STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Viable Plate Count

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A. Scope

This procedure describes how to determine the number of viable bacteria in samples taken from the process.

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.

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

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

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

C. Related Documents and SOPs

1. Autoclave SOP-0504
2. Sampling SOP-0511
3. Growth medium SOP-0520
4. ESCO Airstream Horizontal Laminar Flow Clean Bench Manual (2008)
5. Cell culture incubator Manual
6. Balance Manual

D. Preparation/Materials/Equipment

1. Nitrile gloves
2. Autoclave
3. Laminar flow clean bench
4. Cell culture incubator

5. Laboratory balance
6. Erlenmeyer flasks (1 L)
7. Sterile agar plates of appropriate medium
8. DI water
9. Microcentrifuge tubes (1.5 mL)
10. Pipette tips (1000 µL)
11. Pipette tips (200 µL)
12. Pipettor (200-1000 µL)
13. Pipettor (20-200 µL)
14. Glass beads
15. 70% Ethanol
16. 0.6% bleach solution (10% dilution of commercial bleach)

E. Detailed Procedure

1. Aseptically collect samples for analysis according to Sampling SOP-0511.
2. Prepare the material that will be sterilized such as tip boxes (200 µL and 1000 µL), microcentrifuge tubes, DI water, glass beads, and plating media (Growth Medium SOP-0520).
3. Autoclave the material listed in step E.2. according to Autoclave Operation SOP-0504.
4. Turn on the laminar flow clean bench and wipe the work surface using a paper towel and 70% ethanol.
5. Take out all material from the Autoclave and put it on the laminar flow clean bench.
6. Wait for the material to cool before you start working in the laminar flow clean bench with the autoclaved material.
7. Put on the nitrile protective gloves.
8. Transfer 900 µL of sterile DI water to several sterile microcentrifuge tubes using sterile tips (1000 µL) and pipettor (200-1000 µL).
9. Label each microcentrifuge tube with sample ID.
10. Mix the sample by inversion and transfer 100 µL to the first tube in the dilution series (10-fold dilution or 10^{-1} dilution) using sterile tips (200 µL) and pipettor (20-200 µL).
11. Replace cap and mix.
12. Using a new sterile tip (200 µL), transfer 100 µL of 10^{-1} dilution to the next microcentrifuge tube containing 900 µL of sterile DI water for a 10^{-2} dilution. Continue this serial dilution series to make 10^{-3}, 10^{-4} and 10^{-5} dilutions. The goal is to provide a dilution that yields between 30 and 300 colonies.

13. Label each media plate with sample ID and dilution factor at the bottom of the plate.
14. If the sample is very concentrated, shake the 10^{-3} and 10^{-5} dilutions series and aseptically transfer 100 µL to center of each of two media plates labeled. If the sample is not concentrated, use the 10^{-1} or 10^{0} dilutions for plating.
15. Aseptically place 6-10 sterile glass beads on each plate.
16. Slowly move the plate from side to side in a motion that spreads the cell suspension evenly over the surface of the agar (~15 sec).
17. Remove the glass beads from the plate by inverting the plate above a plastic beaker containing a 0.6% bleach solution.
18. Invert the plates and place into the cell culture incubator at 30 °C for 24 hours.
19. Put the media plates that were not used into a petri dish bag and store them in the refrigerator at 4 °C for no longer than 1 month.
20. Pick up all the material from the laminar flow clean bench and wipe the work surface using a paper towel and freshly prepared 10% bleach, followed by 70% ethanol.
21. Turn off the Laminar flow clean bench.
22. Count the colonies after 24 hours of incubation at 30 °C.
23. Calculate the CFU per milliliter (CFU/mL) with the dilution factor and volume used for plating according to the following formula:

    CFU/mL = (Number of colonies * Dilution factor) / 0.1

F. Data Archival and Analysis

Record the number of colonies and the CFU/mL in the laboratory notebook including the sample name, date, time, vessel and dilution used for plating.