

# Some Industrially Important Microbes and Their Products

## 2.1. ENZYME PRODUCING MICROBES

Type of enzyme	Substrate	Microorganism
Amylase	Starch	<i>Saccharomyces diastaticus</i>
Protease	Proteins	<i>Bacillus</i> sp.
Lipase	Lipids	<i>Aspergillus</i> sp.
Pectinase	Pectin	<i>Aspergillus niger</i> , <i>Penicillium</i> sp.
Cellulase	Cellulose	<i>Cellulomonas</i> sp.
Xylanase	Xylan	<i>Streptomyces</i> sp.
Chitinase	Chitin	<i>Actinomyces</i> , <i>Streptomyces</i> sp.
Sucrase	Sucrose	<i>Streptomyces</i> sp.
Phosphatase	Tricalcium phosphate	<i>Phosphobacterium</i> , <i>Pseudomonas</i> sp.

## 2.2. ORGANIC ACID PRODUCING MICROBES

Type of organic acid	Substrate	Microorganism
Acetic acid	Sugarcane juice	<i>Acetobacter</i> sp.
Citric acid	Beet molasses	<i>Aspergillus niger</i>
Citric acid	Alkane	<i>Saccharomyces cerevisiae</i>
Gluconic acid	Glucose	<i>Aspergillus niger</i>
Lactic acid	Whey	<i>Lactobacillus delbrueckii</i>
Lactic acid	Glucose	<i>Lactobacillus</i> sp.

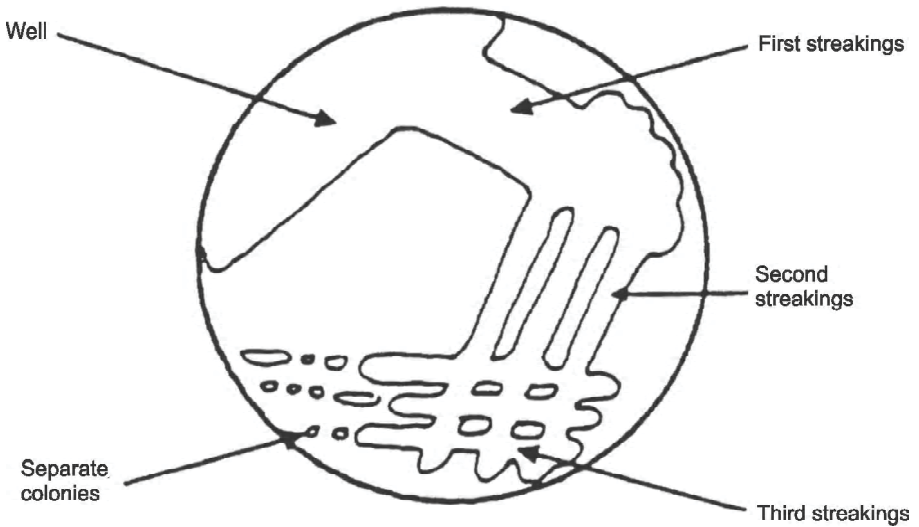


Fig. 1. Streak plate method.

the environment or an infected person. It is therefore necessary to separate microorganisms. Plating or streaking can be used to achieve this. Using sterile inoculation needle, isolate the single colony as inocula further, streak plates, pure cultures can be obtained.

Streaking out a mixed broth culture on an agar plate and incubating it to obtain single colonies of different types of bacteria or yeasts can simulate this.

### 3.3. SERIAL DILUTION

1. Label the test tubes or bottles with the appropriate dilution,  $10^{-1}$  to  $10^{-7}$ .
2. Make sure that the lid of the tube is firmly attached and then shake the culture (soil or water sample) vigorously to separate clumps of cells and to distribute the organisms evenly throughout the liquid.
3. Using aseptic technique (i.e. flaming the neck of the tube or bottle after removal and before replacement of its lid), remove exactly 1 ml of the fluid and transfer to the dilution tube next in the series.
4. Mix the tubes well.
5. Repeat steps 3 and 4 until the last dilution tube is reached.

This process dilutes the organisms in the original sample to a countable number.

### 3.4. VIABLE COUNT: POUR PLATE METHOD

1. Label the bottom of the plates with name, date, sample and dilution. For maximum reliability and precision, each dilution should be plated in triplicate and the average of the three counts used.
2. Use 0.1 ml sterile syringe or pipette tip and start with the highest dilution (i.e.,  $10^{-7}$ ).
3. Using aseptic technique (i.e. flaming the neck of the tube or bottle after removal and before replacement of its lid), remove exactly 0.1 ml of the sample and transfer to the base of a sterile Petri dish.
4. Using aseptic technique, pour 20 ml sterile nutrient agar over the sample and mix gently.
5. Repeat steps 2 to 4 for dilutions  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$ .
6. When the plates are solidified and dry, incubate upside down at the appropriate temperature for appropriate time.
7. After incubation, select plates for counting that contain 30 - 300 colonies (samples which contain  $<30$  colonies/0.1 ml diluent are subject to large fluctuations in numbers or sampling errors, plates which contain  $>300$  colonies are likely to have overlapping colonies).
8. Count accurately and record the number of colonies on each plate.

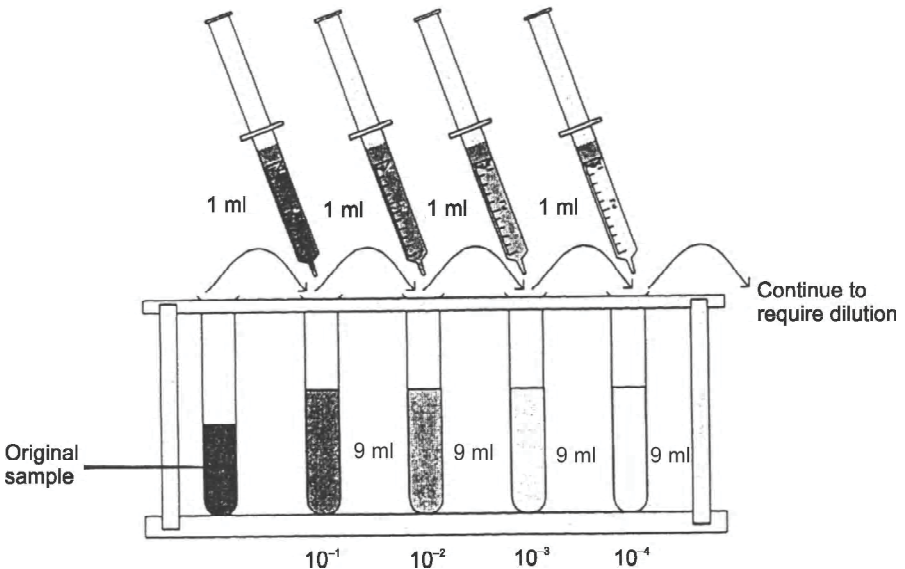


Fig. 2. A representative serial dilution method,

9. Calculate the concentration of viable cells or colony forming units (cfu) in the original suspension using the following formula:

Number of viable cells = dilution factor  $\times$  cfu/ml.

### 3.5. VIABLE COUNT: SPREAD PLATE METHOD

1. Label the bottom of the plates with name, date, sample and dilution. For maximum reliability and precision, each dilution should be plated in triplicate and the average of the three counts used.
2. Use a sterile 0.1 ml syringe or pipette tip. Start with the highest dilution (i.e.  $10^{-7}$ ).
3. Prepare sterile nutrient agar plates.
4. Using aseptic technique (i.e. flaming the neck of the tube or bottle after removal and before replacement of its lid), remove exactly 0.1 ml of the sample and transfer to the surface of an appropriately labelled sterile nutrient agar plate.
5. Using aseptic technique spread the sample evenly across the plate with the glass spreader.
6. Repeat steps 4 and 5 for dilutions  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$ .
7. When the plates are dry, incubate upside down at the appropriate temperature for the appropriate time.
8. After incubation, select plates for counting that contain 30 - 300 colonies (samples which contain  $<30$  colonies/0.1 ml diluent are subject to large fluctuations in numbers or sampling errors, plates which contain  $>300$  colonies are likely to have overlapping colonies).
9. Count accurately and record the number of colonies on each plate.
10. Calculate the concentration of viable cells or colony forming units (cfu) in the original suspension using the following formula:

Number of viable cells = dilution factor  $\times$  cfu/ml.

### 3.6. STORAGE OF ORGANISMS

It is not advisable to maintain bacteria and fungi for long periods, as they become contaminated. Organisms should be sub-cultured and checked for purity every three months or so but only if aseptic technique can be guaranteed.

Cultures, other than those requiring light for their growth and survival, are best stored in the dark at  $10-15^{\circ}\text{C}$ . If it is not possible to achieve a constant, cool temperature, a refrigerator may be used exclusively for this purpose.

growth is limited by one of the three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, called a lack of "biological space".

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce secondary metabolites, such as antibiotics, do so during the stationary phase of the growth cycle (secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria induce or unmask the activity of dozens of genes that may be involved in sporulation process.

- Death phase : Eventually they show loss of viability.

If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines (note: if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Growth is expressed as change in the number viable cells (y-axis) vs time (x-axis). Generation times are calculated during the exponential phase of growth. Time measurements are in hours for bacteria with short generation times.

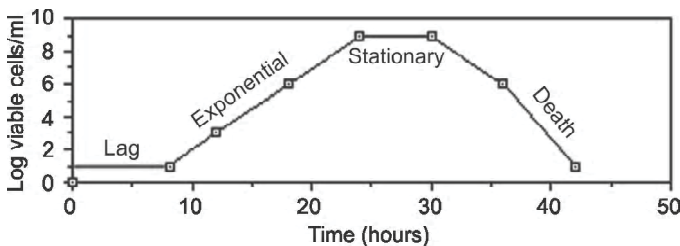


Fig. 3. A representative bacterial growth curve.

## Growth Rate and Generation Time

Bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time

for *E. coli* in the laboratory is 15-20 minutes, but in the intestinal tract, the coliform's generation time is estimated to be 12-24 hours. For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour. Symbionts such as *Rhizobium* tend to have longer generation times. Many lithotrophs, such as the nitrifying bacteria, also have long generation times. Some bacteria that are pathogens, such as *Mycobacterium tuberculosis* and *Treponema pallidum*, have long generation times, and this is thought to be an advantage in their virulence.

#### Generation times for some common bacteria under optimal conditions of growth

Bacterium	Medium	Generation Time (minutes)
<i>Escherichia coli</i>	Glucose-salts	17
<i>Bacillus megaterium</i>	Sucrose-salts	25
<i>Streptococcus lactis</i>	Milk	26
<i>Streptococcus lactis</i>	Lactose broth	48
<i>Staphylococcus aureus</i>	Heart infusion broth	27-30
<i>Lactobacillus acidophilus</i>	Milk	66-87
<i>Rhizobium japonicum</i>	Mannitol-salts-yeast extract	344-461
<i>Mycobacterium tuberculosis</i>	Synthetic	792-932
<i>Treponema pallidum</i>	Rabbit testes	1980

### Calculation of Generation Time

The generation time is the time interval required for the cells (or population) to divide. When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression (If we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on.)

Generation time can be calculated using the formula

$$G = t/n$$

where,

G = generation time (time for the cells to divide)

t = time interval in hours or minutes

n = number of generations (number of times the cell population doubles during the time interval)

'n' can be calculated using the formula

$$n = \frac{\log b - \log B}{\log 2}$$

### 3.8. MEASUREMENT OF CELL BIOMASS

#### Introduction

The cell density can be quantified in two basic ways as follows:

1. Quantitatively expressed as grams of dry or wet weight per litre of sample, or
2. Qualitatively as number of viable/dead cells per ml.

The cells in a sample can be separated from the broth and weighed while they are wet, or the cells may be thoroughly dried before weighing. The dry weight measurement usually gives a much more consistent result than the wet weight. For viable counts, the number of cells can be counted either by successively diluting the original sample and plating on a Petri dish, with the help of a microscope and a counting chamber, or with an automated cell counter such as a Coulter counter or a cytoflowmeter. While, the plating method detects only the viable cells, the automated cell counters can detect the total number of cells.

In reality, the most often used method simply monitors the optical density of the sample. The absorbance of the sample measured in a spectrophotometer is correlated to either the dry weight or the number of cells per volume.

Biomass concentration is one of the most critically needed measurements in microbial growth studies. It is also one of the most difficult and unreliable parameters of study. For example, all the dry/wet weight methods and all the automated counting equipment fail completely if the broth contains other insoluble particulate matter. Similarly, the optical density measurement only has limited usefulness if the broth is not clear. In addition, these methods cannot distinguish the viable cells from the dead ones. On the other hand, the standard plate count can detect viable cells among other particulate matters. However, the method requires elaborate preparations, and it takes 24-48 hours for the cells to be incubated and counted. Consequently, the direct plate count is useless in feedback control of a microbial growth process; it is mainly used industrially to countercheck other measurements, especially the optical density.

In this experiment, the cell density of a given sample is measured with the following five methods: wet weight, dry weight, optical density, direct cell counting with a chamber and successive dilutions followed by plating.