MESOPHILIC AEROBIC BACTERIA (STANDARD PLATE COUNT OR TOTAL PLATE COUNT)

PRINCIPLE

Viable bacteria are quantitated by the fractional gram pour plate technique.

SCOPE

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

MEDIA AND REAGENTS

1. Plate Count Agar (PCA). Prepare according to the manufacturer's directions.

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. Water Dilution Blanks. Fill water dilution bottles to 80, 90, or 99 mL with Butterfield's Phosphate Diluent. Cap bottles and sterilize at 121°C, at 15 pounds pressure for 15 mins. in a steam autoclave (Note 1).

4. Sodium Hydroxide Solution, 1*N*.

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PROCEDURE

Two common fractional gram sample dilution techniques may be used for any given sample. The number of dilutions depends on the individual sample and may be determined by past experience.

A. Factor Five Dilution Series (FFS): Aseptically weigh 20 g of the sample into a sterile 80 mL water blank and homogenize. This is the primary 1:5 dilution blank (PDB). Twenty mL of the PDB can be aseptically transferred to another sterile 80 mL water blank, and the sample is diluted by a factor of 25.

B. Factor Ten Dilution Series (FTS): The same as FFS only 10 g of sample and 90 mL sterile water blanks are used. The sample is diluted by a factor of 10, 100, 1000, etc.

Aseptically dilute the sample by either FFS or FTS (Note 3). Pipet aseptically 1 mL of each dilution to appropriately marked duplicate Petri dishes. Pour 15-20 mL of the PCA agar which has been cooled to 45°C into each dish. Swirl plates and allow to solidify.

Invert the plates and incubate at 35-37 °C for 48 ± 3 hrs. Count the number of colonies on those plates showing 25-250 colonies (Note 4). Average the count of the duplicate plates, multiply by the dilution factor and record as the number of bacteria per gram or as Standard Plate Count per gram. If the lowest dilution shows less than 25 colonies, then these colonies must be counted and reported (Note 5).

CALCULATION

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

NOTES AND PRECAUTIONS

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

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with the sterile diluent. This instruction applies to all methods employing dilution bottles.

2. The amount of sample to be tested and the extent of dilution is best determined by each individual laboratory.

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

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

5. The membrane filter technique (I-B) may be used for sugars and syrups if low counts are anticipated.

REFERENCES

AOAC 2002.07.

Compendium of Methods for the Microbiological Examination of Foods, Third Edition, 1992, Chapter 4, (http://www.apha.org/media/science.htm).