Lab 4 : Enumeration of Microorganisms on Solid Medium

Objective :
Teach the techniques of microbial enumeration in UHT milk using a solid medium, through pour plating (inoculation in mass) and surface spreading.

Materials and Reagents Required

Equipment :

- Sterile Petri dishes
- Sterile pipettes (1 mL and 10 mL)
- Sterile dilution bottles containing 9 mL of physiological saline (0.9% NaCl)
- Sterile spreaders (for surface inoculation)
- Distilled water, bleach, or 70% alcohol for disinfection

Reagents :

- Culture medium : Nutrient Agar (NA) for total flora
- UHT milk sample (opened for 24 hours to maximize contamination likelihood)
- Sterile physiological saline (0.9% NaCl)

Laboratory equipment:

- Incubator at 30°C
- Bunsen burner
- Water bath

Experimental Procedures

I. Preparation of Dilutions

- Aseptically collect 1 mL of UHT milk.
- Transfer it into a tube containing 9 mL of sterile physiological saline.
- Mix vigorously using a vortex or by shaking to obtain the 10^{-1} dilution.
- Repeat until reaching the 10^{-4} dilution.

II. Pour Plate Method (Inoculation in Mass)

1. Prepare sterile Petri dishes.
2. Melt the NA medium in the water bath and allow it to cool to about 45°C (do not exceed this temperature to avoid killing microorganisms).
3. Transfer 1 mL of each dilution into the Petri dishes.
4. Pour the melted NA (about 15–20 mL) into each dish containing the inoculum.
5. Gently mix using figure-eight motions.
6. Allow the medium to solidify at room temperature (10–15 minutes).

III. Surface Inoculation (Spread Plate Method)

1. Pipette 0.1 mL of each dilution.
2. Place this volume at the center of the already solidified Petri dish.
3. Spread the sample using a sterile spatula with gentle, uniform motions.
4. Allow the surface to dry for a few minutes.

IV. Incubation

1. Place the Petri dishes (both pour plates and spread plates) in the incubator at 30°C for 48 hours.
2. Incubate plates upside down to prevent condensation.

Result Analysis

1. After incubation, observe the plates and count visible colonies (between 30 and 300).
2. Calculate the initial concentration of microorganisms in the sample using the formula:

N (CFU/mL) = Mean number of colonies×Dilution / Inoculated volume (mL).

3. the results obtained from the two methods (pour plate and spread plate) and discuss possible differences.