MESOPHILIC AEROBIC BACTERIA (MEMBRANE FILTER METHOD)

PRINCIPLE

Bacteria are quantitated by a membrane filtration technique. Use of the membrane filtration technique allows accurate quantitation of bacteria when low counts are anticipated.

SCOPE

The method is applicable to the sugars and syrups of the corn wet milling industry.

SPECIAL APPARATUS

1. Smooth-tipped, stainless steel forceps

2. 47 mm grid marked, white sterile 0.45 μm membranes and 47 mm absorbent pads (Millipore HAWG 047S0, HAWG S2 or equivalent)

3. Autoclavable 47 mm filtration systems with holder base, funnel assembly and receiver flask (Millipore or equivalent)

4. Vacuum pump capable of 22-27 inches of vacuum

5. Sterile 47 mm plastic petri dishes, tight sealing

MEDIA AND REAGENTS

1. Plate Count Agar (PCA), or Standard Methods Broth if using alternate procedure in Note 1. Prepare and sterilize PCA according to the manufacturer's directions.

2. Ethanol

3. Sodium Hydroxide Solution, 1*N*

4. Butterfield's Phosphate Diluent

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Stock Solution: Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of purified water, adjust to pH 7.2 with about 175 mL of 1*N* NaOH solution and dilute to 1 L volume. Store under refrigeration.

Diluent: Dilute 1.25 mL of stock solution to 1 L volume with purified water. Prepare dilution blanks using this solution.

5. Dilution Blanks: Fill dilution bottles to 50 mL with Butterfield's phosphate diluent. Cap bottles and sterilize at 121°C at 15 pounds of pressure for 15 mins. in a steam autoclave.

6. 47 mm petri dishes plus medium: Prepare 47 mm petri dishes by pouring approximately 2-3 mL of sterile and properly prepared media into each f the needed number of dishes. Allow the media to solidify (Note 1).

PROCEDURE

1. Aseptically weigh 25 g of the sample into a sterile 50 mL phosphate buffer diluent blank and homogenize.

2. Assemble a sterile 47 mm filtration system with a 47 mm Grid marked, white 0.45 μm pore size membrane (Note 2). Connect the receiver flask to the vacuum pump using the vacuum hose.

3. Aseptically pour all of the homogenous sample solution into the filter funnel and cover the funnel top opening. Apply vacuum (22027 psi) and filter the sample solution through the membrane filter. If desired, sterile phosphate buffer can be used for rinsing of the funnel.

4. Using sterile forceps transfer the membrane to a petri dish containing solidified, sterile medium. Roll the filter onto the medium in such a manner to avoid air bubbles forming between the membrane and the medium surface.

5. Invert the petri dish and incubate.

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6. Incubate the petri dish containing the membrane at 35-37°C for 48 hrs. minimum and 72 hrs. maximum. Using a lighted magnifier, count separately all the colonies. The membrane grid can be used as an aid when scanning the filter (low power magnification). The total count of bacteria colonies represent the number of bacteria per 25 g of sample (as is).

CALCULATION

The number of bacteria colonies can be reported either as is or on a dry solids basis:

1. As is basis (per 10g) = $\frac{\text{Total Count}}{2.5}$

2. Dry solids basis (per 10g) = $\frac{\text{As is Count}}{[\frac{\text{Sample D.S.\%}}{100\%}]}$

NOTES AND PRECAUTIONS

1. An alternate method is to prepare Standard Method Broth and disperse 2.2 mL onto an absorbent pad in a 47 mm sterile petri dish. Continue with step 6.

2. The membrane can only be handled with sterile forceps. Sterilize the forceps by keeping the forcep blades in ½" of ethanol and then igniting the alcohol to burn itself out just prior to handling the membrane.

REFERENCE

Standard Method for the examinations of water and waste water (APHA/AWWA) 20th addition 19215A-D 1998