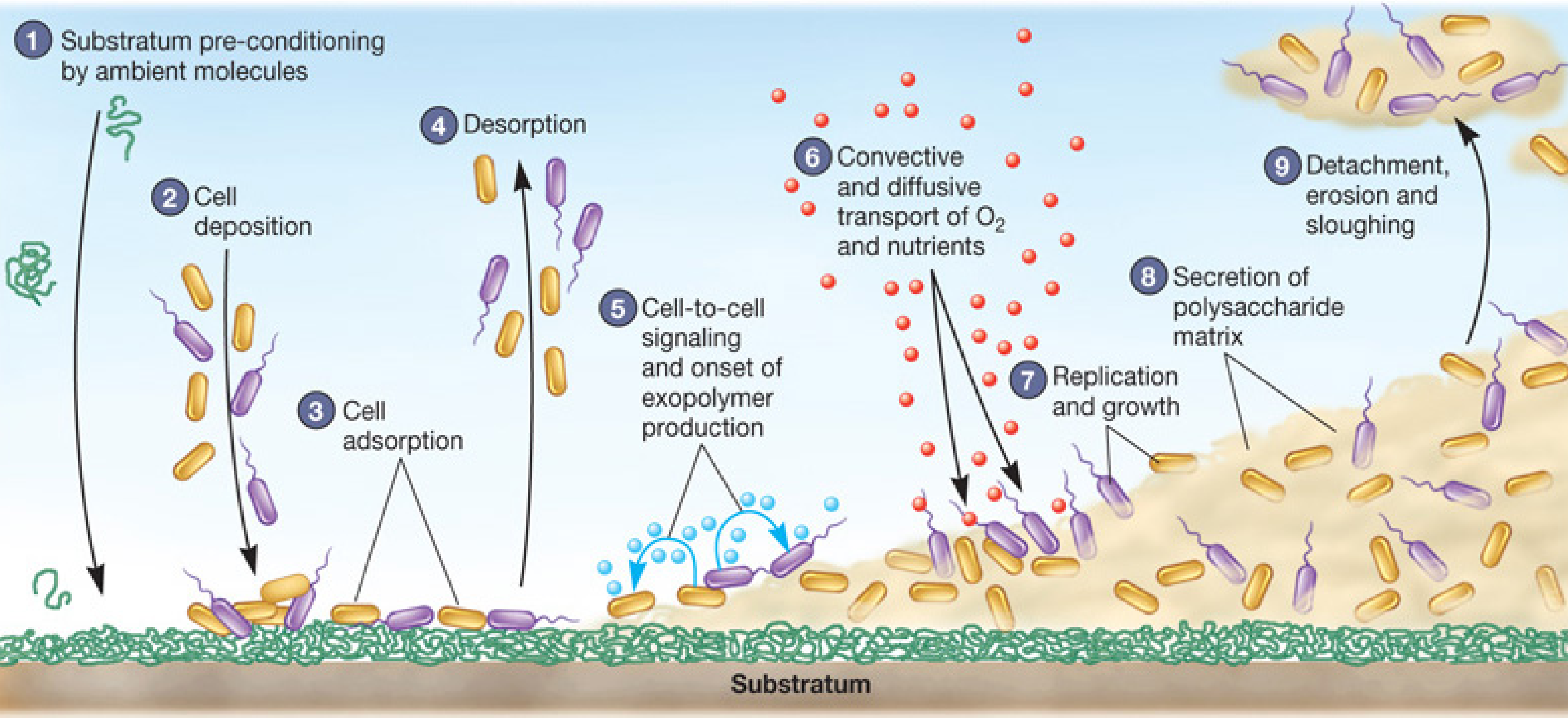
Microbial Nutrition and Growth

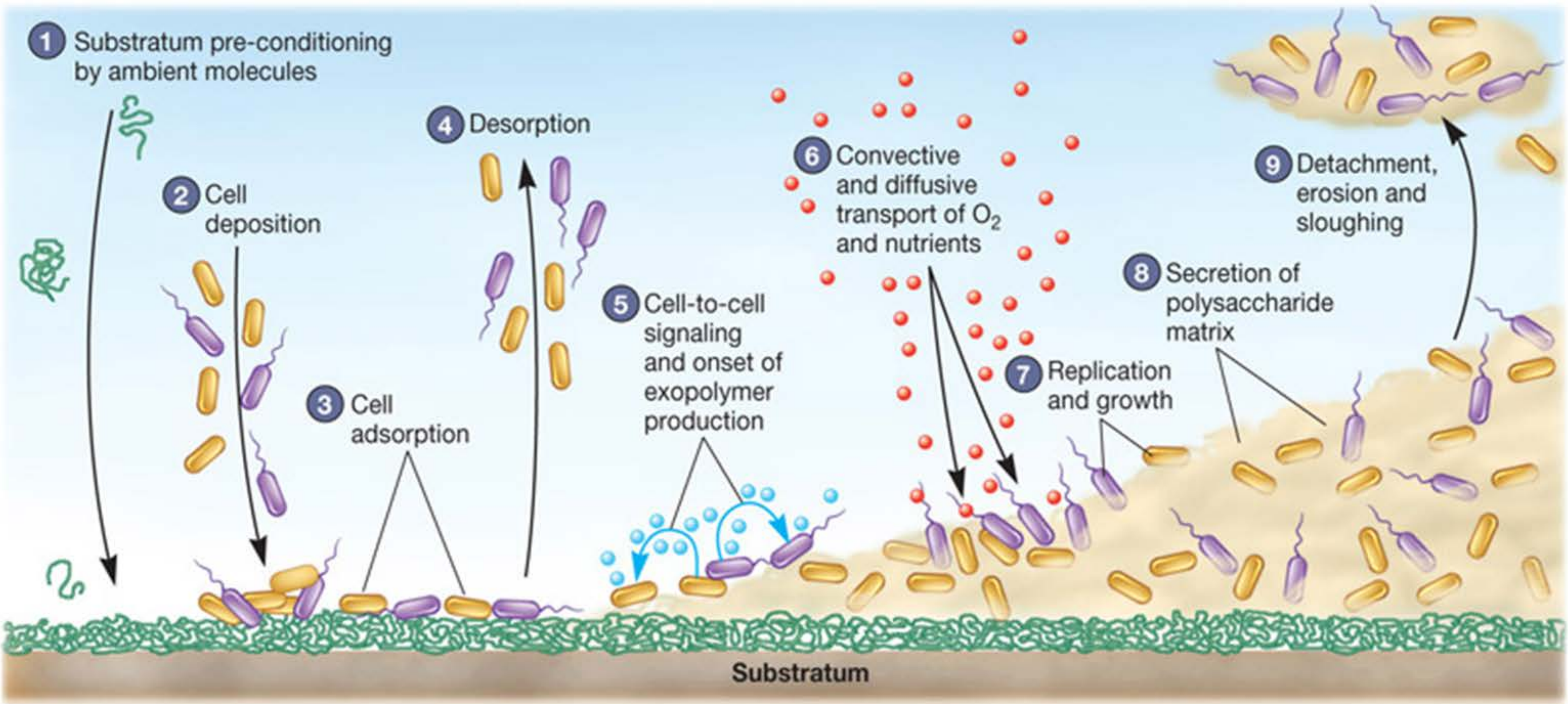


- Knowledge on the requirements of microorganisms regarding **nutritional factors** (carbohydrates, amino acids, vitamins, etc.) and **external factors** (pH, temperature, a_w, O_2, etc.) allows us predict which kinds of microorganisms we can expect in various environments, in other words, which microbiological problems can be expected.

- Predictive microbiology

Microbial Nutrition and Growth

- All forms of life are controlled by **survival** and **propagation**.
- Microorganisms are found in **all** conceivable environments – where there is water, there are microorganisms.

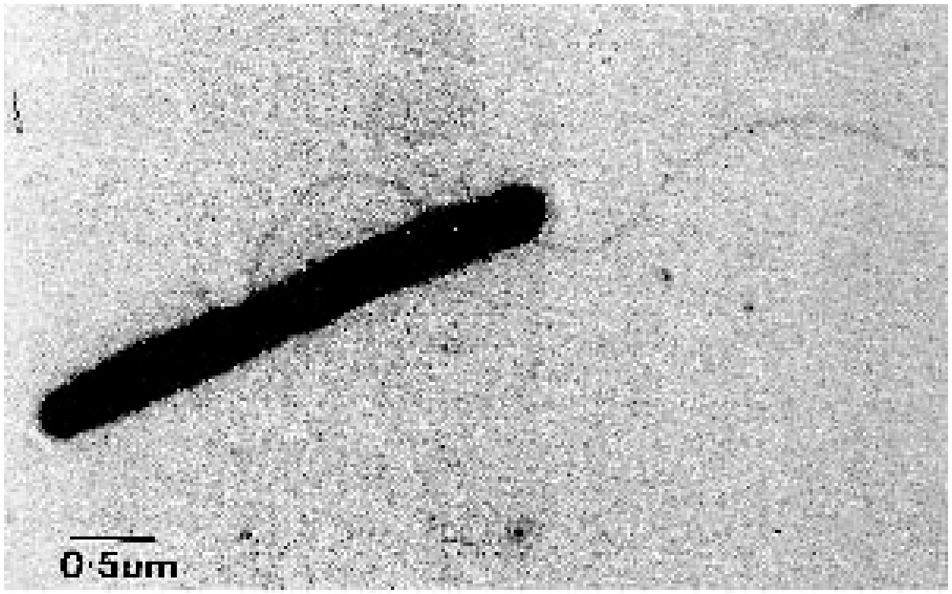


$$pH = -\log a_H \quad \text{or} \quad -\log [H^+]$$

pH = the hydrogen ion activity or the hydrogen ion concentration

Physiological Importance

- Intracellular pH = 7
- < pH 5 leads to cell death due to the destruction of proteins and membranes



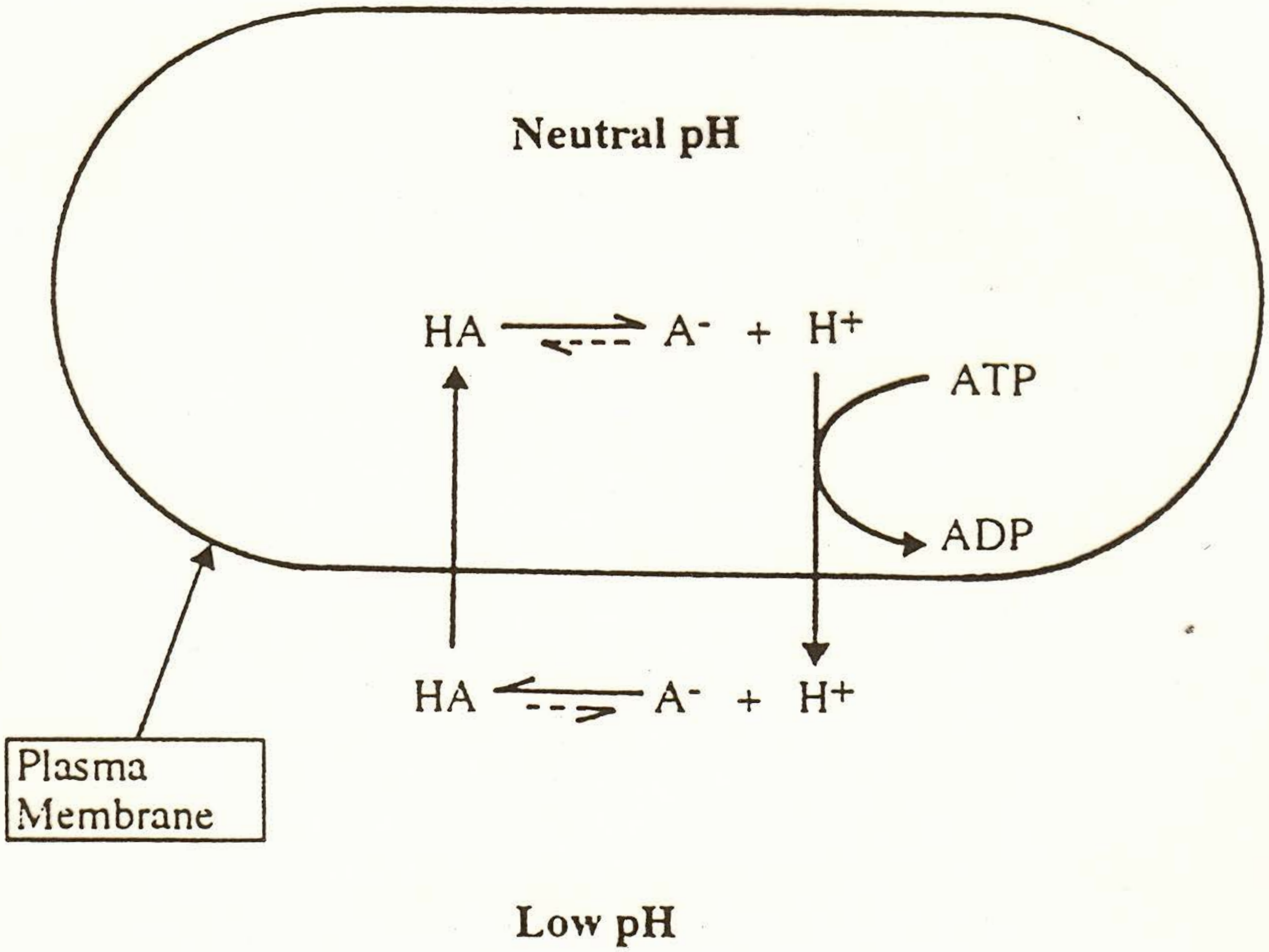
Types

- Acidophiles, pH < 5.5
- Neutrophiles, pH 5.5-8.0
- Alkalophiles, pH > 8.0

Adaptation

- Active transport of protons
- Buffered cell systems
- Chaperones

Weak organic acids



* Microbial inhibition by weak organic acids

* Henderson-Hasselbach

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

$$(HA \leftrightarrow H^+ + A^-)$$

Oxygen – O_2

Physiological importance

- Vital for organisms dependent on oxygen as an external electron acceptor (aerobic respiration)
- Oxygen forms very reactive free radicals (superoxides, peroxides, hydroxyl ions and hydrogen peroxide) that destroy cell components

Types

- Obligate aerobic
- Facultative aerobic
- Aerotolerant anaerobic
- Strictly anaerobic
- Microaerophilic

Adaptation

- In the presence of oxygen, enzymes are required to deal with oxygen radicals, e.g. superoxide dismutase, catalase and peroxidase

Free radicals

- Oxygen can be extremely toxic due to the formation of superoxide (O_2^-), peroxide O_2^{2-}) and hydroxyl ions (OH^-). These free radicals oxidise cell components.

- Oxygen-tolerant organisms convert these toxic substances to oxygen (O_2) and water (H_2O).

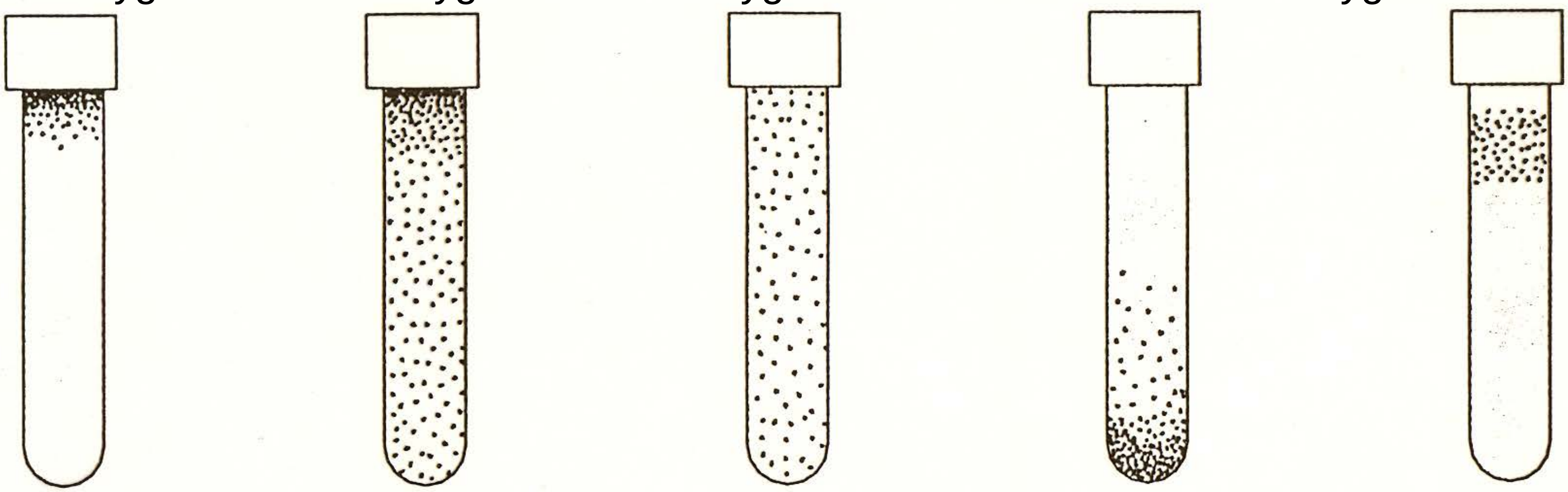
$$2\ O_2^- + 2\ H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{peroxidase}} 2\ H_2O + NAD$$

Oxygen Concentration

need oxygen	prefer oxygen	ignore oxygen	oxygen is toxic	< 2 – 10% oxygen



Obligate aerobe	Facultative anaerobe	Aerotolerant anaerobe	Strict anaerobe	Microaerophile

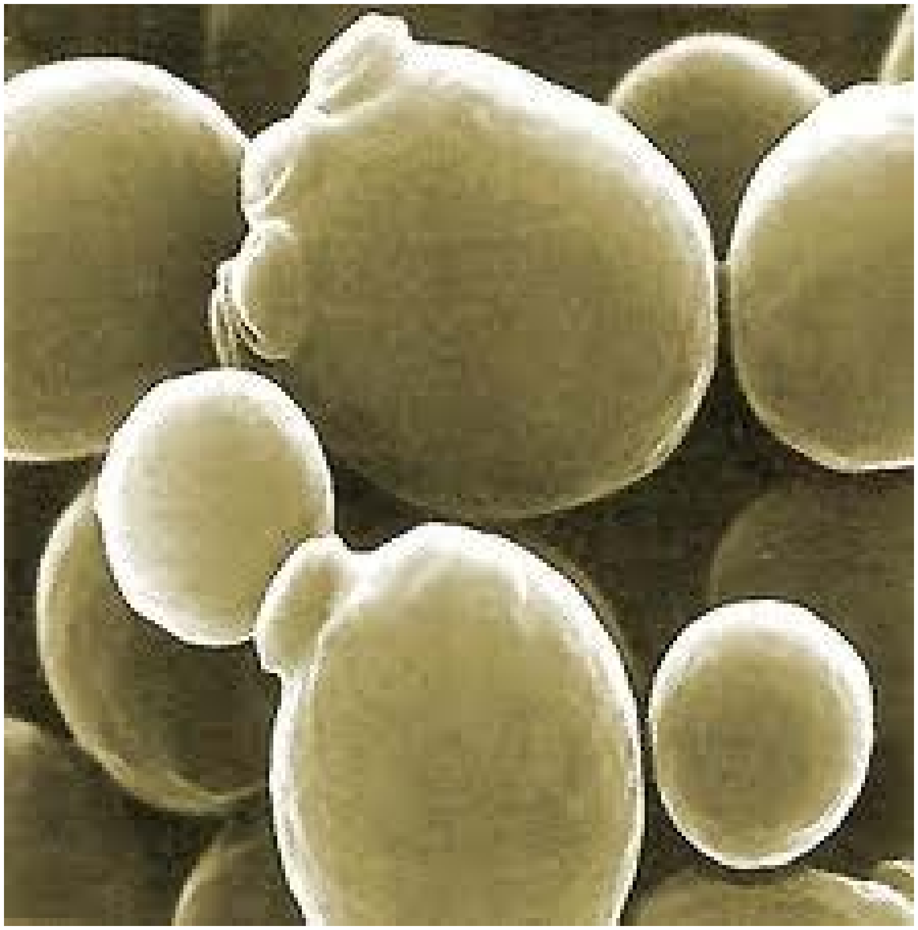
Enzyme content

+ SOD + Catalase	+ SOD + Catalase	+ SOD – Catalase	– SOD – Catalase	+ SOD +/– Catalase (low levels)

Saccharomyces cerevisiae

Examples

- Ethanol production from yeast takes place only in the absence of oxygen (fermentation). In the presence of oxygen large amounts of carbon dioxide are produced (respiration), but not ethanol. Used in the production of beer and wine and bread-making.



Water Activity, $a_w = P_{soln} / P_{water}$

Physiological importance

- Free water molecules are needed for metabolism (chemical reactions in the cell)
- Maintenance of turgor

Types

- Osmo-tolerant organisms
- Halophiles

Adaptation

- Hypotonic solutions – cell wall, inclusion bodies, pressure-sensitive channels
- Hypertonic solutions – compatible solutions (bacteria often amino acids and fungi usually sugar molecules)

Food Preservation

Examples

- Food preservatives with sugar, salt and drying
- Halobacterium, an archaea that grows in a saturated salt solution

Table 3.9 *Minimum water activities at which active growth can occur*

Group of micro-organism	*Minimum a_w*
Most Gram-negative bacteria	0.97
Most Gram-positive bacteria	0.90
Most yeasts	0.88
Most filamentous fungi	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.61

Temperature

Physiological importance

- Mobility/flexibility of the plasma membrane
- Chemical reactions: the rate of enzyme-catalysed reactions doubles for each 10°C increase in temp. (increased rate of growth)
- High temperatures denature enzymes & proteins and melt plasma membranes
- At low temperatures metabolism ceases

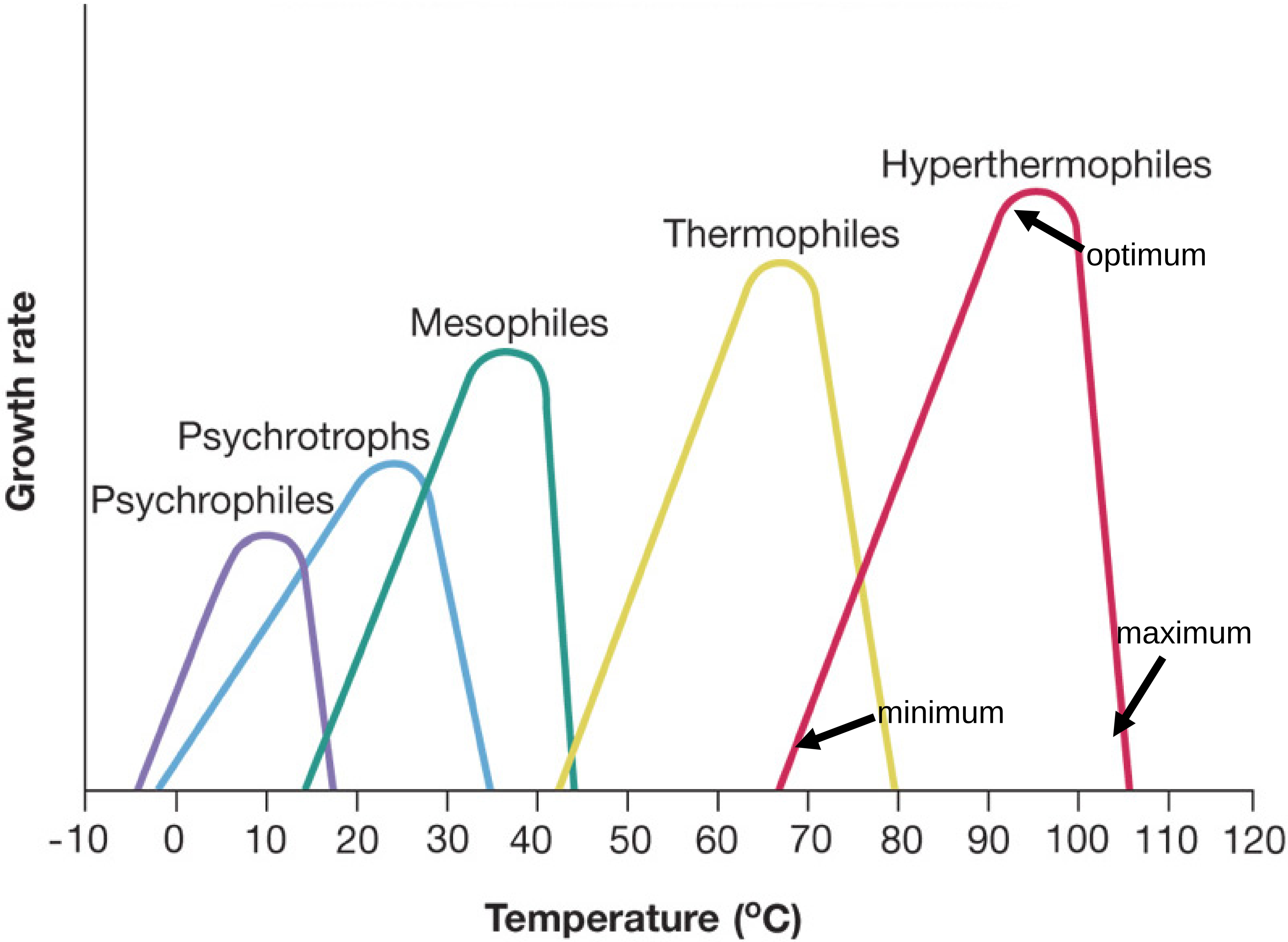
Adaptation

- Fatty acids in the plasma membrane (saturated, unsaturated, branched fatty acids)

Types

- Cryophilic < 0□C
- Psychrophilic 0-20□C
- Psychrotrophic 0-35□C
- Mesophilic 20-45□C
- Thermophilic 40-80□C
- Hyperthermophilic 80-110□C

Microorganisms exhibit distinct cardinal growth temperatures (minimal, maximal, optimal)



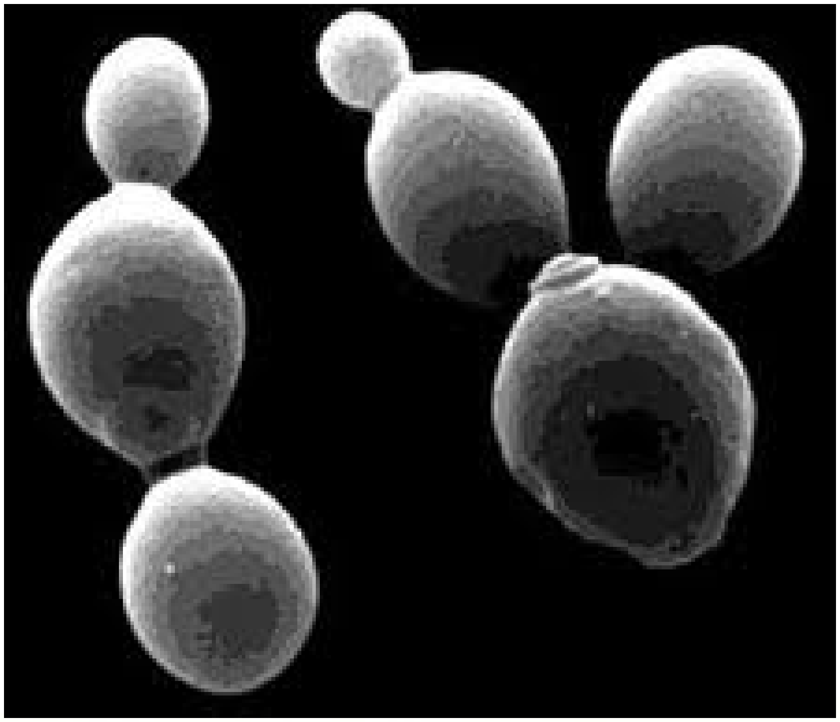
Bioreactors

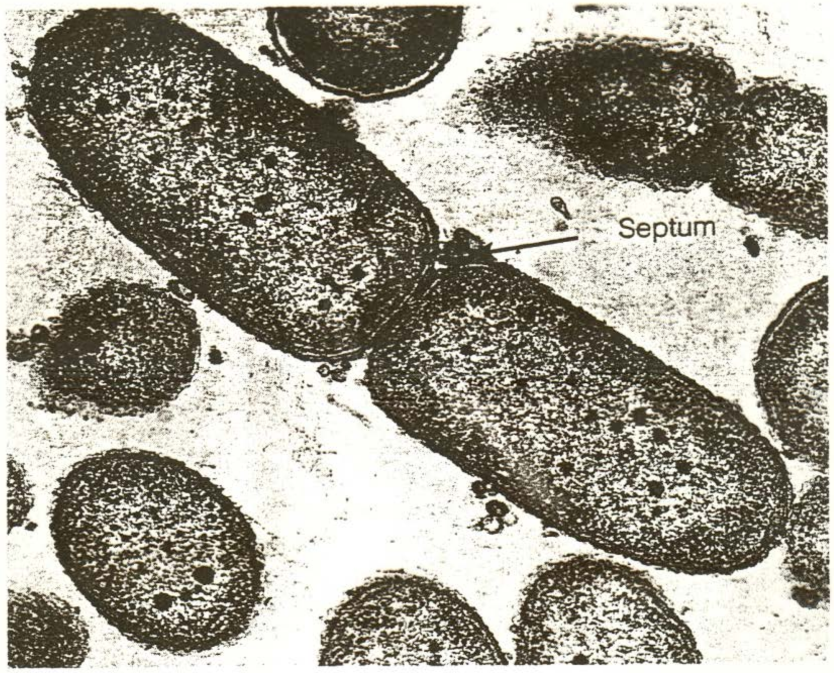
Examples

- The industrial/commercil use of enzymes that can withstand harsh/extreme? environments

Microbial growth

- Budding, e.g. of yeasts
- Binary fission of bacteria
- Instead of measuring the length/weight it is more practical to count the increase in the number of cells





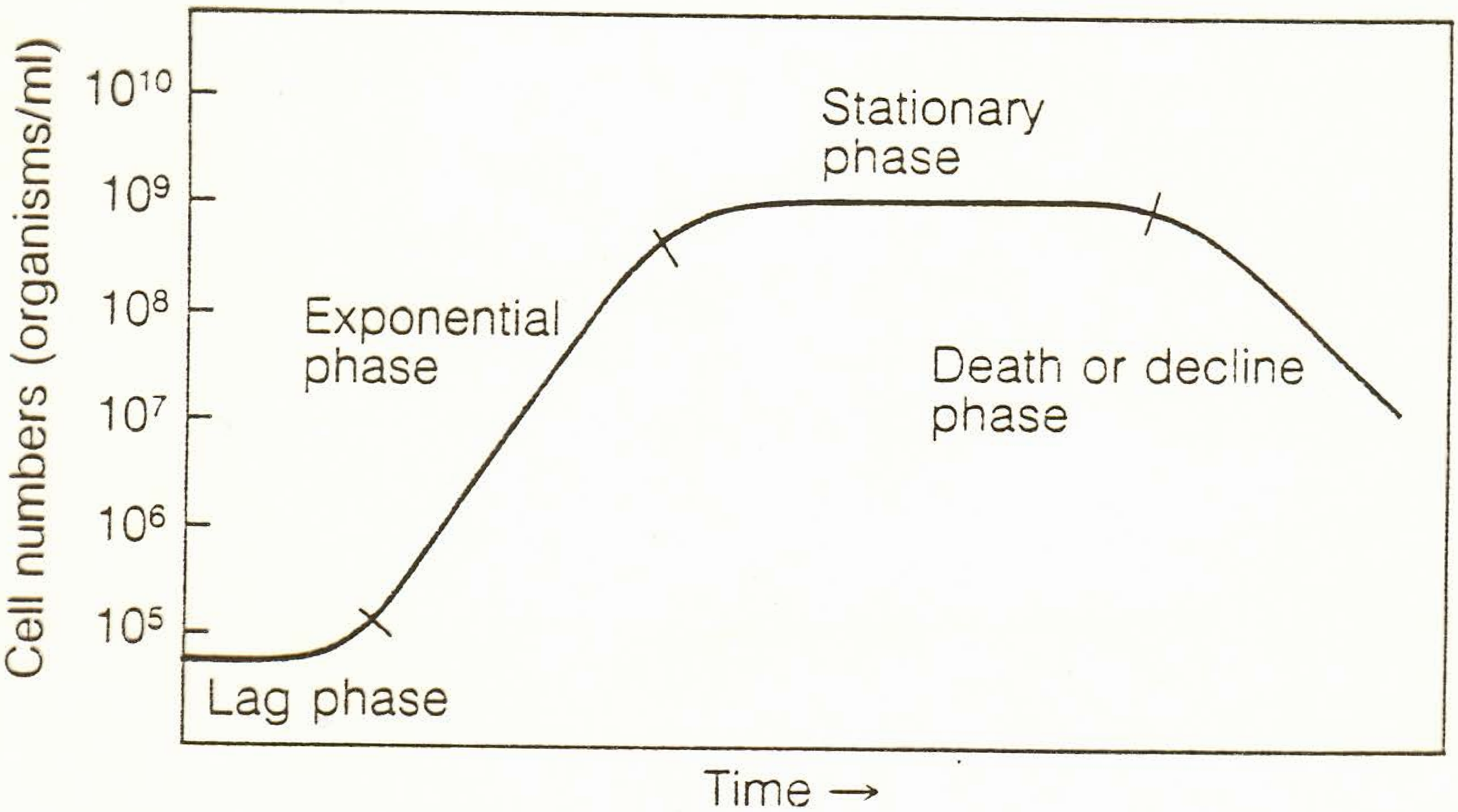


Figure 5–13 Population Growth Curve

A population growth curve consists of four distinct growth phases: lag phase, exponential phase, stationary phase, and death or decline phase. Each growth phase reflects changes in the environment and metabolism of the cells.

Microbial analysis

- Cell growth can be measured using counting chambers, viable counts, MPN and OD measurements

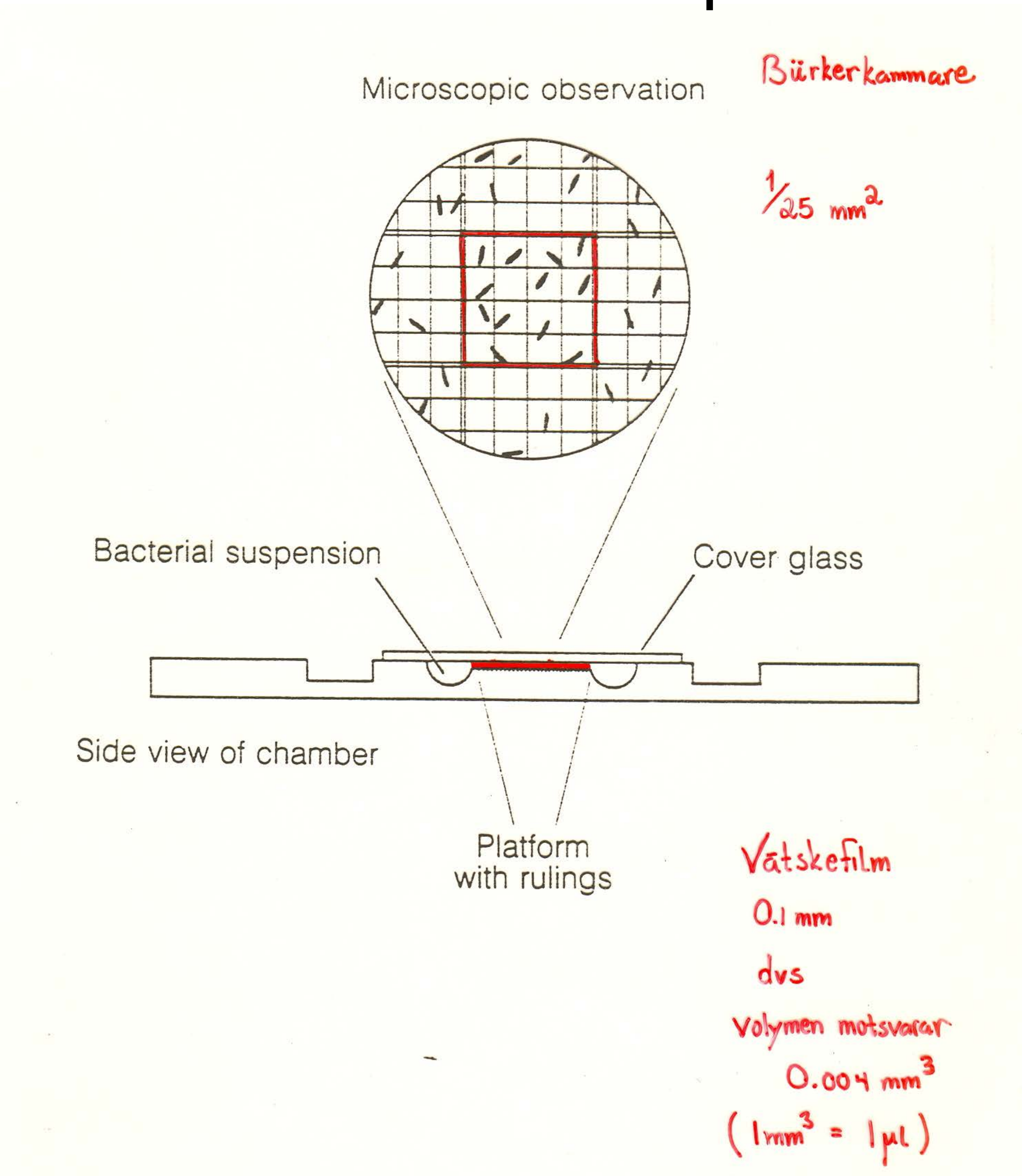
Criteria

- Sensitivity (level of detection)
- Specificity (selectivity)
- Reproducibility
- Ease of use (automation)
- Time
- Acceptance
- Cost
- Quantitative/qualitative analysis

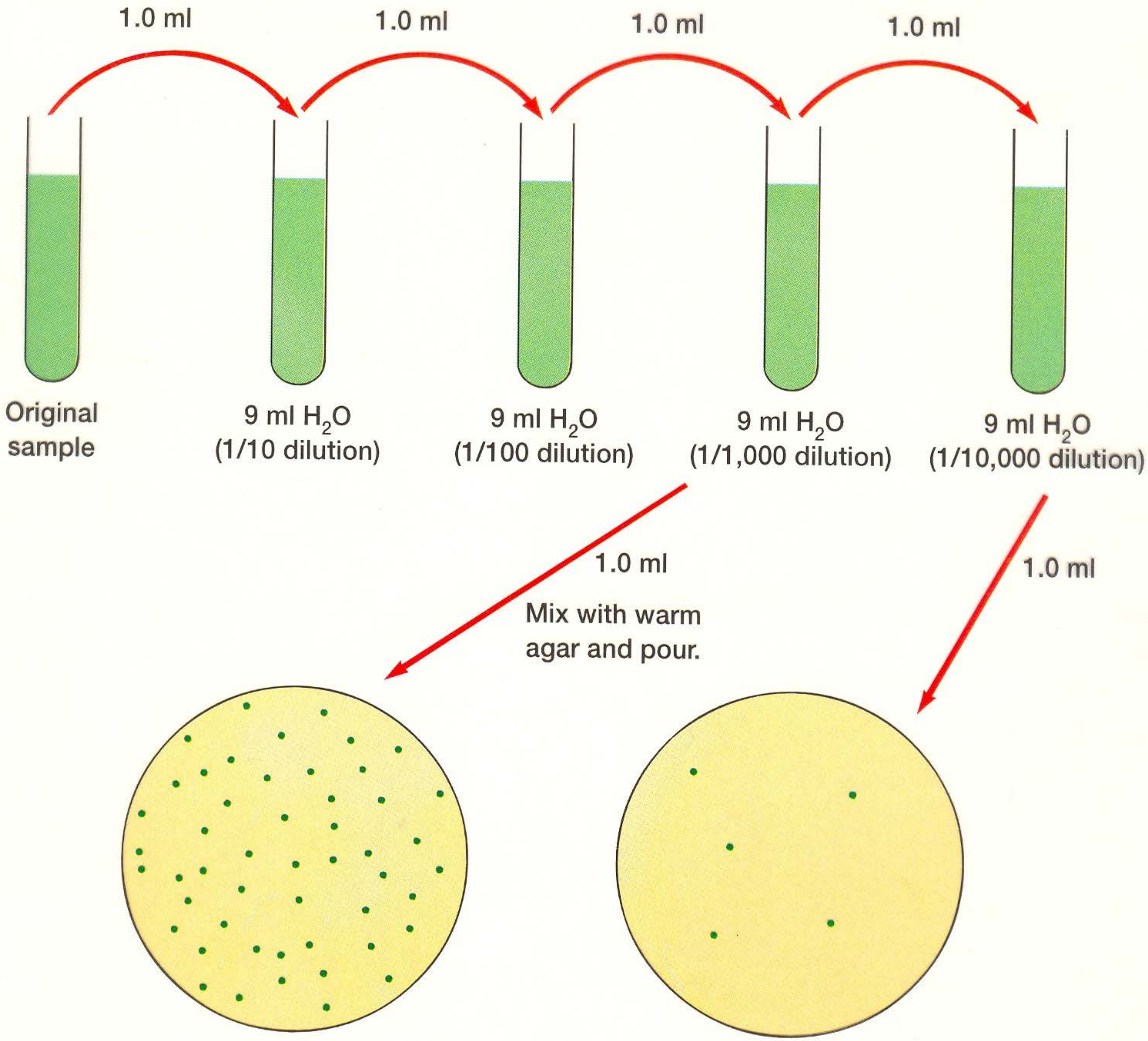
Table 5–2 Summary of Methods for Measurement of Microbial Growth

METHOD	USE	LIMITATIONS
Direct microscopic count	Rapid laboratory enumeration of cell suspension	Requires large number ($\geq 10^6$ cells/ml) of cells for accuracy
Viable count	Enumeration of viable cells in water, milk, and other products	Time-consuming, requires proper medium for growth
Membrance filtration	Enumeration of bacteria from water, milk, and other products, especially when numbers are low	Time-consuming, requires proper medium for growth
Most probable number (MPN)	Enumeration of bacteria from water, milk, and other products	Time-consuming, requires proper medium for growth, provides indirect estimate of numbers
Turbidimetric measurement	Rapid estimation of cell density in a suspension	Does not differentiate between viable and nonviable cells, provides only estimate of cell density, requires $> 10^6$ cells/ml
Dry weight determination	Determination of cell mass for industrial or laboratory applications	Time-consuming, does not differentiate between viable and nonviable cells
Cell activity measurement	Research applications to follow cell metabolism	Involves extensive preparatory time

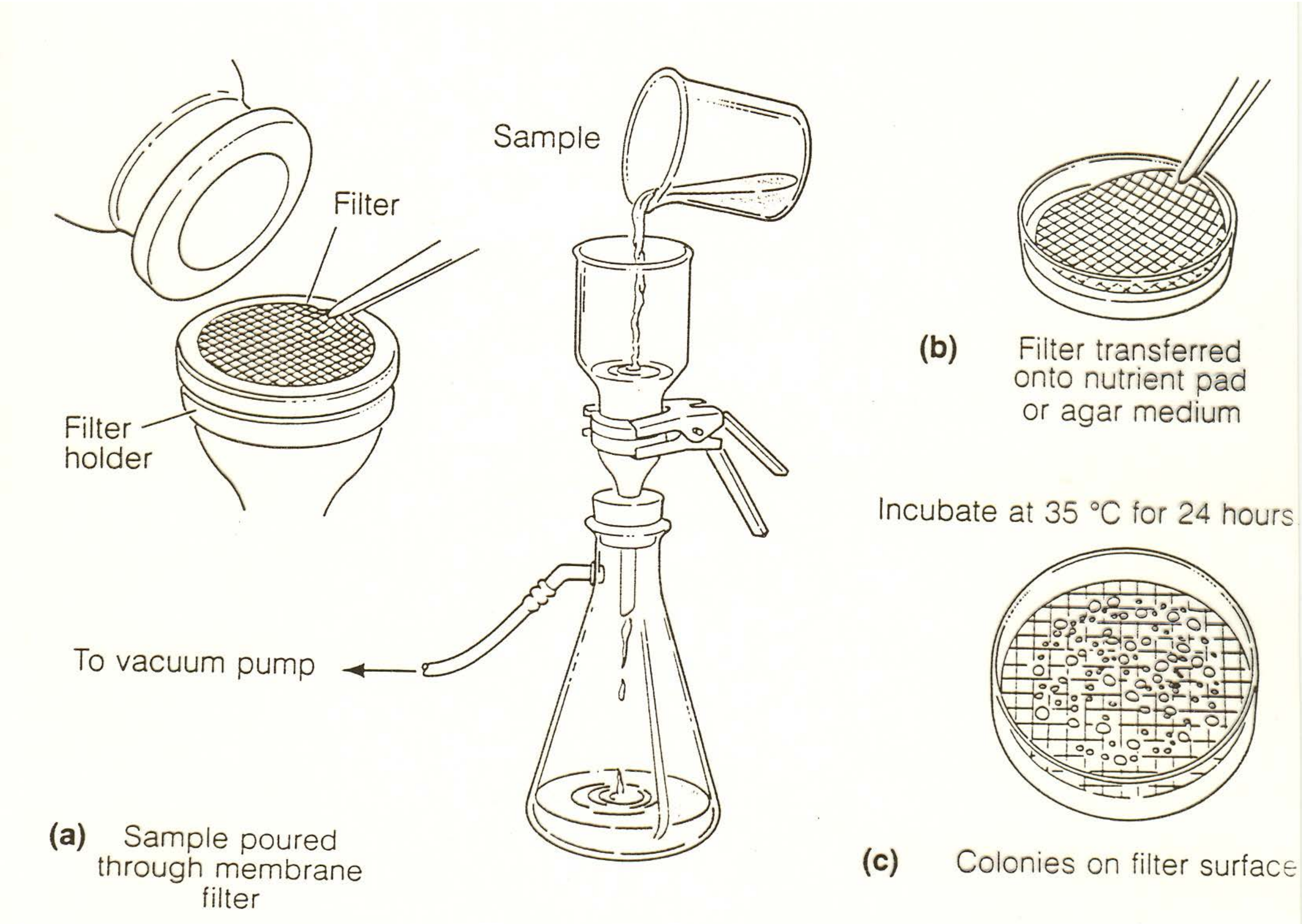
Direct microscopic count



Viable count



Filtration and viable count



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Phylogenetic Identification and In Situ Detection of Individual Microbial Cells without Cultivation

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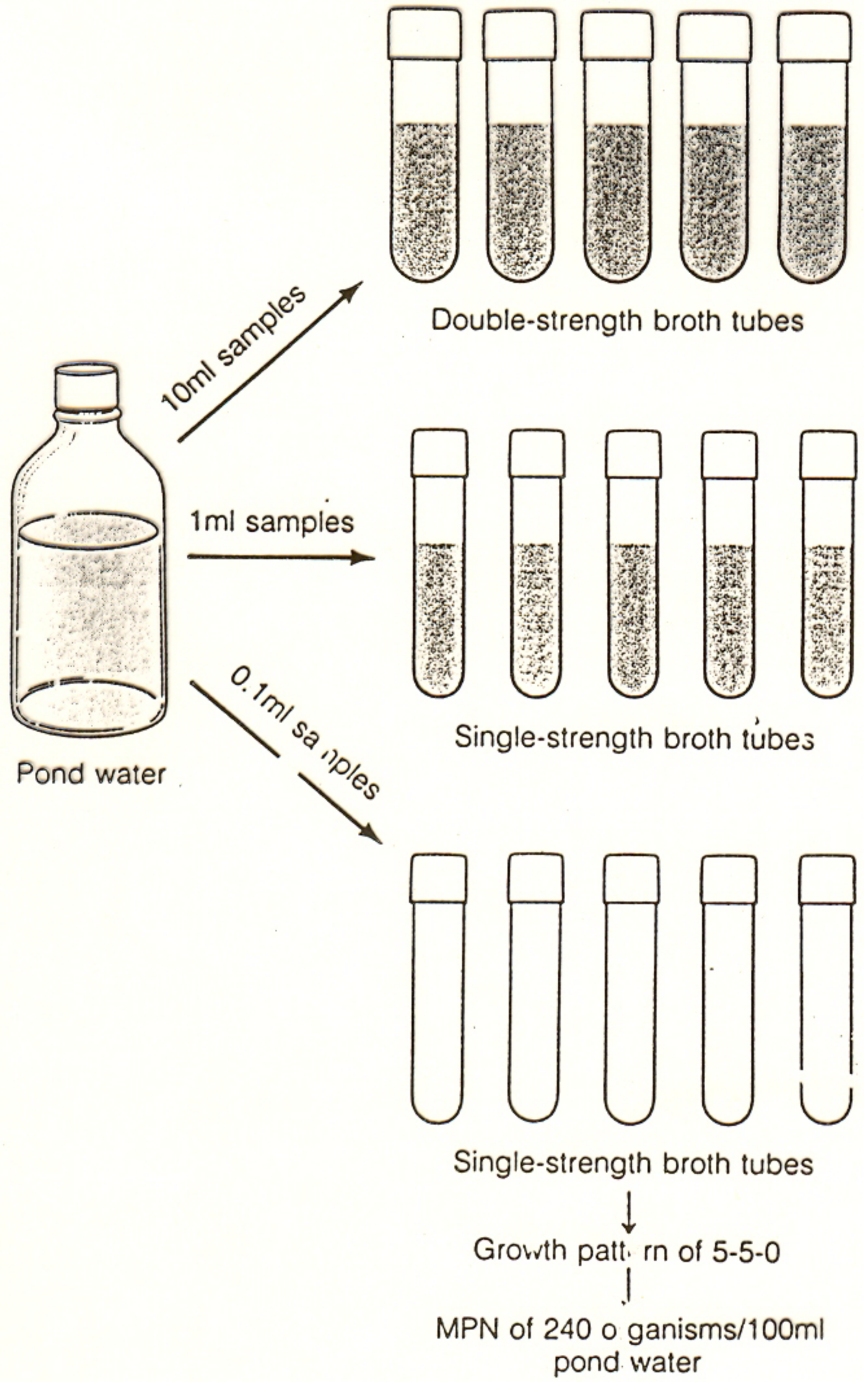
TABLE 1. Culturability determined as a percentage of culturable bacteria in comparison with total cell counts

Habitat	Culturability (%)[a]	Reference(s)
Seawater	0.001–0.1	48, 81, 82
Freshwater	0.25	75
Mesotrophic lake	0.1–1	150
Unpolluted estuarine waters	0.1–3	48
Activated sludge	1–15	160, 161
Sediments	0.25	75
Soil	0.3	153

[a] Culturable bacteria are measured as CFU.

Most Probable Number (MPN)

statistical expression for estimating the number of microorganisms in a volume.



Five-Tube MPN Table

Number of Tubes Giving Positive Reaction			MPN Index per 100 mL	Number of Tubes Giving Positive Reaction			MPN Index per 100 mL
a	*b*	*c*		*a*	*b*	*c*	
0	0	0	<2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
				5	1	2	60
2	0	0	4				
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
				5	3	2	140
3	0	0	8				
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
				5	4	4	350
4	0	0	13	5	5	0	240
4	0	1	17	5	5	1	300
4	1	0	17	5	5	2	500
4	1	1	21	5	5	3	900
4	1	2	26	5	5	4	1,600
4	2	0	22	5	5	5	≥1,600