STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Optical Density Measurement using Spectrophotometer

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APPROVALS: Process Change Committee **DATE:**

A. Scope

This procedure describes the method to prepare a sample and measure the optical density using a spectrophotometer.

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.

D. Preparation/Materials/Equipment

1. Fisherbrand disposable culture tubes (borosilicate glass, 13 X 100 mm, cat. No. 14-961-27)
2. USA Scientific pipet tips (1053-0000, 500-5000 µl range)
3. USA Scientific Tip One pipet tips (1111-2021, 101-1000 µl range)
4. 5 mL pipetor
5. 1 mL pipetor
6. Thermo Electron Corporation Spectronic 20D+ Spectrophotometer

E. Detailed Procedure

1. Turn the spectrophotometer on by using the "0% T" knob on the left for ≥ 15 minutes to allow it to warm up.
2. Set the wavelength to 550 nm by using the "wavelength" knob on top of the spectrophotometer.
3. Close the lid without anything inside the spectrophotometer.
4. Set the spectrophotometer to transmittance mode by toggling with the "mode" button in the front panel of the spectrophotometer.
5. Set the transmittance to "0" by using the "0% T" knob on the left.
6. Put a test tube filled with ≥ 3 ml of a blank (usually deionized water). Make sure the test tube is snug against the groove on the right and close the lid.
7. Use the "100% T/OA" knob on the right to go up to 100% transmittance. This should correspond to an absorbance reading of "0."
8. Dilute the culture sample to be in an absorbance range of 0.1 to 0.6 OD_{550nm}. The culture should be diluted using deionized water.
9. Place ≥ 3 ml of diluted/undiluted culture in a 13 X 100 mm disposable glass culture tube. Make sure the test tube is snug against the groove on the right and close the lid.
10. Close the lid and change to absorbance mode using "mode" button to get a 550 nm absorbance reading.
11. If measuring absorbance of a viable culture, place the tube in the biohazard waste beaker for inactivation.

F. Data Archival and Analysis

Record all OD_{550nm} measurements in batch record and fermentation log sheet. Store all log sheets and batch records in a folder labeled with Run Number.

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