0/ |
| what h | J. I | 1076 | 20.0 | 1.5 | J.1 | 1.00 | 1.50 | -14/0 |
| What if | 9.1 | 15% | 20.0 | 7.2 | 5.7 | 1.60 | 1.26 | -21% |

Summary:

- 1. Yeast biomass can impact on TC (corn)/Ash of AC sample for D3RINs application
- 2. We need to have more accurate data on yeast biomass percentage in AC samples and total cellulose level in yeast biomass in AC samples



Results of TC (yeast) Tested at NCERC

| Yeast source | TC (yeast) (DWB, %) | TC (total) (DWB, %) | TC (corn) (DWB, %) |
|--|------------------------|------------------------|-----------------------|
| Commercial yeast donated to NCERC | 16.8 | 8.0 | 7.0 |
| | | | |
| Dry yeast from Ethanol Plant A | 14.2 | 8.1 | 7.0 |
| | | | |
| Propagated yeast from Ethanol Plant B | 11.6 | 9.1 | 8.8 |



Trials on Estimating Yeast%

- a. Run parallel flask fermentation using BC material to make AC, and use corn starch to make pure yeast biomass
- b. Compare the yeast counts (counts/ml) in final beer among the flasks
- c. Dried the final beer, and compared the dried mass weight between the DDGS and yeast biomass
- d. Estimate the yeast biomass percentage in AC
- e. So far, we got the estimate of around 10% yeast biomass in AC sample from two independent trials



Summary

- The yeast biomass in the AC samples likely has higher cellulose reading than the corn matrix in the dried AC samples
- We need to estimate the yeast biomass percentage in the AC sample, and the total cellulose level in the yeast biomass, in order to calculate the impact of the yeast biomass on TC (corn)/Ash
- Our data showed the impact on the TC (corn)/Ash could be as high as relatively 10% or higher

