

**STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PLANT**

TITLE: Ethanol Concentration Determination by Gas Chromatography Operating Procedure

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APPROVALS: Process Change Committee
UF EH&S

DATE: 4/1/11
DATE:
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A. Scope

This procedure describes the method to prepare a culture for an ethanol measurement by gas chromatography.

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the plant, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles or Face Shield
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

Refer to the UF BioSafety Manual for information on how to handle cultures, compressed gas cylinders, and chemicals listed in D below.

- Agilent Technologies 6890N Network GC System operating manual
- Culture Sampling SOP

D. Preparation/Materials/Equipment

1. USA Scientific Tip One pipet tips (1111-2021, 101-1000 µl range)
2. Biohit m1000 pipetor
3. 100 ml volumetric flask
4. 2 L volumetric flask
5. Ethyl alcohol, absolute, 200 proof 99.5+% A.C.S. reagent (Acros, 61509-0040, 4 L)
6. National Scientific Target KP Vials (C4000-1)
7. National Scientific DP Green Vial Caps (C4000-51G, PTEF/RR SEPTA, 100/PK, 100/CS)
8. 1-Propanol (Fisher-Scientific, A414-4, 4 L)
9. Agilent Technologies 6890N Network GC System G1530N (autosampler with 100 sample capacity, wide bore column and split injection)
10. Compressed hydrogen tank
11. Compressed air tank
12. Compressed helium tank

E. Detailed Procedure

1. Make ethanol standards having the following concentrations: 5, 10, 20, 30, 40, and 60 grams per liter of solution) using 100 ml volumetric flasks.
2. Make 2% (w/v) 1-propanol in a large 2 L volumetric flask. Store this in the flammables cabinet after use for use in future GC analyses.
3. Prepare the ethanol standards for GC analysis by pipeting 300 μ l of the standard and 300 μ l of the 2% (w/v) 1-propanol solution into a glass vial and cap.
4. Mix contents by shaking gently for 2 seconds.
5. Place vial(s) in GC sample holder.
6. Run GC by following the instructions given in the Agilent Technologies 6890N Network GC System operating manual.
7. Read the ethanol and propanol area on the GC chromatogram.
8. Plot the ethanol area divided by the 1-propanol area obtained from the GC chromatogram on the y-axis and the corresponding ethanol concentration of the standard on the x-axis.
9. Draw a linear regression line and measure the slope.
10. Run the sample to be analyzed by GC following the same steps above (Steps 3-7).
11. Divide the ethanol area by the 1-propanol area and divide that number by the linear regression line slope (from Step 9). This is the sample's ethanol concentration in grams per liter.

F. Data Archival and Analysis

Record all ethanol measurements in batch record and fermentation log sheet. Store all log sheets and batch records in a folder labeled with Run Number.

G. Fermentation Log Sheet

Start date and time: _____

Hz concentration of seed: _____

EtOH concentration at time of inoculation: _____

Total hours the seed was grown: _____

Total volume of the seed: _____

Total volume of the inoculum: _____

Vessel used for growing the seed: _____

RPM of the seed: _____

RPM of the fermentation: _____

Comments: _____

Experiment				
Time	5 N NH ₄ OH	pH	OD ₅₅₀	EtOH g/L
0				
24				
48				
72				
96				

Experiment				
Time	5 N NH ₄ OH	pH	OD ₅₅₀	EtOH g/L
0				
24				
48				
72				
96				

120				
144				

120				
144				

Experiment				
Time	5 N NH ₄ OH	pH	OD ₅₅₀	EtOH g/L
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24				
48				
72				
96				
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