MESOPHILIC AEROBIC SPORE-FORMERS POUR PLATE METHOD

PRINCIPLE

Vegetative cells present in the sample are thermally destroyed at 80°C which concurrently activates the mesophilic endospores. The aerobic spores are quantitated by a pour plate technique.

SCOPE

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

SPECIAL APPARATUS

An 80°C water bath of sufficient size to hold 250 mL Erlenmeyer flasks (three per sample).

MEDIA AND REAGENTS

1. Dextrose Tryptone Agar (DTA). Prepare medium according to manufacturer's directions.

2. Tryptone Glucose Extract Agar (TGEA). Prepare medium according to manufacturer's directions.

3. Butterfield's Buffered Phosphate (BBP) 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 at 4°C.

   Diluent: Dilute 1.25 mL of stock solution to 1 L volume with purified water.

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4. Sterile Water Dilution Blanks. Fill water dilution bottles to 80 mL or desired volume with Butterfield's Phosphate Diluent (Note 1).

PROCEDURE

Aseptically weigh 20 g of the sample into a sterile 80 mL water blank and homogenize (Note 2). Pipet 10 mL of the suspension into a flask containing 100 mL of sterile DTA or TGEA at a temperature of 50-60°C (Note 3). After the suspension has been added to the DTA, shake the flask to resuspend the sample. Immediately place the flasks in a preheated 80°C water bath (Note 4). Flasks are held in the water bath with the water level above the sample level in the flasks for 30 mins. Occasionally agitate the flasks gently to assist heat distribution.

After 30 mins., remove the flask and gently agitate by swirling the mixture. Evenly distribute the mixture into a set of 5 sterile plates and allow to solidify.

Incubate the inverted plates at 35-37°C for 48 ± 2 hrs.

CALCULATIONS

Mesophilic spore count is obtained by counting the total of surface and subsurface colonies from all plates. Multiply by 5 to express in terms of spores per 10 g of sample.

NOTES AND PRECAUTIONS

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

2. When testing pregelatinized starch, weigh 5 g of sample per 95 mL of diluent, and pipet 40 mL of homogenized solution into a flask containing 80 mL of DTA.

3. If high counts are anticipated, pipet 1.0 mL instead of 10 mL onto the plate.

4. The flask caps should be loosened when placing the flasks into the 80°C water bath.

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REFERENCES

1. FDA Bacteriological Analytical Manual (BAM) 8th Edition, www.cfsan.fda.gov/~ebam/bam-toc.html.

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