

Lab 3: Direct Enumeration of Microorganisms Using the Light Microscope

Introduction

Direct enumeration of microorganisms using a light microscope is a rapid method that allows observation and quantification of both living and dead cells in a sample. Commonly used in the food industry (milk control, fermented products), environmental microbiology (water quality assessment), and biotechnology (monitoring microbial cultures), it provides a direct and reliable estimation of microbial load.

Objective

To directly count the number of cells in a sample through microscopic observation.

Required Material

- Baker's yeast (*Saccharomyces cerevisiae*)
- Pasteur pipettes or sterile micropipettes
- Beaker
- Spatula
- Filter paper
- Microbiological stain (e.g., methylene blue)
- Light microscope
- Sterile distilled water
- Immersion oil
- Bunsen burner

Method

1. **Preparation of the yeast suspension:**
 - Dissolve a small amount of baker's yeast in warm sterile water and mix until a homogeneous suspension is obtained.
 - Allow the suspension to rest for a few minutes to activate the cells.
2. **Preparation of the slide:**
 - Mark a 1 cm² area on the microscope slide.
 - Place a small drop (10 µl) of the yeast suspension onto a clean microscope slide.
 - Spread the drop evenly to form a thin layer on the slide.
3. **Fixing the sample:**
 - Fix the preparation by quickly passing the slide through the flame of a Bunsen burner (3–4 quick passes).
4. **Staining:**
 - Apply a few drops of stain (e.g., methylene blue) to the preparation and allow it to act for 1–2 minutes.
 - Rinse the slide gently with distilled water and let it dry.
5. **Microscopic observation:**
 - Place the slide on the microscope stage.
 - Observe the sample at an appropriate magnification (40x).
6. **Cell counting:**
 - Select several fields of view on the slide by slightly moving the stage to observe different areas.
 - Count the number of cells in each field. Carefully note the number of cells observed

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for each selected field.

- Repeat the operation for 5 fields to obtain a representative average.

Calculation of cell concentration:

- Estimate the total cell concentration using the following formula:

Cell concentration = average number of cells per field × field area / volume of sample on the slide

The cell concentration is expressed as: **cells/cm²/μl**.