COLIFORM GROUP OF BACTERIA (MEMBRANE FILTER METHOD)

PRINCIPLE

Coliform bacteria can be quantitated by a membrane filtration technique, using a selective differential media followed by identification. Use of the membrane filtration technique allows accurate quantification of coliforms 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 seal

6. Test tubes containing gas collector tubes (Durham tubes)**.**

MEDIA AND REAGENTS

1. Media

   A. M-Endo Broth. Prepare media according to the manufacturer's directions. After heating to a boil, cool the media. Do not autoclave the media, use media within 96 hours of preparation, and store

unused portion in refrigerator. Prepared broth is available commercially.

B. Brilliant green lactose bile broth (BGLB), 2%

2. Ethanol

3. Sodium Hydroxide Solution (NaOH), 1*N*

4. Butterfield's Phosphate Diluent

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 minutes in a steam autoclave.

6. 47 mm petri dishes plus medium: Prepare 47 mm petri dishes by pipeting 2.0 mL of media onto absorbent pad.

PROCEDURE

A. Quantitative Procedure for Total Coliforms

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 1). Connect the receiver flask to the vacuum pump using the vacuum hose.

3. Aseptically pour all of the homogenized sample solution into the filter funnel and then cover the funnel top opening. Apply vacuum (22-27 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 sterile medium. Slide the filter carefully onto the medium in such a manner to avoid air bubbles forming between the membrane and the pad.

5. Invert and incubate the petri dish containing the membrane at 35-37°C for 18-24 hours. Using a lighted magnifier, count all magenta-red colonies with a metallic sheen. Record the results and calculate the number of coliforms per gram of sample. Confirm the colonies, if it is deemed necessary by the qualitative tests described below.

B. Coliform Confirmation

Select two or more typical colonies from the membrane. Innoculate each colony into a separate tube containing 10 mL of BGLB broth plus a gas collector tube. Incubate the tubes at 35-37°C and examine tubes for gas production at 24 and 48 hours. All gassing BGLB tubes are considered positive for the presence of coliforms.

CALCULATION

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

$$\text{As is basis (per g)} = \frac{\text{Total Coliform Count}}{25}$$

$$\text{Percentage confirmed} = \frac{\text{Number of Confirmed Colonies x 100}}{\text{Total Coliform Count}}$$

NOTES AND PRECAUTIONS

1. The membrane can only be handled with sterile forceps. Sterilize the forceps by keeping the forcep blades in 1/2" of ethanol and then igniting the alcohol to burn itself out just prior to handling the membrane.

REFERENCES

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.