CHAPTER 4 : Microorganism Enumeration Techniques

I. Introduction

Microbial enumeration is a fundamental step in microbiology, used in food, pharmaceutical, environmental, and clinical laboratories.
It allows estimating the number of microorganisms in a sample and evaluating sanitary quality, regulatory compliance, and the effectiveness of preservation or disinfection processes.

1. Objectives of microbial enumeration

1.1. Determine the total or viable concentration of microorganisms in various samples:

- Food samples (milk, meat, canned food…)
- Water (drinking water, swimming pool water, wastewater)
- Surfaces (worktops, equipment, workers' hands)

1.2. Evaluate sanitary quality:

- Identify contaminations
- Verify compliance with microbiological standards
- Validate cleaning/disinfection procedures

II. Classification of Enumeration Techniques

Microbial enumeration techniques are grouped into two major categories based on how cells are detected:

Direct Methods

Based on observing or measuring cells directly without culturing.

- Microscopic counting
- Counting chambers (Thoma/Neubauer)
- Filtration + direct microscopic count
- Electronic counters (Coulter counter)

Indirect Methods

Based on detecting the growth, metabolism, or activity of viable microorganisms.

- Plate Count Method (CFU)
- Most Probable Number (MPN)
- Turbidity measurement

This classification forms the basis of all enumeration protocols and allows selecting the appropriate method according to the sample type and purpose.

1. Direct Methods

a. Microscopic Counting (Thoma/Neubauer Chamber)

Principle

A special counting chamber with a precisely known depth (0.1 mm) and grid area is used to count cells in a fixed volume.
Each counted square corresponds to a known volume, allowing calculation of cell concentration.

Steps

1. Prepare homogeneous suspension
2. Place a drop on the counting chamber
3. Lower the cover slip to obtain the fixed height
4. Observe under the microscope (usually 40× objective)
5. Count cells in several small squares (e.g., 5 big squares)
6. Apply the standard formula

Advantages

- Rapid and does not require incubation
- Suitable for dense cultures (e.g., fermenters)
- Can distinguish morphology (shape, size)

Limitations

- Counts both live and dead cells
- Requires a skilled operator
- Not suitable for motile bacteria unless immobilized
- Time-consuming for dilute samples

Example

Direct microscopic counting of somatic cells in raw milk

2. Indirect Methods

Indirect methods estimate microbial load based on statistical probability, observable growth, or microbial activity, rather than counting cells directly.

a. Plate Count Method (CFU)

Principle

1 living cell = 1 visible colony (CFU) after incubation on a solid medium.
Only viable cells capable of dividing form colonies.

Steps

1. Serial decimal dilutions
2. Inoculation on agar (pour plate / spread plate)
3. Incubation
4. Counting colonies (30–300 CFU)
5. Calculate CFU/mL or CFU/g

Advantages

- Quantitative and accurate
- Detects only viable cells
- Widely applicable to food, water, surfaces

Limitations

- Time-consuming (24–72h)
- Underestimates cells in clumps

Example: Total flora count in yogurt

1. Homogenize yogurt sample
2. Prepare serial dilutions
3. Spread 1 mL or 0.1 mL on Plate Count Agar (PCA)
4. Incubate at 30°C for 48–72 h
5. Count colonies and calculate total microbial load

b. Most Probable Number (MPN / NPP)

Principle

Statistical method based on detecting the presence or absence of microbial growth in a set of tubes with serial dilutions.
It assumes that microorganisms are randomly distributed in the sample.
The pattern of positive/negative tubes is compared to a standard MPN table to estimate cell concentration.

Steps

1. Prepare serial dilutions
2. Inoculate 3 or 5 tubes per dilution
3. Incubate

4. Observe growth (turbidity, gas, color change)
5. Compare the pattern to MPN table

Advantages

- Useful for samples with low microbial load
- Suitable for turbid or particulate samples

Limitations

- Less precise than plate count
- Results take longer

Example: Enumeration of coliforms in wastewater

1. Prepare dilutions: 10^0, 10^{-1}, 10^{-2}
2. Inoculate 5 tubes per dilution with lactose broth + Durham tube
3. Incubate at 35–37°C for 24–48 h
4. Positive tubes show gas + turbidity
5. Using the combination (e.g., 5–3–1), estimate MPN from tables

Conclusion

Microbial enumeration is an essential tool in microbiology for assessing the quantity and viability of microorganisms in various samples. Both direct and indirect methods have specific advantages and limitations, and the choice of method depends on the type of sample, the required accuracy, and the intended application. Understanding these techniques allows microbiologists to monitor food safety, water quality, clinical diagnostics, and environmental hygiene effectively. Mastery of these methods is crucial for ensuring public health and maintaining high standards in laboratory practice.

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