STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Ethanol Concentration by Gas Chromatography

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APPROVALS: Process Change Committee **DATE: October 3rd, 2012**

A. Scope

This procedure describes the method to prepare a sample 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.

Refer to UF Biosafety guidelines and the NIH Guidelines whenever handling biological cultures/genetically modified organisms.

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
- 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.

1. Agilent Technologies 6890N Network GC System operating manual
2. Culture Sampling SOP-0511
3. Gas Chromatography Calibration for Ethanol SOP-0518

D. Preparation/Materials/Equipment

1. USA Scientific Tip One pipet tips (1111-2021, 101-1000 µl range)
2. 1 mL pipetor

3. National Scientific Target KP Vials (C4000-1)
4. National Scientific DP Green Vial Caps (C4000-51G, PTEF/RR SEPTA, 100/PK, 100/CS)
5. 1-Propanol (Fisher-Scientific, A414-4, 4 L), 2% solution in DI water.
6. Agilent Technologies 6890N Network GC System G1530N (autosampler with150 sample capacity, wide bore column and split injection)
7. GC Column (HP-PLT/Q, 15m x 0.53mm)
8. Compressed hydrogen tank
9. Compressed air tank
10. Compressed helium tank

E. Detailed Procedure

1. Make sure the GC has been calibrated according to SOP-0518 and the slope of the calibration curve is available.
2. Assure the sample to be tested is well mixed.
3. Prepare the sample to be analyzed by pipeting 300 µl of the sample and 300 µl of the 2% (w/v) 1-propanol solution into a glass vial using a 1 mL pipetor and 1000 µL tips.
4. Seal the vial using the appropriate screw-caps for the vials used.
5. Mix contents by shaking gently for 2 seconds.
6. Place vial(s) in GC sample holder.
7. Run GC by following the instructions given in the Agilent Technologies 6890N Network GC System operating manual.
8. Read the ethanol and propanol area on the GC chromatogram.
9. Divide the ethanol area by the 1-propanol area and divide that number by the linear regression line slope. 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.