STANDARD OPERATING PROCEDURE
FOLEY PILOT PLANT

TITLE: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

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APPROVALS:	**Process Change Committee**	**DATE:**
	HHSM	**DATE:**

Scope

This plate count method describes the procedure for determining the number of cultural ethanologenic *Escherichia coli* in a fermentation sample using Luria Britani/2% xylose plates.

Safety Requirements

Always where gloves, safety glasses, and lab coat when handling bacterial preparations and fermentation samples.

Related Documents

Refer to the UF biosafety manual regarding BSL-1 microorganisms.

Equipment list

1. Gloves
2. Safety glasses
3. Labcoat
4. 70% Ethanol
5. LB/xylose plates
6. Pipettes and tips
7. Dilution Tubes
8. Glass Beads
9. LB diluent
10. Agar
11. Autoclave

Procedure

1. Aseptically collect samples for analysis. All preparations must be made so that samples can be analyzed within 1 hour of collection.
2. Prepare LB/xylose plates according to the following recipe: 10g/L tryptone, 5g/L yeast extract, 5g/L sodium chloride, 20g/L d-xylose, 15g/L agar. Mix tryptone, yeast extract, sodium chloride and agar in 1L flask with 500mL working volume and autoclave at 121C for 30 minutes. Cool to 45C in a water bath and pipette 20mL of 50% w/v filter sterilized xylose solution (dissolve 500g/L d-xylose in distilled water and filter sterilize) into flask using the aseptic technique. Pour 20mL of agar solution into petri plates and allow them to solidify (about 20 minutes). Invert plates and allow them to cool and dry for at least 24h.

3. For LB diluent, use the following recipe: 10g/L tryptone, 5g/L yeast extract, 5g/L sodium chloride. Make 500mL and autoclave at 121C for 30 minutes.
4. Allow LB diluent to cool to room temperature before diluting and plating.
5. Wipe bench top with ethanol in order to provide a clean environment.
6. Label each plate with sample ID and dilution factor.
7. Ensure incubator is at 37C

Preparing Dilution Series

1. Transfer 9.9mL of LB diluent to sterile 30mL tubes.
2. Using pipette, transfer 0.1mL of fermentation sample to the first tube in the dilution series (100 fold dilution). Replace cap and mix with a vortex mixer.
3. Using a new sterile pipette, transfer 0.1mL of 10^{-2} dilution to the next tube for a 10^{-4} dilution, and repeat in a third tube for a 10^{-6} dilution. The goal is to provide a dilution that yields between 30 and 300 colonies.

Spreading Plates

1. Mix the first tube in the dilution series using a vortex mixer and aseptically transfer 0.1mL to center of each of two LB/xylose plates.
2. Aseptically place 4-6 sterile glass beads on plate, cover and shake in a motion that spreads the cell suspension evenly over the surface of the agar. Continue this process until all dilutions are plated.
3. Invert the plates and place into the incubator at 37C for 24 hours.

Counting

1. Count the colonies after 24 hours and record the numbers in the appropriate log book.
2. Calculate the colony forming units per milliliter for plates yielding between 30 and 300 colony and record in appropriate log book.