MESOPHILIC AEROBIC BACTERIA (PETRIFILM AEROBIC COUNT PLATE METHOD)

PRINCIPLE

Mesophilic aerobic bacteria are quantitated by the selective Petrifilm Aerobic Count Plate method facilitated by the presence of a tetrazolium indicator dye.

SCOPE

The method is applicable to starches, sugars, syrups and most co-products of the corn wet milling industry (Note 1).

REAGENTS

1. 3M Petrifilm Aerobic Count Plates (ACP). Carefully read manufacturer's instructions prior to use.

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 1.0 L volume. Store under refrigeration at 4°C.

   Diluent: Dilute 1.25 mL of stock solution to 1.0 L volume with purified water.

3. Sterile Water Dilution Blanks

   Fill water dilution bottles to 90 mL or desired volume with Butterfield's Phosphate Diluent (Note 2).

PROCEDURE

A. Quantitative Procedure for Mesophilic Aerobic Bacteria

1. Prepare decimal dilutions by aseptically weighing 10 g of sample into 90 mL sterile diluent (Note 3). The sample is further diluted by factor 10 dilution series, if high counts of mesophilic aerobic bacteria are anticipated. Adjust pH of the diluted sample between 6.6 and 7.2. For acid products use 1*N* NaOH, for alkaline products use 1*N* HCI per manufacturer’s instructions.

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2. Place the ACPs on a flat surface. Lift top film and dispense carefully 1 mL of the sample dilution to the center of bottom film. Carefully roll down the top film onto the sample to prevent the entrapment of air bubbles. Distribute sample evenly within the circular well using the plastic spreader. Let plate stand undisturbed for one minute to permit gel hydration and solidification.

3. Incubate the plates in stacks (20 maximum) at 35-37°C in a horizontal position with the clear side up for 48 hrs.

4. Count all the red colonies on the ACP plate and multiply by a dilution factor to quantify the total mesophilic aerobic bacteria.

CALCULATION

Number of aerobic bacteria per g = Average number of aerobic bacteria × Dilution Factor.

NOTES AND PRECAUTIONS

1. Although the Petrifilm method is approved by AOAC for all foods, use of the method for highly osmophilic samples should be carefully evaluated against the conventional method for performance verification. A suggested procedure would be to prewet as follows: Place the plate on a flat surface. Carefully lift the top film and wet the center of the bottom film with 1.0 ml of sterile water. Release the top film onto the bottom film with a "rolling down" motion to prevent air bubbles from forming within the plate. Place the plate spreader on the top film and gently press in a downward motion to evenly distribute the sterile water on the bottom film. Allow the plate to gel for 1-2 hours. To support reliable results for highly osmophilic samples, a corresponding aerobic plate count test should be also run for performance verification.

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

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

REFERENCES

1. Association of Official Analytical Chemistry, www.aoac.org.

2. Aerobic Plate Count in Foods, Dry Rehydratable Film (Petrifilm Aerobic Count Plate) Method (Medical-Surgical Division/3M, 225-55 3M Center, St. Paul, MN 55144, USA), Ref. JAOAC 73, 242 (1990).