MESOPHILIC ANAEROBIC BACTERIA (POUR PLATE METHOD)

PRINCIPLE

Viable anaerobic bacteria are quantitated by the fractional gram pour plate technique under an anaerobic atmosphere. Caution must be exercised when applying the method since isolates may be pathogenic.

SCOPE

The method is applicable to starches, syrups, sugars and most coproducts of the corn wet milling industry.

SPECIAL APPARATUS

Anaerobic jars, BBL GasPak or equivalent, equipped with GasPak hydrogen and CO_2 generator envelopes with an anaerobic indicator.

MEDIA AND REAGENTS

1. Anaerobic Agar (ANA). Prepare according to manufacturer's directions (Note 1).

2. 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.

3. Dilution Blanks. Fill dilution bottles to appropriate volume with Butterfield's Phosphate Diluent (Note 2).

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PROCEDURE

Aseptically weigh 20 g of sample into a sterile 80 mL water blank and homogenize (Note 3). This is the primary dilution and represents a sample dilution factor of 5. Twenty mL of the primary dilution can be aseptically transferred to another 80 mL water blank, and the sample is diluted by a factor of 25. The number of dilutions depends on the individual sample and may be determined by past experience.

Pipet 1.0 mL (Note 4) of each sample dilution into duplicate Petri dishes. Pour 20-25 mL of ANA agar which has been cooled to 45°C into each dish. Swirl plates and allow to solidify. Immediately after solidification invert the plates and place them in an anaerobe jar. Following manufacturer's directions, generate the anaerobic atmosphere.

Incubate the anaerobe jar at 35-37°C for 48-72 hrs. (2-3 days). Count the number of colonies on those plates showing 25-250 colonies (Note 5). Average the count of the duplicate plates, multiply by the dilution factor and record as the number of anaerobic bacteria per gram. If the lowest dilution shows less than 25 colonies, then these colonies must be counted and reported.

CALCULATION

Number of anaerobic bacteria per gram = Average number of anaerobic bacteria x Dilution factor

NOTES AND PRECAUTIONS

1. The anaerobic agar must be freshly prepared each time the procedure is performed.

2. When sterilizing dilution blanks, a portion of the diluent may be lost. If this occurs, the sterilized blanks are brought to the proper volume with the sterile diluent.

3. When running counts on pregelatinized starches, no more than 5 g of sample per 95 mL of diluent may be used.

4. If high counts are anticipated, pipet 0.1 or 0.5 mL instead of 1.0 mL.

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5. Plates containing granular samples should be examined with a stereoscopic microscope to aid in counting small colonies.

REFERENCE

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