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Workshop on Basic Microbiology for the Mineral Industry

Atelier de travail sur la microbiologie fondamentale pour le compte de l'industrie minérale

R.G.L. McCready,
V. Sanmugasunderam,
and W.D. Gould

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R.G.L. McCREADY, V. SANMUGASUNDERAM, and W.D. GOULD

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WORKSHOP ON BASIC MICROBIOLOGY FOR THE MINERAL INDUSTRY

FOREWORD

The following worksnop has been prepared by the Biotechnology Section of CANMET, the Department of Energy, Mines and Resources, for presentation to the members of BIOMINET who wish to gain an understanding of basic microbiology and, hopefully, an awareness of present and potential applications of biotechnology in the mineral industry.

The primary goal of this two-day workshop is to provide a basic understanding of the terminology, cell structure and function, the present and potential applications, as well as tne limitations of the biological processes in the mineral industry. It is hoped that the workshop will result in better dissemination of ideas between biologists and members of the mining community.

ATELIER DE TRAVAIL SUR LA MICROBIOLOGIE FONDAMENTALE POUR LE COMPTE DE L'INDUSTRIE MINÉRALE

AVANT-PROPOS

L'atelier de travail présenté ci-dessous a été préparé par la Section de biotechnologie du CANMET et le ministère de l'Énergie, des Mines et des Ressources en vue d'être offert aux membres du BIOMINET désireux d'améliorer leurs connaissances de la microbiologie fondamentale et, si possible, de se renseigner sur les applications actuelles et potentielles de la biotechnologie dans l'industrie minérale.

Le but principal de cet atelier d'une durée de deux jours est d'aider les membres à mieux comprendre la structure et les fonctions de la cellule, à améliorer leur connaissance de la terminologie pertinente et des applications actuelles et potentielles des procédés biologiques. Il vise également à les renseigner sur les facteurs qui limitent l'application des procédés biologiques dans l'industrie minérale. On espère que cet atelier de travail stimulera l'échange d'information entre les membres de la collectivité minière.

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BACTERIA

Figure 1.1 presents a simple diagram of a bacterial cell. The shape of bacterial cells can vary from a rod-shaped cylinder (bacilli), to spherical cells (cocci), to corkscrew-shaped cells (vibrio or spirochetes). The cellular shape, size, and location of spores and flagella are used as parameters for identifying a particular organism.

The nucleus of a bacterial cell is composed of two complementary strands of DNA in one long continuous loop. The bacterial nucleus does not have a nuclear membrane and this is one of the distinguishing features of the unicellular bacteria (prokaryotes). Higher life forms such as yeast, fungi, plants, and animals (eukaryotes) have nuclear membranes; in even higher life forms, e.g., insects and above, the nuclear DNA is divided into distinct chromosomes.

Surrounding the nucleus is a clear viscous material, similar to raw egg white. This solution contains soluble enzymes, cell nutrients, and cellular biosynthetic precursors for the synthesis of protein, RNA, and DNA. This material is referred to as the cellular cytoplasm and is surrounded by a membranous sac called the cytoplasmic membrane.

The cytoplasmic membrane is a lipo-protein complex producing a selective, semi-permeable barrier that controls the entrance and exit of metabolites into or out of the cell. This regulation is, to a great extent, a result of the enzymes contained within the membrane or closely associated with the membrane. Figure 1.2 shows the structure and complexity of the double-membrane of a Gram-negative bacterium.

Figure 1.3 presents the proposed mechanism for protein (enzyme) secretion through tne cytoplasmic membrane.

GRAM-POSITIVE VS GRAM-NEGATIVE

On the external side of the cytoplasmic membrane is the cell wall. Bacteria are divided into two groups, based on the structure and chemical composition of the cell wall, that are reflected in their staining characteristics. The first group is the Gram-negative organisms that have the cytoplasmic membrane surrounded by a peptidoglycan layer (cell wall), which in turn is surrounded by the outer membrane (Fig. 1.2, 1.4, and 1.5). The second major group of bacteria is the Gram-positives; they have no outer membrane and the cell wall is a complex of n-acetylglucosamine (NAG) and n-acetylmuramic acid (NAM), which are cross-linked by a penta-amino acid chain. Cell walls are generally 10-25 nm thick and represent about 25% of the cellular dry weight. The cell wall provides structural rigidity and, to a lesser degree, selective permeability, since the entrance of nutrients and excretion of wastes are controlled somewhat by the molecular diameter of the material. The cell wall is resistant to most chemical reagents except strong inorganic acids and alka-

lies. The cell wall of Gram (+) bacteria can be selectively removed using the enzyme, lysozyme; this produces a protoplast (the cytoplasmic membrane and all intracellular components. A protoplast can only be maintained intact in a hypertonic solution, usually in 25% w/v sucrose). Lysozyme treatment of a Gram (-) organism produces a spheroplast with small pieces of the cell wall remaining attached to the cytoplasmic membrane.

"Plasts" are capable of remaining viable, can carry on reproduction, sporulation, and if nutrients are added to the hypertonic medium, they will resynthesize a new cell wall. Penicillin, which inhibits cell wall synthesis, can also be used to produce protoplasts in Gram (+) organisms.

FLAGELLA

Arising from the basal granule or blepharoplast within the cytoplasmic membrane is the flagellum, which is utilized by some bacteria to provide mobility. The flagellum is 99% protein and contains no free amino acids. The flagellum is 0.012 µm in diameter and varies in length from 0.5 µm to 3-4 µm. Locomotion is believed due to the passage of spiral waves along the length of the flagellum. The number and cell surface location of flagella are used as parameters in bacterial identification (Fig. 1.5).

Because of their small size, flagella can only be directly observed under the electron microscope. However, by precipitating tannic acid or metallic ions on the surface of the flagellum, they may be seen under the light microscope once their diameter has been increased to about 0.4 µm.

FIMBRIAE

Fimbriae (pili) are small hair-like appendages found on the outer surface of Gram (-) bacteria. They are protein (pilin) in composition, and their presence or absence is genetically controlled. Pili or fimbriae aid in the attachment of organisms to surfaces. They may also provide the cell with some degree of protection from bacteriophage (Fig. 1.6).

CAPSULE

The external layer on all bacteria is the capsule layer. The chemical composition and the type of capsule produced by a particular organism is genetically controlled. Capsules may be solely composed of polysaccharides or they may be more complex chemically, being a mixture of polysaccharides and peptides (mucopeptides). Three types of bacterial capsules exist (Fig. 1.1). The first is the microcapsule, which may be less than 1 µm in thickness, but is believed to be universal to all bacteria. The second type is the capsule proper, varying from 1-3 µm in thickness. The third type is the slime layer, which is composed of high molecular weight polysaccharides; it may be loosely bound to the cell or may be secreted freely into the medium. The slime layer may be up to 10 µm in thickness.

The capsule has several functions:

1. It prevents a sudden influx or efflux of water into and out of the cell, thus acting as an osmotic barrier and preventing dehydration of the cell.
2. The latter two types of capsule prevent phagocytosis of the bacterium, mainly with pathogenic organisms.
3. The thicker capsules may also protect the bacterium from bacteriophage infection.

BACTERIAL SPORES

Spores are bacterial protective mechanisms to sustain the bacterial genome in a "suspended state" under conditions unsuitable for vegetative cell growth. Only a few genera of bacteria are capable of sporulation, which is under genetic regulation. Bacterial genera capable of sporulation are:

Bacilli-facultative aerobe, Gram (+)
Clostridium-obligate anaerobe, Gram (+)
Desulfotomaculum-obligate anaerobe, Gram (+)
Sporosarcina-aerobe, Gram (+)
Sporovibrio, Gram (+).

Most bacteria produce one spore per vegetative cell. Sporulation is genetically controlled but is favoured by certain environmental and nutrient conditions, including the following:

1. Sporulation is most prevalent at the end of a period of intense cellular growth.
2. Gradual dehydration of a culture favours sporulation.
3. Nutrient deprivation of cells favours sporulation, e.g., suspension of cells in distilled water.
4. Reduction of O_2 tension for anerobes or exposure to low O_2 tensions for anaerobes often induces sporulation.

Some workers believe that depletion of the carbon or nitrogen source for the particular bacterium induces sporulation, and Mn^{2+} is required during sporulation as a co-factor for synthesis of the temperature-tolerant spore coat.

Sporulation is not a means of reproduction but is the protective mechanism by which the cell protects its nuclear material within a heat-resistant, non-stainable, spore coat or coats. The spore contains up to 15% dipicolinic acid and a higher proportion of Ca^{2+} than the vegetative cell. Figure 1.7 shows the structure of a bacterial spore.

Again, the intracellular location of a spore will assist in the identification of a particular organism. For example:

Clostridium tetani - terminal spore
Clostridium perfringens - subterminal spore
Clostridium welchii - central spore.

SPORE RESISTANCE

The vegetative forms of spore-forming organisms are as susceptible to heat, chemicals, etc., as are non-spore-forming organisms. But the spores are resistant to heat, drying or dessication, and chemicals (e.g., spores of some bacteria will resist boiling water for several hours). Vegetative bacterial cells are generally killed by a 1:80 dilution of phenol or carbolic acid in 10-15 min whereas, in many instances, spores are unaffected by 5% phenol, which is five times the strength required to kill vegetative cells. Spores have been dried on a piece of thread, which was immersed in a nutrient medium 35 years later, and most of the spores were capable of germination (producing a vegetative cell which is an exact replica of the cell that formed the spore).

It should be remembered that spores are a resistant and possibly a protective state for the vegetative cell, but they are not a form of reproduction.

AUTOTROPHS VS HETEROTROPHS

Autotrophic bacteria can synthesize all their cellular components from inorganic compounds. Their cellular carbon is obtained through the fixation of atmospheric CO_2 or by the fixation of aqueous HCO^{3-} ions from the medium. Because they require atmospheric CO_2 as a carbon source, most of the autotrophs are aerobic.

In contrast, heterotrophic bacteria require an organic carbon source. For optimal growth, most organisms require monosaccharides such as glucose (dextrose), fructose, arabinose, or disaccharides such as sucrose (table sugar). Many of the heterotrophs, particularly pathogenic (disease-causing) bacteria, are very fastidious in their growth requirements. Some require specific metal ions, such as Zn^{2+} or Co^{2+}, in their medium in order to synthesize specific enzymes essential for their growth. Some heterotrophs will not grow unless the medium is supplemented with specific growth factors such as various B vitamins, particularly vitamin B_{12}, which is required for the growth of *Mycobacterium tuberculosis* which can cause tuberculosis. The Streptococci that can cause strep throat require hemoglobin (red blood cells) for optimal growth. Some organisms have lost the enzymes to synthesize specific amino acids, vitamins, or metabolic intermediates and cannot grow unless these compounds are provided in the medium.

PATHOGENIC BACTERIA

Some bacteria cause illness and disease in plants, animals, and people because they produce a cellular product that damages the host tissue, allowing an infection to develop and causing the symptoms of illness. Pathogenesis can occur through many bacterial factors:

- bacterial secretion of tissue-damaging enzymes - e.g., β hemolysin Streptococci;

- bacterial secretion of an extracellular toxin
  - e.g., Staphylotoxin - *Staphylococcus aureus*
  Diptheria toxin - *Pneumonococcus diptheriae*
  Tetanus toxin - *Clostridium tetani*;

- production of a bacterial endotoxin (intracellular) that is released when the cell dies or is digested in the G.I.T. (gastrointestinal tract)
  e.g., typhoid toxin - *Salmonella typhi*
  enterotoxin - *Escherichia coli* &
  Salmonella;

- release of cytotoxic cellular products on cell death or digestion.

In contrast to the release of enzymes or endotoxins, these components tend to be released from cellular components such as the cell wall, cytoplasmic membrane, or nucleic acids that have been partially degraded or metabolized by the host.

Because organic compounds are generally toxic to autotrophic bacteria, they cannot live in plants, animals, or people; thus, they are non-pathogenic. All of the pathogenic bacteria are heterotrophs, but not all heterotrophic bacteria are pathogens.

BACTERIAL IDENTIFICATION

Many parameters are used to aid in the identification of bacterial isolates, including:

1. Cell morphology
   (a) The size and shape of the cell - is it a bacillus, cocci, or vibrio? - and the Gram stain reaction.
   (b) Are the cells singular, in pairs, or in characteristic clusters?
   e.g., Staphylococci - grape-like cluster
   Streptococci - long bead-like chains
   (c) Presence and location of flagella
   (d) Presence and location of the spore.

2. Metabolic Requirements
   (a) Required carbon source; does it produce acid, gas, or both during growth?
   (b) Does it grow aerobically, anaerobically, or facultatively?
   (c) Does it produce H_2S from protein medium?
   (d) Electron acceptor: O_2, NO_3, SO_4.

3. Colony Morphology
   (a) Size and shape of the colony
   (b) Pigmentation

(c) Effect on the medium: examine plates for secreted enzymes:
e.g., i) Green halo on BAP (Blood Agar Plate) indicates alpha hemolysis (partial oxidation of hemoglobin), suggesting staphylococci of less pathogenic streptococci.
ii) Clear halo around the colonies on BAP - beta hemolytic streptococci.
iii) Clear halo around colonies on a lipid medium - lipase activity - Bacilli.
iv) Addition of hydrogen peroxide on the colony surface produces fizzing, which indicates the presence of the enzyme catalase and suggests the culture is a pathogenic staphylococcus.

By testing for various metabolic reactions, combined with cellular and colony morphology, each organism can be identified and classified by comparison to *Bergey's Manual of Determinative Microbiology* (1).

A similar approach is taken in the identification of fungi and yeast.

FUNGI AND YEAST: CELL STRUCTURE

Both fungi and yeast are eukaryotic cells and are much more complex than the prokaryotic bacteria. The major differences in yeast and fungal cellular structure from that of bacteria are that the eukaryotes have nuclear membranes; they may be single-celled or multicellular; they contain mitochondria (small cytoplasmic inclusions containing the respiratory enzyme complex) and a nucleolus; they may contain glycogen or starch granules; and they reproduce by either asexual or sexual spore production. Simple fungi produce fuzzy growth seen with bread mold, decaying fruits, or on moist leather, to the more complex structures exhibited by mushrooms, wood-rot, fungi, etc. (Fig. 1.7, 1.8).

Yeast cells are generally singular but much larger than bacteria, and are generally oval cells ranging from 2-5 µm in width and from 5-30 µm in length. They can reproduce asexually by budding or by producing asexual spores. Some species of yeasts are capable of both sexual and asexual reproduction (Fig. 1.9, 1.10).

REFERENCE

1. Buchanan, R.E., and Gibbons, N.E., Co-editors. *Bergey's Manual of Determinative Bacteriology,* 8th Edition; Williams and Wilkins Company; Baltimore, Maryland; 1974.

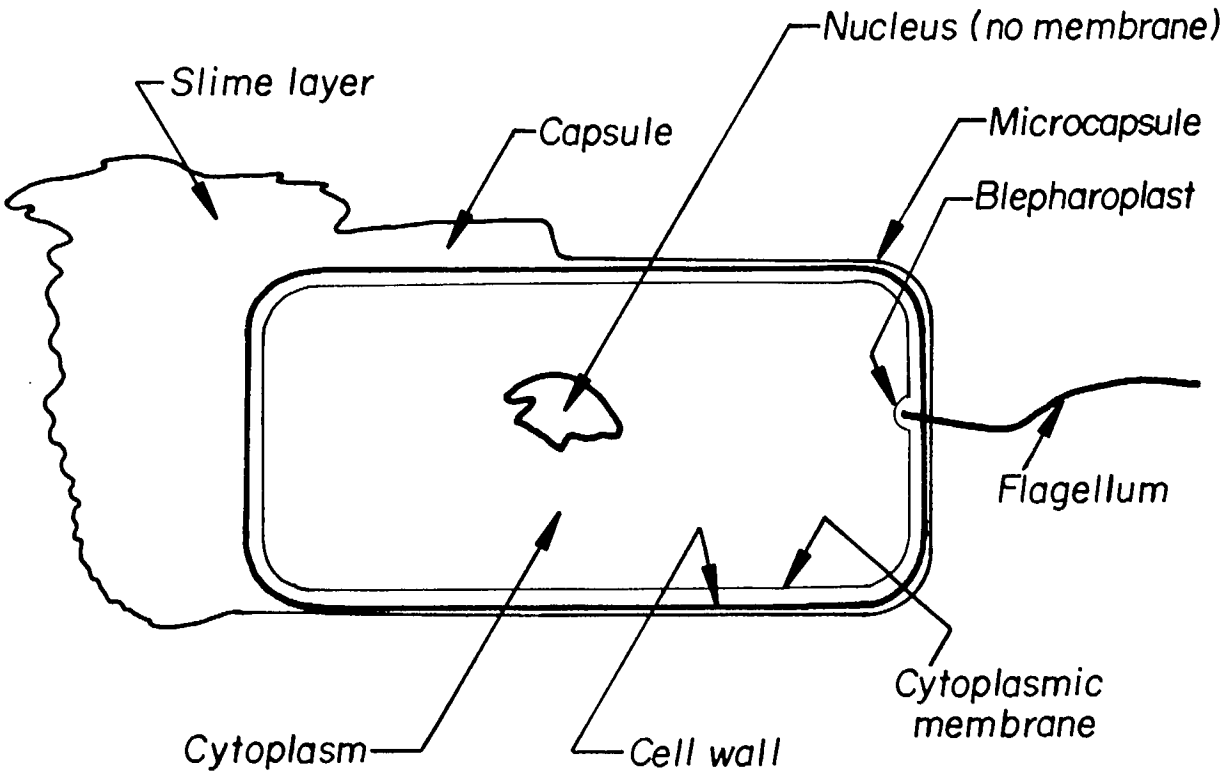

Fig. 1.1 - A bacterial cell

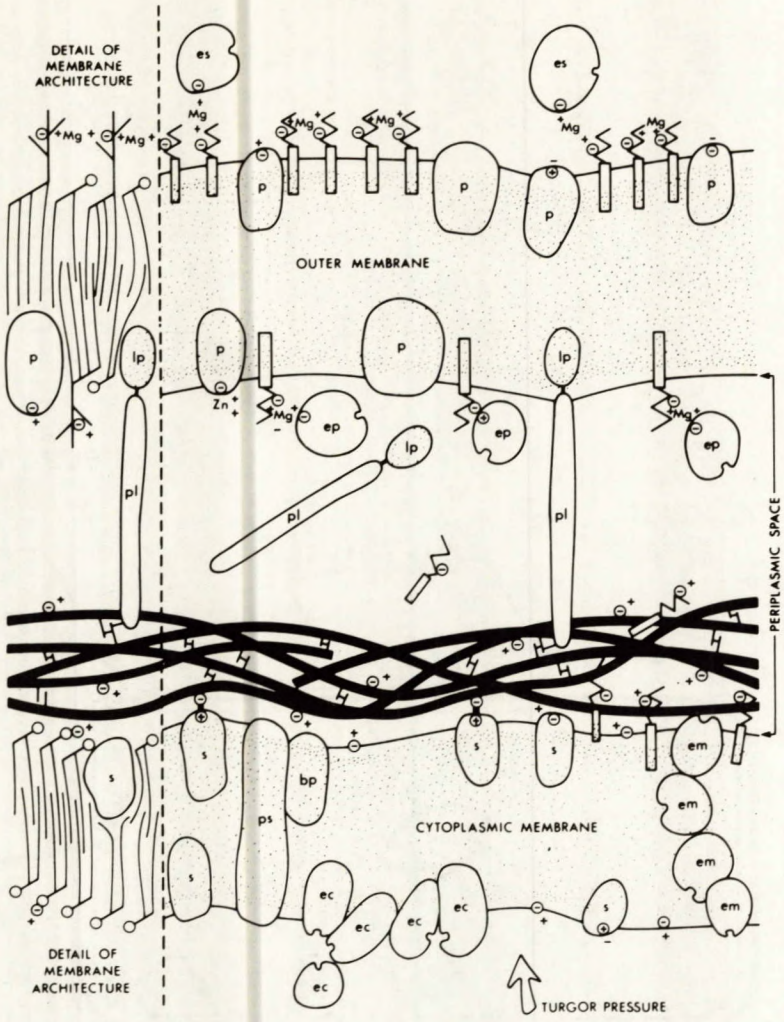

Fig. 1.2 - Schematic diagram of gram-negative cell envelope +, Free cation;- free anion; ⊕ bound cation; ⊖ bound anion; ⊕⊖ adhesion point produced by ionic bonding; hydrophobic zone; covalent bond; cross-linking polypeptide in the peptidoglycan; polysaccharide portion of peptidoglycan; enzymatically active protein; phospholipid; lipopolysaccharide; lipopolysaccharide (schematic); bp, binding protein; ec, enzymes associated with the cytoplasmic membrane whose function is directed to the cytoplasm; em, enzymes associated with the cytoplasmic membrane which synthesize macromo- lecular components of the cell wall; ep, enzymes localized in the periplasmic zone; es, enzymes localized at the cell surface; lp, lipid portion of Braun's lipoprotein; p, structural and enzymatic proteins of the outer membrane; pl, protein portion of Braun's lipoprotein; ps, permease; s, structural protein of cytoplasmic membrane

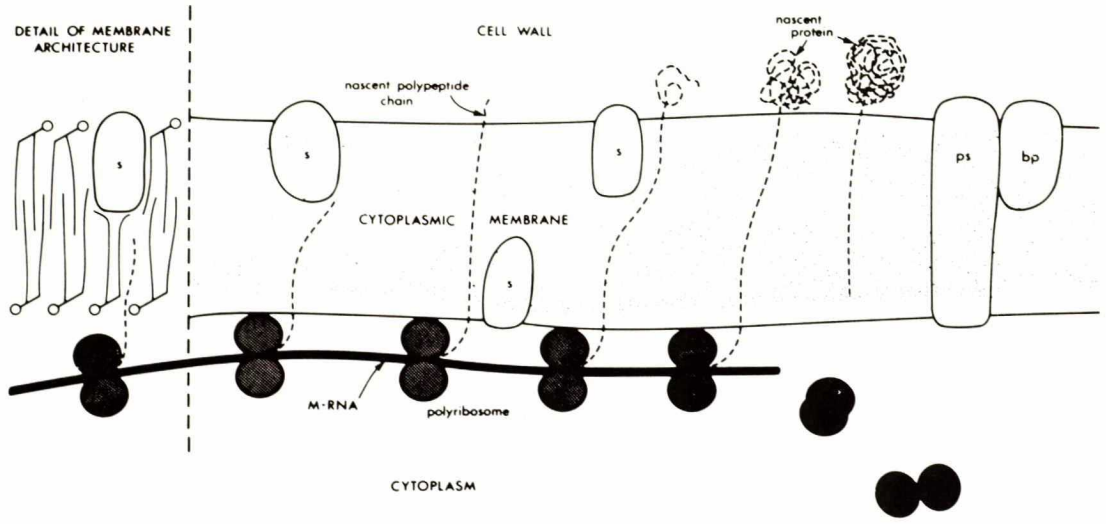

Fig. 1.3 - Proposed mechanism of protein secretion of the cytoplasmic membrane. , Phospholipid; , hydrophobic area; polypeptide chain; bp, binding protein; ps, permease; s, structural protein of cytoplasmic membrane

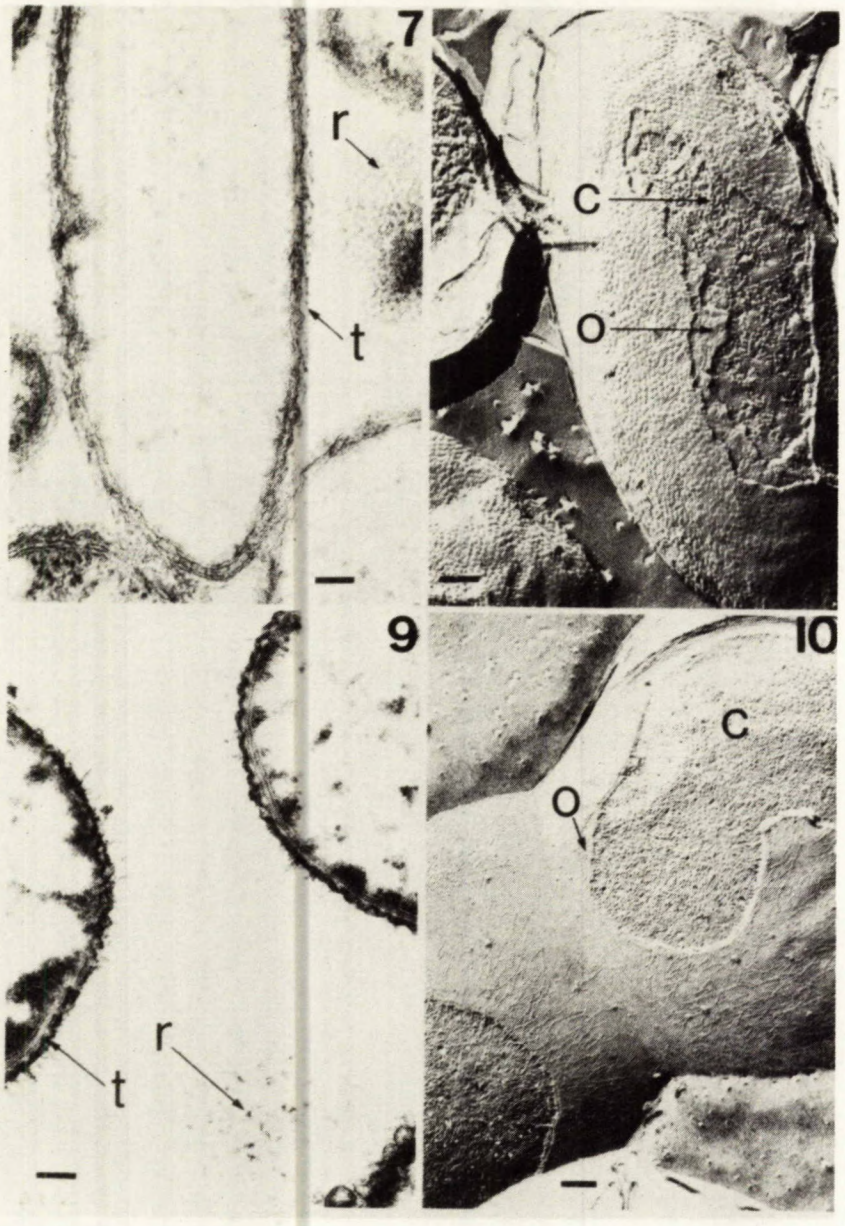

Fig. 1.4a) - Electron micrograph of gram-negative bacteria showing the cell wall structure

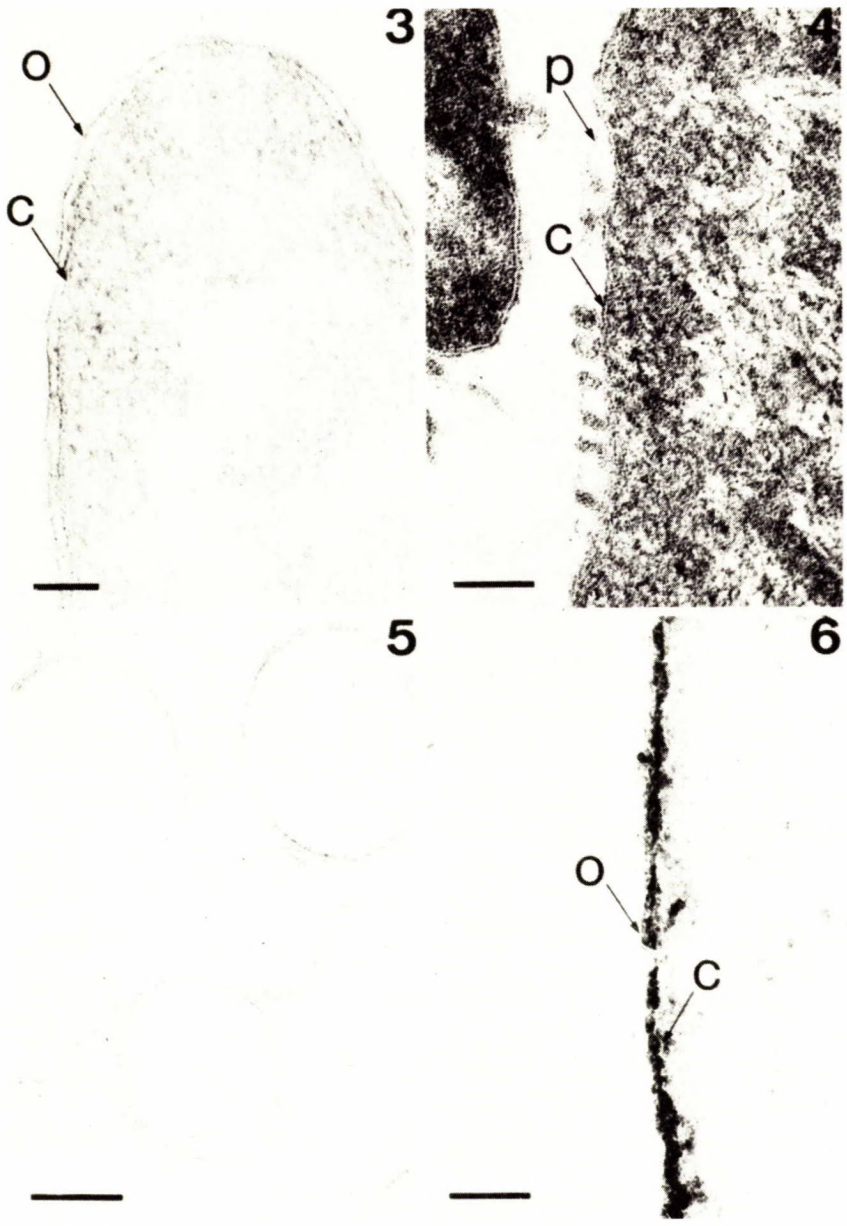

Fig. 1.4b) - Electron micrograph of a section of a cell of the marine pseudomonad (B-16) showing the cytoplasmic membrane (c) and the outer membrane of the cell wall (o). The bar in this and subsequent micrographs indicates 0.1 μm

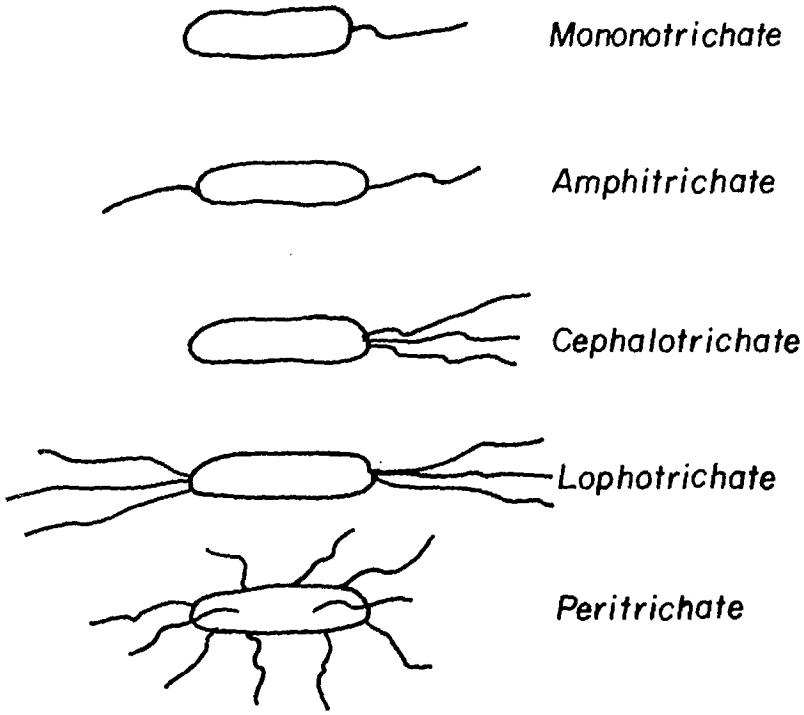

Fig. 1.5 - Location of flagella

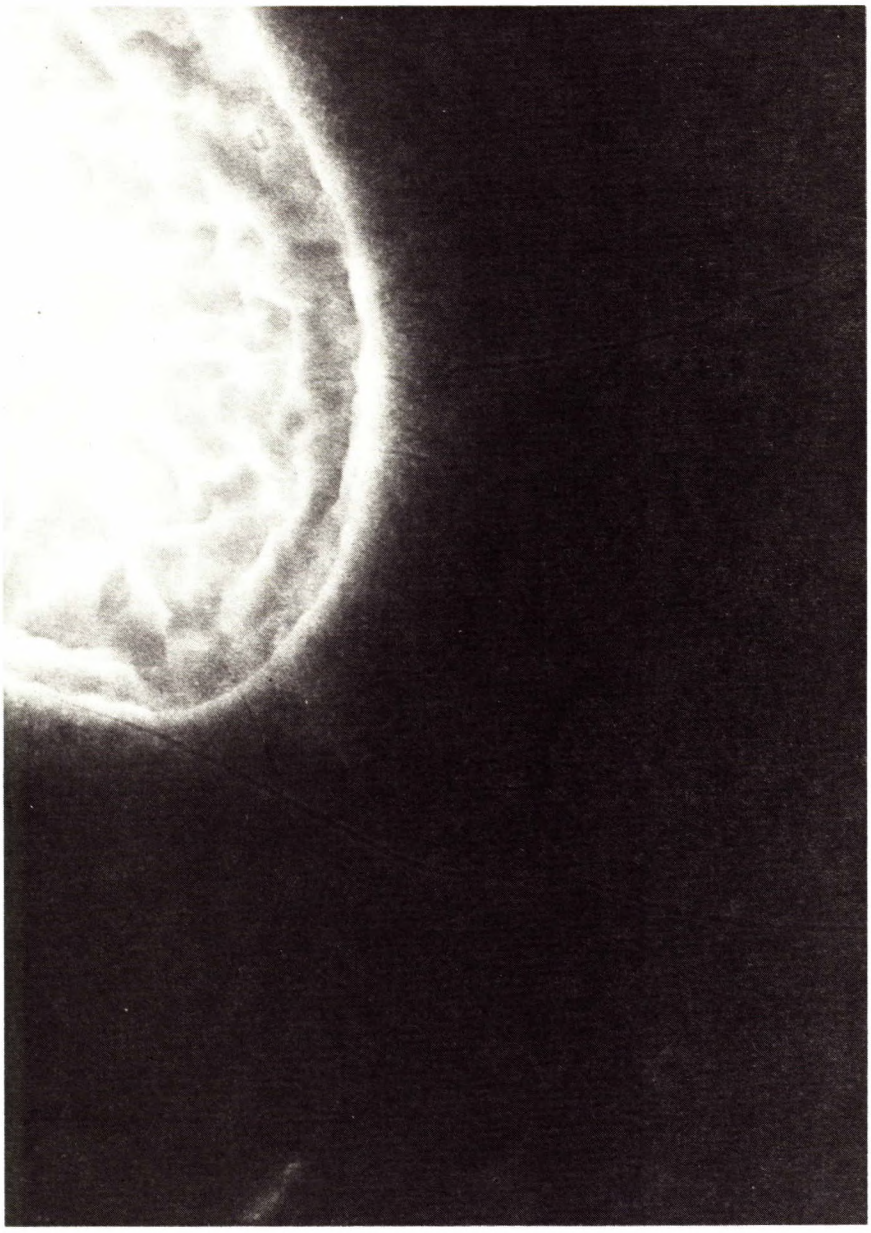

Fig. 1.6 - Electron micrograph of fimbriae on cell surface

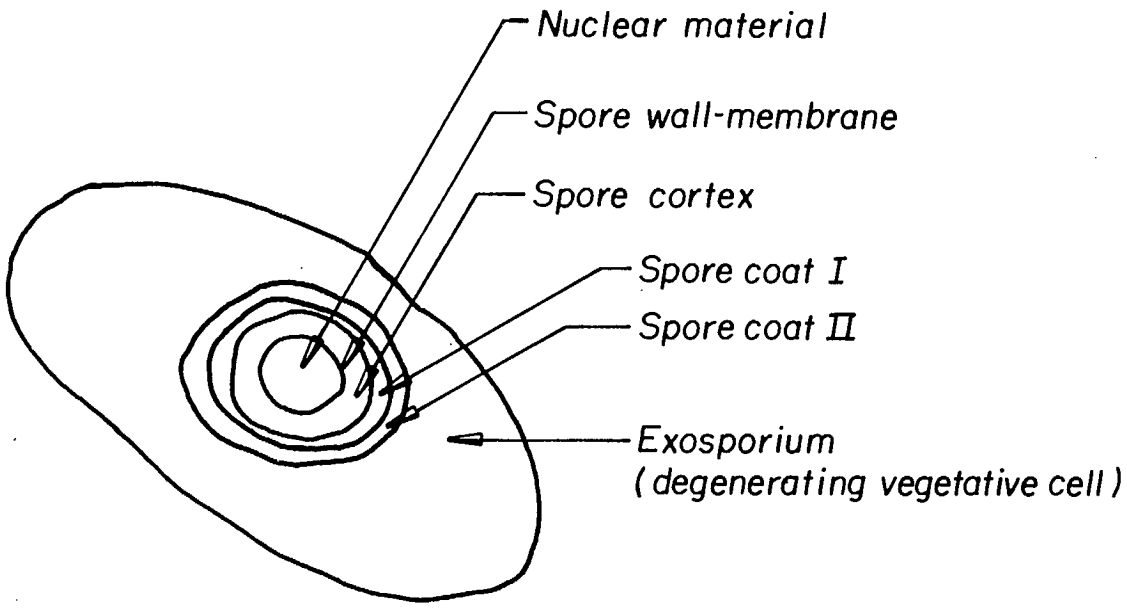

Fig. 1.7 - Structure of a bacterial spore

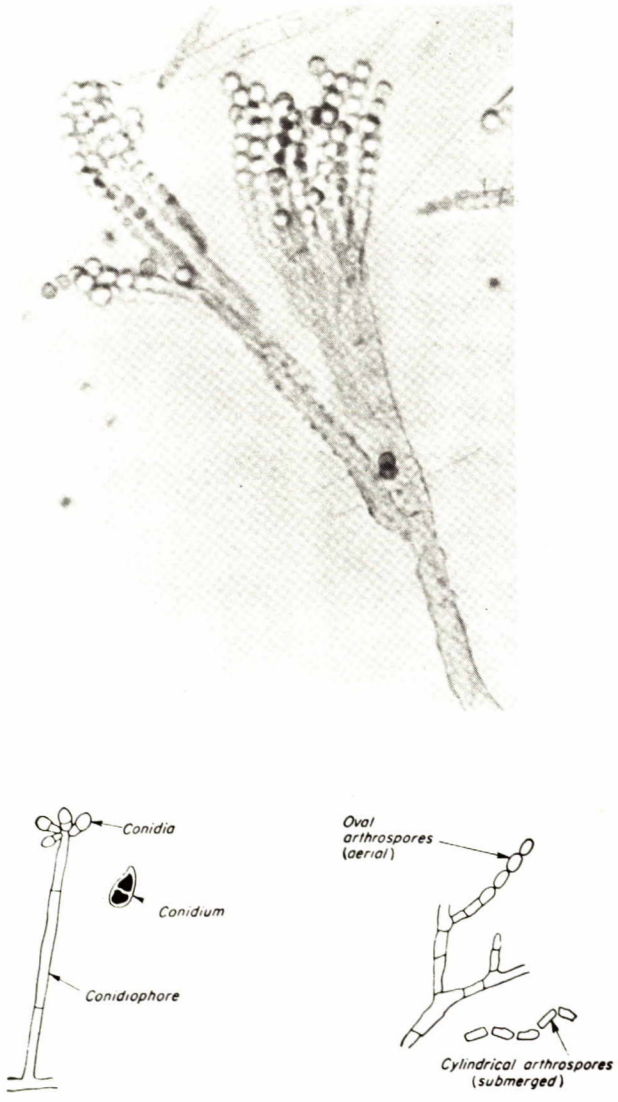

Fig. 1.8 - Photomicrograph showing sporulation

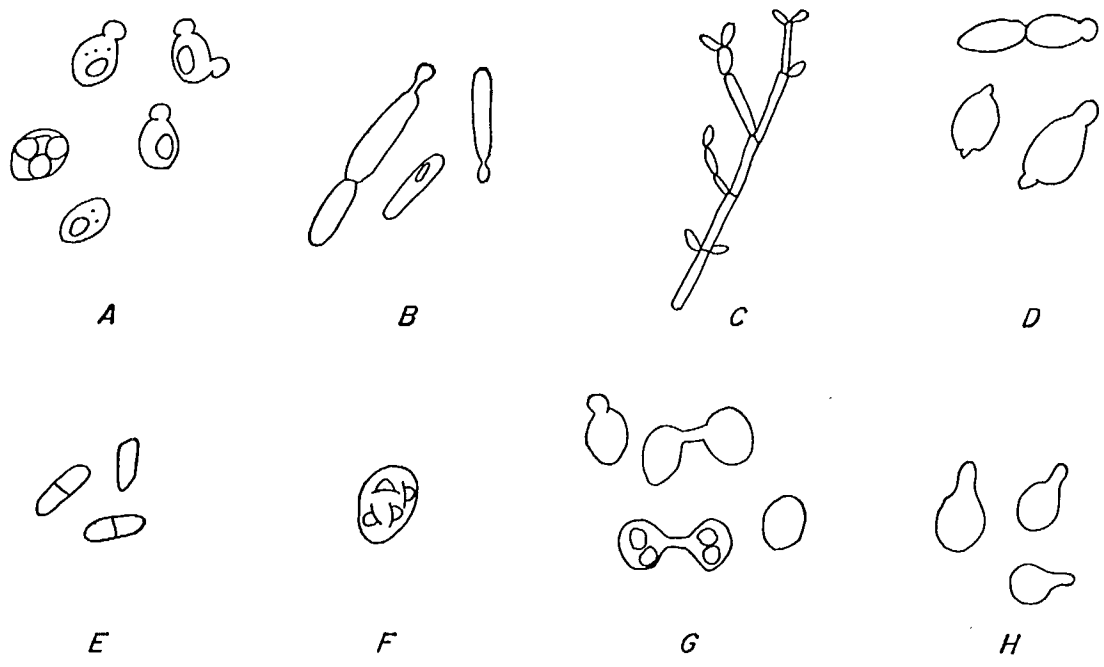

Fig. 1.9 - Yeasts of different shapes: (A) *Saccharomyces cerevisiae* with budding cells and one ascus with four ascospores; (B) *Candida* yeast with elongated cells; (C) *Candida* yeast showing pseudomycelium; (D) apiculate (lemon-shaped) yeast; (E) *Schizosaccharomyces* yeast, multiplying by fission; (F) *Hansenula* yeast with ascospores shaped like derby hats; (G) *Zygosaccharomyces* yeast showing conjugation and ascus with four ascospores; (H) flask-shaped yeasts

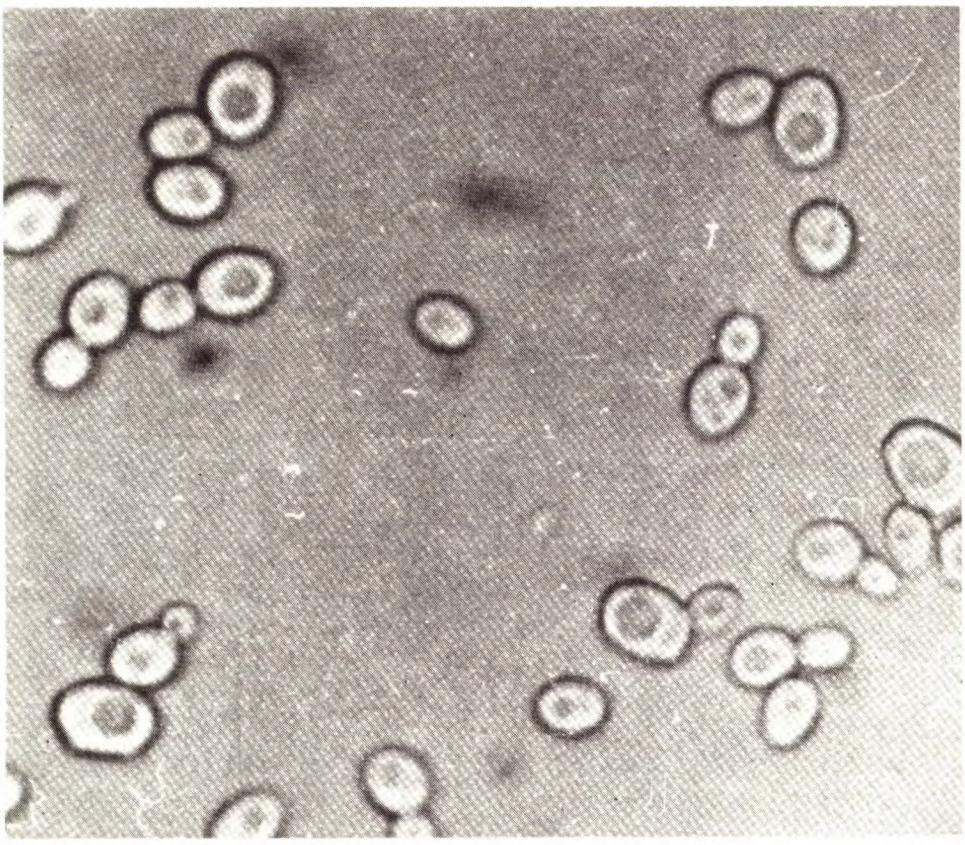

Fig. 1.10 - Photomicrograph of budding *Saccharomyces cerevisiae* yeasts (bread yeasts)

CHAPTER 2 - MEDIA AND GROWTH REQUIREMENTS

INTRODUCTION

All biological systems, from unicellular organisms to the highest order - man - require nutrients (food) for their growth and normal cellular functions. Nutrients are extracellular substances that, on entering a cell after passing the cell membrane, can be used by the cell to build cellular material or to obtain energy.

Almost any material can nourish one microorganism or another. The list of materials utilized by biological systems ranges from the usual growth substances - proteins, sugars, and amines - to the unusual, such as rubber, paper, leatner, oil, carbon monoxide, iron, or elemental sulphur. No one organism is capable of utilizing all nutrients, and some nutrients can be utilized by a limited number of species.

The selection and transport of nutrients into the cell, and the disposal of waste products out of the cell, are the main functions of the cell membrane. Some compounds enter the cell by diffusion, but in most instances the nutrients are transported across the cell membrane by a process called "active transport". Active transport is the ability of an organism to accumulate substances within the cell in high concentration, from an external environment in which the substances are low in concentration (movement against the concentration gradient).

Differences in the ability to utilize nutrients constitute part of the basis for the identification of microorganisms. Nearly all species, and in some cases even genera, are classified according to their ability to utilize certain compounds or the products formed from them.

It is claimed that some 35-40 elements in the periodic table are essential nutrients for bacteria and fungi. Although the majority of these requirements are metals, six non-metals (C, O, H, N, P, S) and two metals (K, Mg) constitute over 95% of the dry weight of bacteria and fungi. These eight elements are usually referred to as "macronutrients" (the concentration needed in growth media being greater than 10^{-4} moles per litre). All other elemental nutrients are called "micronutrients" or trace elements, and are usually required at concentrations less than 10^{-4} moles per litre. The eight macronutrients and their physiological functions are outlined in Table 2.1. Bacteria can be divided into four main categories according to their carbon source and the manner in which they secure their energy requirements (Table 2.2).

Autotrophic bacteria have the ability to grow solely on inorganic materials, with carbon dioxide as the carbon source, and sunlight (photosynthesis) or the oxidation of inorganic compounds (chemosynthesis), as the energy source.

Obligatory autotrophs use only inorganic carbon, growth usually being inhibited by organic compounds. *Mixotrophic* autotrophs, on the other hand, can use both organic and inorganic carbon sources. All autotrophs incorporate carbon dioxide into the cell material.

Among the *chemosynthetic* microorganisms, the thiobacilli are of significance to those involved in mining. In particular, the organism *Thiobacillus ferrooxidans* is capable of oxidizing metal sulphides to metal sulphates and sulphuric acid. It is also the major cause of acidity in mine waters and tailings.

Heterotrophic bacteria require complex organic compounds as a main carbon source, although some of them may use carbon dioxide to a lesser extent. The energy source may be photosynthetic or chemosynthetic. Chemosynthetic heterotrophs constitute the vast majority of bacteria commonly dealt with in general or applied microbiology.

MEDIA FOR AUTOTROPHS

A culture is any growth or cultivation of microorganisms. The term is usually employed with reference to deliberate growth of a microorganism in the laboratory. A culture medium is a substrate or nutrient solution upon which microorganisms are cultivated in the laboratory.

The chemical composition of the medium used for the cultivation of autotrophs is simple. For example, the so-called 9K medium of Silverman and Lundgren (1), which is used for growing *Thiobacillus ferrooxidans,* has the following composition:

$FeSO_4$	9.0 g
$(NH_4)_2SO_4$	3.0 g
K_2HPO_4	0.5 g
$MgSO_4\ 7H_2O$	0.5 g
KCl	0.05 g
$Ca(NO_3)_2\ 4H_2O$	0.01 g
Distilled Water	1000 mL

The ability of the organism to grow in such a mixture of simple chemical compounds clearly shows that it has an intricate mechanism for synthesizing complex carbohydrates, fats, proteins, vitamins, and other substances that comprise the cell constituents. The elaborate capacity for synthesis of such complex molecules from a few simple chemicals in a tiny cell is a remarkable feat of nature!

The media used for cultivation of other autotrophs are very similar to the 9K medium, except that certain other specific chemicals may have to be added or substituted. Such media composed of known chemical substances are known as chemically defined or synthetic media.

MEDIA FOR HETEROTROPHS

The diversity of nutritional types among bacteria is so great that a large number of different media are available for their cultivation, and these media are compiled in a book of over 1000 pages (2). Heterotrophic bacteria may have relatively simple or extremely complex nutritional requirements. Those with simple nutritional requirements can be grown on a defined or synthetic medium. Chemically defined media can be reproduced exactly at any time, and by workers in different laboratories, because their constituents are known exactly. They are essential for the study of nutritional requirements of organisms and are used in the manufacture of microbial products in which a minimum of extraneous organic material is required. Other microorganisms require one or more vitamin-like substances or *growth factors*. Certain nutritionally fastidious organisms require a dozen or more of these accessory substances and were originally supplied in the form of meat infusions that were, and still are, widely used in culture media. Other complex raw materials such as peptones, meat extracts, yeast extract, etc., are added to growth medium. Agar (agar-agar), a polysaccharide (galactan) extracted from *Gelidium* and other seaweeds, is included when a solid medium is desired. A description of the raw materials used in complex media is given in Table 2.3. Many of these required substances for individual bacteria are listed in *Bergey's Manual of Determinative Bacteriology* (3).

Microbiologists use many special-purpose media to facilitate recognition, enumeration, and isolation of certain types of bacteria.

Enriched media are used to support growth of species with elaborate nutritional requirements, such as blood, serum, or extracts of plant or animal tissues.

Differential media permit the observer to grow several species of organisms on one medium and to differentiate between the various organisms by their distinctive appearance, or by their ability to utilize or alter the reagents or chemicals added to the medium. For example, if a mixed culture were inoculated onto a blood-agar plate, one of the bacteria may hemolyze (destroy) the red blood cells while the others do not. Thus, one can differentiate between the bacteria capable of hemolysis and those that are not.

Selective media contain certain chemical substances that inhibit the growth of one type of bacteria without inhibiting others. The addition of a low concentration (1 in 50 000, i.e., 0.002%) of crystal violet to blood agar inhibits the growth of some bacteria, notably staphylococci, while allowing the growth of *Streptococcus pyogenes*. Crystal violet blood agar is therefore a selective medium for *Streptococcus pyogenes*.

PHYSICAL CONDITIONS REQUIRED FOR GROWTH

In addition to supplying the proper nutrients, it is also necessary to provide the proper physical conditions conducive for optimum growth. Since bacteria vary greatly in their nutritional requirements, they also exhibit diversity in their response to changes in the physical conditions of their environment.

Gaseous Atmosphere: Most bacteria are capable of growth under ambient oxygen tension. Certain types, however, are capable of deriving their oxygen requirements from various substrates. Aerobic organisms require the free admission of air, while anaerobes grow only when atmospheric oxygen is excluded. Between these two groups are the microaerophiles, which develop best under partial aerobic conditions (<0.1 atmosphere) and the facultative anaerobes, which can grow under both aerobic and anaerobic conditions. A complete description of various methods for maintaining anaerobic conditions in cultures can be found in *Laboratory Methods in Anaerobic Bacteriology CDC Laboratory Manual* (4).

Temperature: Since all the processes of growth are dependent on chemical reactions, and since the rates of these reactions are influenced by temperature, the growth of bacteria is influenced by temperature. Temperature affects both the rate of growth and the total amount of growth (yield of biomass) of the organism. Variations in temperature can affect certain metabolic processes and may even alter the cell morphology. (See the following section for more details.)

pH: The pH of the culture medium is extremely important for growth of microorganisms. The majority of microorganisms prefer culture media that are approximately neutral. However, there are organisms that can withstand extremes. The pH of a culture medium should always be checked and adjusted. It should be noted that the pH always rises as the temperature falls and allowance must be made if the pH is tested when the medium is hot, as is the case with agar, which must be melted for the adjustment of pH. During autoclaving, solutions that have been adjusted to be a little on the alkaline side of neutrality tend to fall about 0.1 pH unit.

Although temperature, pH, and the gaseous environment are the major physical conditions affecting growth, there are other important factors. They will be dealt with in detail in the next section.

The media upon which microorganisms are grown must be sterile or free from possible contamination with other organisms, which might influence or prevent the normal growth of the inoculated type. Sterilization is the freeing of an article from all living organisms, including bacteria and their spores. Sterilization can be effected in a variety of ways:

1. Physical Methods
   - (a) Heat
     - i) Dry heat (including infra-red radiation)
     - ii) Moist heat
   - (b) Radiation
     - i) Ultra-violet radiation
     - ii) Ionizing radiation
   - (c) Filtration

2. Chemical Methods

Heat is most often employed, since it is generally the simplest and most reliable means of sterilization.

Autoclave sterilization for 15 min at 15 lb/sq in. pressure (121°C) is recommended for quantities of liquid media up to 1 L. If larger volumes are to be sterilized in one container, a longer period should be employed. The medium is prepared, distributed in tubes or flasks plugged with non-absorbent cotton, and placed in the autoclave. Flasks are never filled more than two-thirds full. After sterilization, the autoclave is allowed to return to atmospheric pressure. Pressure should not drop too rapidly or the media will boil over, blowing the plugs.

MEDIA FOR YEASTS AND FUNGI

Molds obtain their nutrients by diffusion or transport of soluble matter across the cell membrane, and they utilize relatively simple substrates. Most molds secure carbon and energy from carbohydrates, and some can utilize alcohols or organic acids. In the absence of a readily available source of carbon, they can also satisfy their carbon requirement from protein or from products of protein digestion. Sources of nitrogen include organic compounds such as peptones, peptides, and amino acids. Some species can utilize ammonia or nitrates. Many molds can synthesize all the vitamins and other growth factors they require, but some must be supplied with preformed thiamin, biotin, or their precursors.

Yeasts require the same chemical elements as other forms of life: carbon, hydrogen, nitrogen, oxygen, phosphorus, etc. Carbon is ordinarily obtained from sugars, organic acids, aldehydes, or glycerin. Nitrogen is procured from products of protein hydrolysis (such as proteoses, peptones, amino acids, and ammonia) or from urea or amides. In the laboratory and in industrial processes, ammonium sulphate, phosphate, or chloride is often used as the source of nitrogen. Phosphorus is essential for growth, playing an essential role in carbohydrate metabolism. It is usually supplied as a phosphate salt. Yeasts also require growth factors, such as biotin (which has a significant role in nitrogen metabolism); pyridoxine, thiamin, niacin (precursors of certain coenzymes); and insitol (which is apparently built into the cell structure).

Molds grow more slowly than bacteria. If a medium, which supports both bacteria and molds, were to be inoculated with a culture of mold contaminated with bacteria, the bacteria will overgrow the mold. Acidic media with a relatively high concentration of sugar would be tolerated by molds, but would be inhibitory or unfavorable to most bacteria.

The most common types of media used for growth of molds and yeasts are:

1. Natural media, such as pieces or infusions of fruits, vegetables, or animal tissues.
2. Complex media consisting of peptones, plant extracts, agar, and other compounds of unknown or variable composition.
3. Synthetic media from defined chemicals so that the composition is known and the medium is exactly reproducible.

Sabouraud Agar is commonly used in the laboratory for cultivating fungi and certain yeasts; its main disadvantage is that it does not inhibit the growth of bacteria.

TYPES OF MEDIA AND USES

There are two broad groups of media: liquid and solid. Many liquid media containing different nutrients have been devised and most bacteria will grow in one of them. All large-scale fermentations are carried out using liquid media. Liquid media have two disadvantages:

1. Cells do not have a characteristic appearance in liquid media and, except when used in a medium designed for a specific biochemical test, liquid media are of limited use in identifying the organisms.
2. Moreover, organisms cannot be separated with certainty from mixtures by growth in liquid media. However, if liquid media are made solid (gelatinous) by the addition of gelatin or agar, these disadvantages are overcome.

On solid media, the colonies of different bacteria have characteristic appearances so as to enable their identification. Solid media are indispensable for the isolation of pure cultures.

The classifications of media according to their physical state are: solid, solid-reversible-to-liquid, semi-solid, and liquid. Examples of solid media are pieces of potato impregnated with nutrients, serum, or egg coagulated by heating. Agar (about 2 to 4%), when added to aqueous solutions, gives a firm gel that remains unmelted at all normal incubation temperatures. Agar is generally inert, being decomposed or liquified by only a few species of marine bacteria. In these respects, it is more suitable than gelatin. A 15% solution of gelatin melts at 24°C and gelatin is decomposed by many proteolytic bacteria. Agar does not add to the nutritive properties of a medium and generally does not promote or inhibit growth. The melting and solidifying temperatures of agar solutions are not the same. At normal concentrations used in the laboratory, most bacteriological agars melt at about 95°C and solidify only when cooled to about 42°C. This ability (reversal of the physical state of agar media) is an advantage compared with the inability of coagulated serum or egg to be melted. Furthermore, the low solidifying point of agar allows heat-sensitive nutrients to be added to it in the molten state at temperatures as low as 45°C.

For special purposes, agar is added to media in concentrations that are too low to solidify. At 0.2-0.5%, it yields a semi-solid medium, with a "custard"-like consistency ideal for studying motile bacteria. Motility tests using semi-solid media are more efficient than the "hanging drop" method. At 0.05-.01% agar, convection currents within the medium are prevented and diffusion of air into the medium is retarded. Hence, semi-solid media are used for cultivating anaerobic and microaerophilic organisms.

Solid media may be distributed in test tubes with slip-on metal caps or cotton-wool plugs. The shape in which the medium is allowed to solidify depends on the method of inoculation for which it is used. The commonest shape is the "slope" or "slant", which provides a large surface area of medium for inoculation. If the medium is to be used for a "stab" or "shake" culture, the test tube is half-filled with the medium, which is allowed to solidify in the upright position. Where a large surface is necessary, as in the separation of organisms from mixtures, the medium is allowed to solidify in the form of a thin layer in a petri dish. Solid media in petri dishes are often referred to as "plates".

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TABLES

Table 2.1 - The macronutrient elements, their major physiological functions, and growth requirements

Element	Physiological function	Required concentration
Carbon	Constituent of organic cellular material. Often the energy source.	$>10^{-2}$
Nitrogen	Constituent of proteins, nucleic acids, and coenzymes.	10^{-3}
Hydrogen	Constituent of organic cellular material and water.	-
Oxygen	Constituent of organic cellular material and water.	-
Sulphur	Constituent of proteins and certain coenzymes.	10^{-4}
Phosphorus	Constituent of nucleic acids, phospholipids, nucleotides, and certain coenzymes.	10^{-4} to 10^{-3}
Potassium	Principal inorganic cation in the cell and co-factor for certain enzymes.	10^{-4} to 10^{-3}
Magnesium	Co-factor for many enzymes, chlorophylls (photosynthetic microbes), and present in cell walls and membranes.	10^{-4} to 10^{-3}

Table 2.2 - Classification of bacteria by their carbon and energy sources

Carbon Source	Energy source		
	Chemical	Light	
	Chemoorganotroph	Photoorganotroph	
Organic Heterotrophs	All higher animals, most microorganisms, nonphotosynthetic plant cells, also photosynthetic cells in the dark	Nonsulphur purple bacteria	Species
	Organic compounds (e.g., glucose)	Organic compounds	Electron donors
Carbon dioxide Autotrophs	Chemolithotroph	Photolithotroph	
	Hydrogen, sulphur, iron, denitrifying bacteria	Green cells of higher plants (in the light), blue green algae, photosynthetic bacteria	Species
	Inorganic compounds (H_2, S, H_2S, Fe(II), NH_3, etc.)	Inorganic compounds (H_2O, H_2S, S, etc.)	Electron donors

Table 2.3 - The nature of some complex materials used as ingredients in media for heterotrophs

Substance	Nature of substance	Nutritional value
Beef extract	An aqueous extract of lean beef tissue concentrated to a paste.	Contains the water soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water soluble vitamins, and salts.
Peptone	The product resulting from the digestion of proteinaceous materials, such as meat, casein, and gelatin. Digestion of the protein is accomplished with acids or enzymes. Different proteins and different methods of digestion result in different kinds of peptones.	Principal source of organic nitrogen. May also contain some vitamins and sometimes carbohydrates, depending upon the kind of proteinaceous material digested.
Yeast extract	An aqueous extract of yeast cells, commercially available as a powder.	A very rich source of the B vitamins. Also contains organic nitrogen and carbon compounds.

CHAPTER 3 - FACTORS AFFECTING MICROBIAL GROWTH

TEMPERATURE

Three terms are used to describe the effect of temperature on growth. At the optimum temperature, growth and replication are a maximum. The optimum for most pathogenic organisms is 37°C or body temperature. The minimum temperature is the lowest temperature at which growth occurs. The maximum temperature is the one at which there is inhibition due to the high temperature interfering with metabolic processes. Generally, between the minimum and optimum temperature the growth rate doubles with every 10°C rise in temperature (Fig. 3.1). Organisms can be divided into three groups with regard to their response to temperature. Psychrophilic or cryophilic organisms tolerate lower temperatures and are inhibited by moderate temperatures (Table 3.1).

The organisms of this group are found in cold lakes, salt water, spring water, and in freezing plants. Many of these organisms produce bright pigments. The mesophilic organisms are adapted to moderate temperatures, and most of the pathogens are in this group. The thermophilic organisms are found in manure piles, decaying material, hot springs, and occasionally in soil. The maximum temperatures that fungi are able to tolerate are between 56-60°C. However, some species of bacteria are able to grow at temperatures close to 100°C. As a general rule, in more extreme environments (i.e., very high or low temperatures, pH values, high salinity) fewer numbers and species of microorganisms will be found. However, almost every habitat on earth is likely to support at least one species of microorganism. This generalization can be applied to many industrial situations. For example, a chemical plant in New Jersey that manufactured liquid fungicides had severe problems due to organic deposits fouling their process lines. The organic deposit was identified as a fungus able to grow in all their fungicide preparations.

pH

The pH of a particular environment has a significant effect on the types of microorganisms capable of growing in that environment. In general, bacteria are inhibited below pH 5.3 or above pH 7.6. Fungi are capable of growth over a greater pH range than bacteria, typically from pH 3.0 to pH 8.0. At neutral pH, however, fungi are unable to compete with the more rapidly growing bacteria for nutrients, and thus fungi are usually found only in acidic habitats. A number of microorganisms are able to tolerate extremes of pH. A bacterium, *Thiobacillus thiooxidans*, can grow at pH 2.0, and a soil fungus, *Scytalidium acidophilicum*, can grow in nutrient broth containing 1.0 N H_3PO_4. *Thiobacillus thiooxidans* tolerates low external pH by maintaining a normal pH (approximately 6.5) within the bacterial cell. Soil fungi have been isolated that are able to grow at pH values between 9.0 and 10.0.

BAROMETRIC PRESSURE

Pressures of several atmospheres have no effect on bacteria, but hydrostatic pressures of several hundred atmospheres will kill most bacteria. Bacteria in deep ocean deposits are adapted to extremely high pressures. Many of them are unable to grow at atmospheric pressure, requiring pressures of over a hundred atmospheres for growth to occur.

TOXIC COMPONENTS

A large number of chemicals are toxic to bacteria if they are present at sufficient concentrations. The mechanisms of inhibition vary widely, such as inhibition of respiration ($\bar{C}N$, $\bar{N}_3$, dinitrophenol); inhibition of enzymes essential for metabolic processes (heavy metals, some organic chemicals, and antibiotics); and the destruction of cellular integrity (organic solvents, detergents, and some antibiotics). Some chemicals are very non-selective in their mode of action. NaOCl, I_2, creosote, phenol, strong alkalies, and acid react indiscriminantly with most enzymes and proteins. Antibiotics usually act in very low concentrations, entering the cell and specifically combining with a metabolite or enzyme. Some microorganisms have evolved means of tolerating or detoxifying various chemicals. Several species of fungi and bacteria are able to degrade cyanide to either formamide or ammonia and carbon dioxide. Many of the pesticides used in agriculture can be degraded by soil bacteria. Some bacteria produce metallothioneins, low-molecular-weight proteins that combine specifically with heavy metals to prevent their toxic action on the cell.

OSMOTIC PRESSURE

The bacterial cell has a higher concentration of solutes than many aqueous environments. This results in an osmotic pressure within the cell that is called turgor. The mechanically rigid cell wall of most bacteria is responsible for the cellular integrity of bacteria under a wide range of external osmotic pressures. When the osmotic pressure of the medium equals that of the cell, the environment is said to be isotonic with the cell. If the environment has a higher osmotic pressure than the cell, it is said to be hypertonic. If the environment has a lower osmotic pressure than the cell, it is said to be hypotonic. This is the normal condition for most bacteria and results in the maintenance of turgor.

Many bacteria can adjust to wide ranges in the environmental osmotic pressures. For example, *Aerobacter aerogenes* can grow in media with salt concentrations ranging from less than 0.1% to approximately 12%. A number of bacteria, such as the marine bacteria, require high salt concentrations for growth. These organisms are called halophiles. They usually are unable to grow in media containing less than 1% salt and some are even capable of growth in solutions containing 29% salt.

FREEZE-THAW

Most bacteria can survive at temperatures far below the minimal temperature for growth. If a suspension of bacterial cells is frozen rapidly, it can be kept at temperatures as low as -194°C for long periods with almost no loss in viability. However, freezing does kill some bacteria immediately. In particular, if bacteria are frozen slowly, the formation of ice crystals within the cell results in the disruption of cellular structure.

One means of preserving bacterial cultures is lyophilization. A suspension of bacterial cells is rapidly frozen at the temperature of dry ice, and then dried under high vacuum. These cells, if protected from air, remain viable for many years.

IRRADIATION

The effect of radiation on bacterial cells is dependent on wavelength. Light is essential for the growth of the photosynthetic green and purple bacteria, but radiation is either useless or harmful to most other bacteria. Radiation of wave-lengths greater than 9000 Å (infrared) simply generates heat in the absorbing matter. Wavelengths between 2000 and 9000 Å are sufficiently energetic to produce photochemical reactions (photosynthesis, etc.). The ultraviolet region of the spectrum can be either bacteriocidal or detrimental to bacterial growth. Nucleic acids absorb radiation around 2600 Å, and cell death from exposure to this wavelength can be attributed to the destruction of the nucleic acids. At sublethal intensities of ultraviolet light, mutations may occur. Ionizing radiation (X-ray, alpha particles, etc.) also has both lethal and mutagenic effects on bacteria. Peroxides produced from water and cell constituents by ionizing radiation are primarily responsible for the observed cell damage.

OXYGEN

Bacteria can be divided into four groups in their response to free oxygen. *Aerobic bacteria* require oxygen for respiration, although some aerobes are able to use alternate "electron-acceptors" in place of oxygen (i.e., NO_3^-, NO_2^-, SO_4^{-2}, and Fe^{-3}). Denitrifying bacteria, which are able to reduce oxidized nitrogen compounds to nitrogen gas, are used to remove nitrogen from wastewater (municipal effluents, fertilizer manufacturing waste, and some mine effluents).

Anaerobic bacteria grow in the absence of oxygen and, in fact, some anaerobes are killed by exposure to oxygen. Anaerobes are unique in that they can ferment sugars and amino acids to organic acid or alcohols.

Facultative anaerobic bacteria can grow under either aerobic or anaerobic conditions. Microaerophilic bacteria grow in the presence of small amounts of atmospheric oxygen.

TABLE

Table 3.1 - The effect of temperature on the three major groups of organisms

	Optimum	Minimum	Maximum
Psychrophilic	15-20°	0°	30°
Mesophilic	25-40°	5-25°	43°
Thermophilic	50-55°	25-45°	60-90°

FIGURE

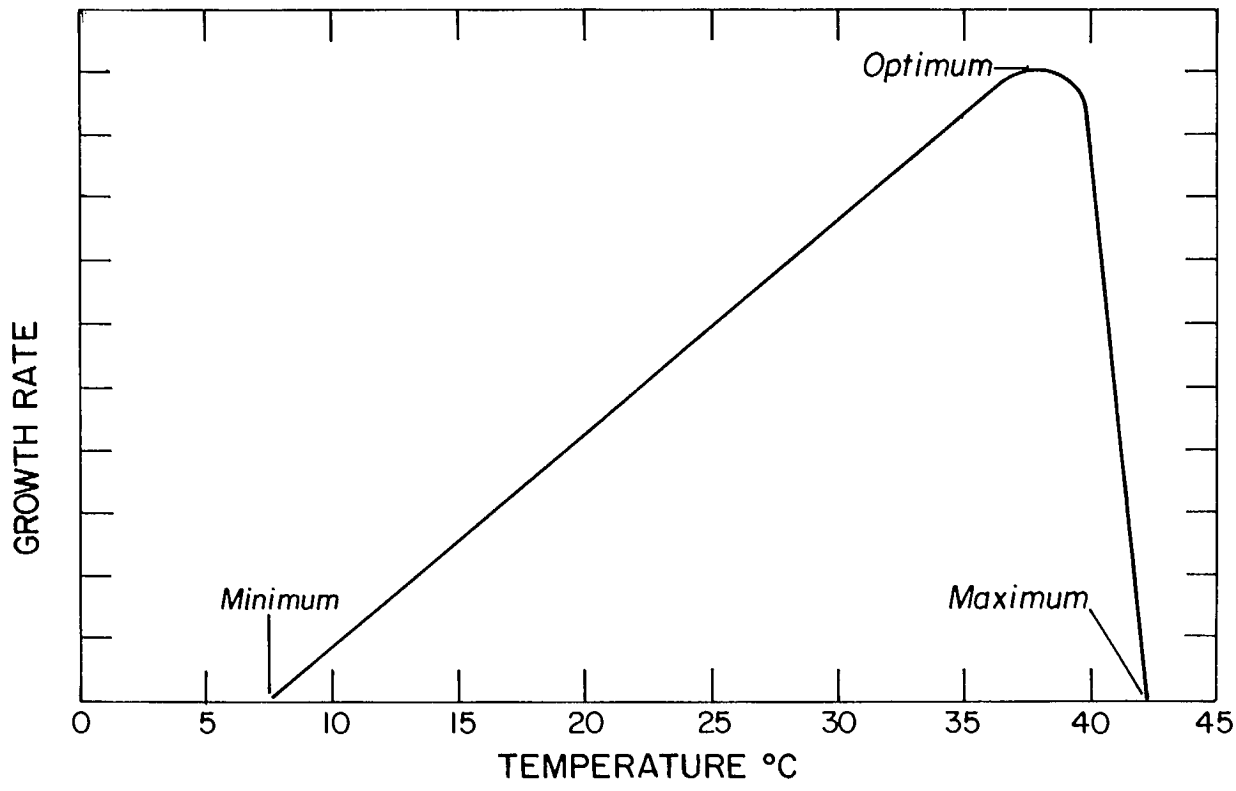


Fig. 3.1 - Growth curve showing effect of temperature on growth rate

CHAPTER 4 - GROWTH CHARACTERISTICS

Microorganisms can be divided into two groups: phototrophs (which obtain their energy from light) and chemotrophs (which obtain their energy from the oxidation of chemical compounds). They can be further divided into autotrophs and heterotrophs depending on whether they obtain their carbon for cellular structure from CO_2 (autotrophs) or organic carbon (heterotrophs) (Table 4.1).

AUTOTROPHS

Autotrophs obtain their energy from either light (photoautotrophs) or from the oxidation of inorganic compounds (chemoautotrophs). Autotrophs can synthesize all their cellular constituents from CO_2 and inorganic chemicals. The oxidation of inorganic compounds provides less energy than the oxidation of organic compounds, and the autotrophs must expend a great deal of energy to synthesize cellular constituents from CO_2 and simple inorganic compounds. Thus, the growth rates of the autotrophs are usually much lower than most of the heterotrophs (Fig. 4.1).

One of the most important bacteria in the mining industry is *Thiobacillus ferrooxidans*. *T. ferrooxidans* oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) in order to obtain energy. A number of species in the genus *Thiobacillus* (*T. thioparus*, *T. ferrooxidans*, and *T. denitrificans*) are also capable of oxidizing elemental sulphur to sulphuric acid. *T. denitrificans* is unique in that it can oxidize sulphur under anaerobic conditions using nitrate in place of oxygen. It is very difficult to enumerate *Thiobacillus ferrooxidans*, due to its low growth rate and its inability to form observable colonies on agar plates. Organic compounds are toxic to many of the autotrophic bacteria.

Another important group of autotrophs is the nitrifying bacteria, which oxidize ammonium to nitrate in the soil. *Nitrosomonas* sp. oxidizes ammonium to nitrite, and *Nitrobacter* sp. oxidizes nitrite to nitrate.

HETEROTROPHS

Heterotrophs oxidize a wide range of organic compounds to obtain energy. Sugar, amino acids, aromatic compounds, hydrocarbons, and some heterocyclic compounds can be oxidized by heterotrophic bacteria. Also, heterotrophs utilize many organic compounds directly for the synthesis of cell components. Sugars, amino acids, nucleosides, and vitamins can be taken up and incorporated into cell constituents by heterotrophs. Most heterotrophs require certain vitamins and amino acids because they are unable to synthesize these compounds themselves.

CO-METABOLISM

Co-metabolism is that partial degradation of an unusual compound (usually synthetic) by a microorganism because the compound resembles a natural substrate

for one of the microorganism's enzymes. The organism is usually unable to degrade the compound further or to use it as a carbon or nitrogen source. Resistant compounds can be degraded completely in soil via co-metabolism due to the large variety of microorganisms in soil, each of which may carry out one or two steps in the sequence of degradative reactions. Co-metabolism plays a role in the degradation of many pesticides and petroleum products in soil.

SEQUENTIAL METABOLISM

During sequential metabolism two or more microorganisms carry out a series of reactions, with each microorganism completing one or more of the reactions in the sequence. The nitrifying bacteria are one example of sequential metabolism. *Nitrosomonas* sp. oxidizes ammonium to nitrite, and *Nitrobacter* sp. oxidizes nitrite to nitrate. Sequential metabolism is often necessary for the degradation of complex organic compounds in soil.

TABLE

Table 4.1 - Major nutritional types of bacteria

Type	Source of energy for growth	Source of carbon for growth
Phototrophs		
- autotroph	light	CO_2
- heterotroph	light	organic compound
Chemotroph		
- autotroph	oxidation of inorganic compound	CO_2
- heterotroph	oxidation of organic compound	organic compound

FIGURE

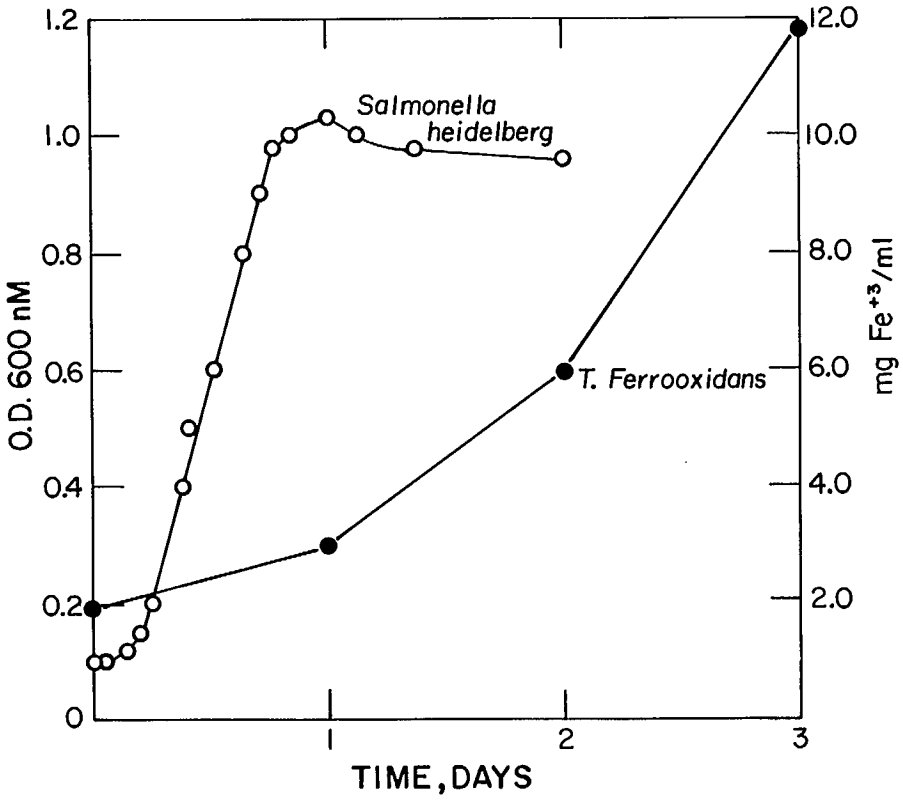


Fig. 4.1 - Growth rate comparison of autotrophs and heterotrophs

CHAPTER 5 - SIMPLE STERILIZATION TECHNIQUES

INTRODUCTION

Species of microorganisms vary in the ease with which they may be destroyed, removed, or inhibited; the situations in which they occur also vary greatly (e.g., blood, tissue, food, water, sewage, and soil, etc.).

There are four main reasons for killing, removing, or inhibiting organisms:

1. Prevent infection of humans, animals, or plants.
2. Prevent spoilage of foodstuffs.
3. Prevent contamination of an industrial process that requires a pure culture.
4. Prevent contamination in microbial research.

DEFINITION OF TERMS

Sterilization: The freeing of an object or substance from all life of any kind. Sterilization is accomplished mainly with heat, gases, chemicals, gamma or u.v. irradiation, or by filtration.

Disinfection: The killing or removal of all organisms capable of causing infections, i.e., the removal or death of all pathogenic organisms. Disinfection does not necessarily mean sterilization, although some processes of disinfection do accomplish sterilization. Disinfection is generally accomplished with chemicals, and is usually effective against vegetative cells but not spores.

Microbistasis: Microbistatic conditions do not immediately kill microorganisms but inhibit multiplication so that the microorganisms die only after a few hours. Important microbistatic agents are dessication, low temperature, antibiotics (particularly in low concentration), sulfonamide drugs, and dyes such as crystal violet.

With the exception of filtration, each sterilization method can be characterized by the rate of the killing process. Sterilization is a probability function, since bacterial cells usually die at an exponential (logarithmic) rate (Fig. 5.1).

Factors influencing microbial death that must be considered in the choice of a sterilization procedure include the following:

1. The design and chemical composition of the item.
2. The biological and physical state of the microorganism prior to sterilization.
3. The inherent resistance of the microorganism to the sterilizing process and the resulting death rate of the microorganism.

4. The initial population of the microorganisms to be sterilized.
5. The intensity of the sterilizing agent.
6. The duration of exposure to the sterilizing environment.

HEAT STERILIZATION

Heat is the most common method employed for the sterilization of material. Both moist heat and dry heat are used. Steam sterilization in an autoclave is the most common technique used to sterilize samples for microbiological research. The standard conditions for autoclaving are to use saturated steam under 15 psi pressure at 121°C for 15 min. In many cases, the sample configuration will affect the time required for complete sterilization. If a number of large samples are loaded in an autoclave, longer autoclaving times are necessary. To determine the effectiveness of an autoclave under a particular set of conditions, indicator organisms that exhibit a predictable death rate when exposed to a defined treatment can be used: spores of either *Bacillus* spp. or *Clostridium* spp. can be used as these biological indicators. Indicator strips or tape that changes colour when exposed to a certain temperature for a defined period of time can also be used. Moist heat is used for the sterilization of culture solutions, glassware, and equipment that is insensitive to heat or moisture.

Dry heat can be used for sterilizing glassware, oils, instruments, and materials that are moisture sensitive. Hot forced air and infrared energy have been used for dry-heat sterilization. Dry heat is less efficient than moist heat, requiring higher temperatures for longer durations. Dry heat inactivates microorganisms by oxidation of intracellular constituents. Bacterial spores are more resistant to dry heat than vegetative bacteria.

GASES

Gases are used for the sterilization of materials that are heat sensitive. Ethylene oxide is the most commonly used gas because of its wide compatibility with various materials. The major disadvantage of ethylene oxide is that it is very flammable and explosive (explosive limits in air are 3-100%). The diffusion of ethylene oxide, moisture, and heat into materials can be a limiting factor. Ethylene oxide effectively kills all vegetative cells and spores. Propylene oxide is identical to ethylene oxide in its mode of action but is not as widely used.

Gaseous formaldehyde can be used as a space and surface sterilant. Gaseous formaldehyde can be generated by heating paraformaldehyde, which is a mixture of polyoxymethylene glycols containing 90-99% formaldehyde. Formaldehyde is active against vegetative cells, spores, and viruses. After sterilization, formaldehyde gas can be neutralized by ammonia gas to form hexamethylene tetramine.

RADIATION

Ionizing or nonionizing radiation may be used. Nonionizing radiation includes infrared, ultraviolet, and radiofrequency. Ionizing radiation can be particle beams (beta particles or electrons) or electromagnetic radiation (X rays and gamma rays). Gamma rays can be generated from nuclides such as cobalt-60 or cesium-137. Ionizing radiation is a reproducible and consistent means of sterilization. Electron beams are currently used for industrial sterilization but have the disadvantage of a lower penetrating power than electromagnetic radiation.

Ultraviolet radiation in the 254-nm wavelength range has been used for the sterilization of surfaces, but has limited application due to its low penetrating power. Vegetative cells are far more susceptible to ultraviolet radiation than are bacterial spores.

Although radiofrequency electromagnetic radiation (i.e., microwave) has been considered for use in the food industry, it has not been accepted as a means of sterilization. The killing action of radiofrequency radiation is species-specific and different frequencies may be required to kill all types of microorganisms. However, radio frequency radiation may have potential if it can be used in conjunction with disinfectants.

FILTRATION

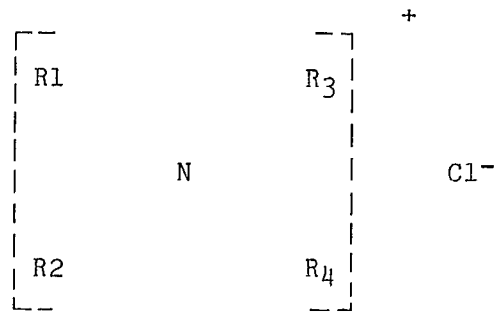
Filters function by entrapping microorganisms within the porous structure of the filter matrix. Vacuum or pressure is required to move the solution through the filter. Filters are primarily used for solutions that are heat-labile. The filter most commonly used for sterilization removes all particles above 0.2 μm in size, which includes all fungi, most bacteria, and bacterial spores. However, it does not remove viruses.

DISINFECTANTS

Disinfectants may be bacteriocidal or bacteriostatic in their action. The most well-known use of disinfectants is to provide sterile conditions in hospitals. However, industrial uses of disinfectants or antimicrobial agents are considerable, and during 1973, 238 million pounds of antimicrobial agents were sold in the United States. Microorganisms are responsible for a large number of industrial problems such as biofouling, biocorrosion, and the microbial decomposition of various products.

QUATERNARY AMMONIUM COMPOUNDS

Quaternary ammonium compounds have the following general structure:



R_1, R_2, R_3, and R_4 are alkyl groups that may be all identical or different. There are a large number of structural possibilities for these compounds, but certain structures appear to be more effective than others as antimicrobial agents. The most useful antimicrobial agents, in terms of cost and effectiveness, have several features in common: a long, straight chain alkyl group and a molecular weight between 380 and 420. Substitutions in the structure of the quaternary ammonium compounds with methyl, ethyl, or chlorobenzyl groups significantly improve antimicrobial activity. The polymeric quaternary ammonium salts are a new class of disinfectants that have excellent antimicrobial activities as well as being relatively non-toxic. Two examples of this type of product are: WSCP poly [Oxyethylene (dimethylamino) ethylene (dimethylimino) ethylene dichloride]; and AM2046-4-[1-tris (2-hydroxyethyl) ammonium chloride-2-butenyl] poly (1-dimethyl ammonium chloride-2-butenyl)-w-tris (2-hydroxyethyl) ammonium chloride.

PHENOLS

Phenols have generally been the largest-selling class of antimicrobial products. Phenolics derive their antimicrobial activity from several modes of action: (a) coagulation of cell proteins; (b) increased hydrogen ion concentration around the cell; (c) lower surface tension that disrupts the cell wall as well as some metabolic processes; and (d) phenols have been shown to be capable of inactivating various cellular enzymes.

The chlorophenols constitute the greatest proportion of phenol derivatives used as industrial disinfectants. Pentachlorophenol has excellent antifungal activity, has good antibacterial activity (particularly against Gram-positive bacteria), is water soluble, and is relatively inexpensive. Pentachlorophenol is used as a wood preservative, for water treatment, as a slimicide in pulp and paper manufacture, for the preservation of leather, and as a preservative for drilling muds. A number of other substituted chlorophenols such as 2, 4, 5 trichlorophenol and hexachlorophene (2,2-methylenebis-3, 4, 6-trichlorophenol) are used for particular applications.

MERCURY COMPOUNDS

Mercury compounds have been used to protect a variety of coatings from microbiological attack for many years. In the past, mercury compounds have been banned for some applications (i.e., preservatives for oil-based paints), and in the future may be banned for many other applications due to their extreme toxicity.

HETEROCYCLIC COMPOUNDS

Heterocyclic compounds possess a cyclic structure with two or more types of atom in the ring, one of which is carbon. The number and variety of heterocyclic compounds with antimicrobial activity is extensive, and many of these have been discovered relatively recently. Only the industrially useful biocides in this group will be discussed. The first heterocyclic compound to be evaluated for biocidal activity was 8-hydroxyquinoline. Heterocyclic compounds comprise a large number of the fungicides used in agriculture but few of these are used as industrial biocides.

Mixtures of heterocyclic compounds [5-chloro-2-methyl-4-isothiazolin-3-one; and 2-methyl-4-isothiazolin-3-one (trade name Kathon 886)] have been shown to inhibit bacteria, fungi, and algae in cooling-tower waters. Microbial fouling of cooling-tower waters interferes with both water flow and heat transfer.

Organoborate esters have been used to prevent the growth of microorganisms in petroleum products, particularly in jet fuel. The product currently marketed for this purpose, Biobor JF™, is a mixture of organoborinanes.

FIGURE

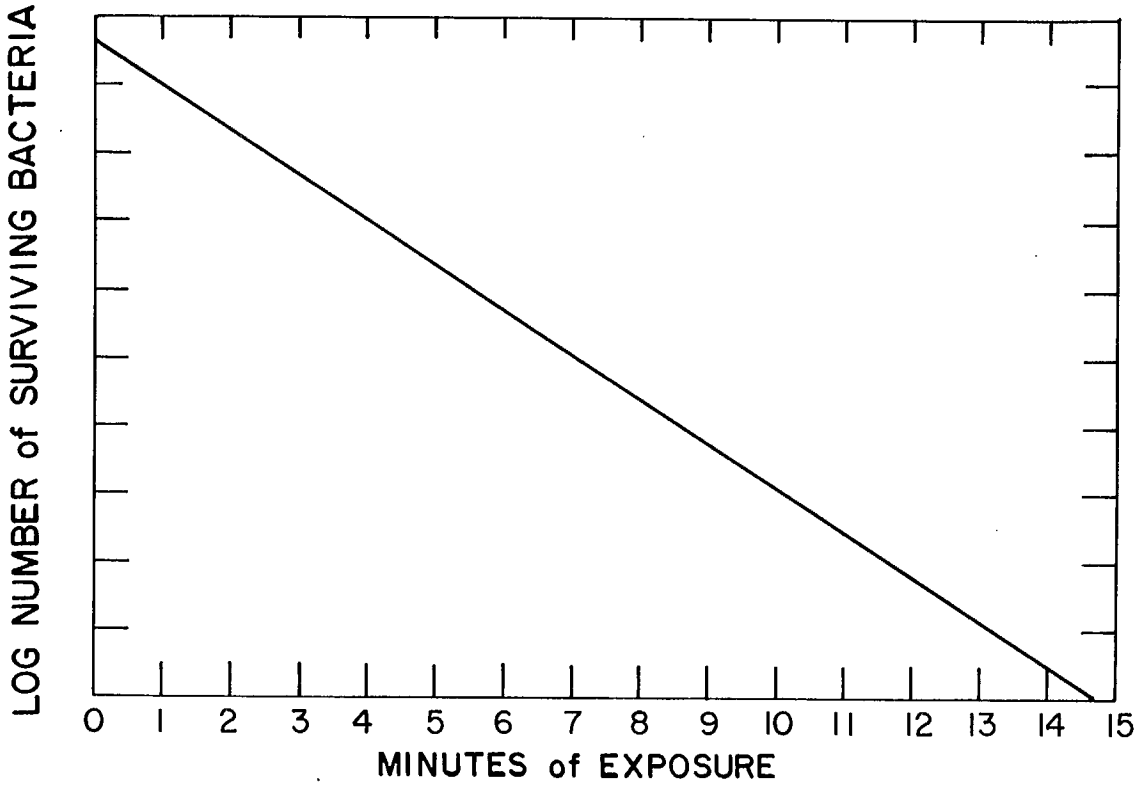


Fig. 5.1 - Sterilization as a probability function of exposure time

CHAPTER 6 - COLLECTION OF SAMPLES FOR MICROBIOLOGICAL ANALYSIS

OBJECTIVES

The two most important objectives in collecting samples for microbiological analyses are: (1) to bring samples to the laboratory with a minimal loss in viability; and (2) to avoid contamination of the sample with other microorganisms.

SAMPLE COLLECTION

Liquid samples should be obtained in sterile, hard plastic bottles. Glass bottles are subject to breakage, and soft plastic bottles tend to release toxic organic compounds into the solution. Solid samples should be taken with a sterile spatula and placed in a sterile container (sterile plastic bag or a sterile, wide-mouth plastic bottle). Spatulas can be sterilized simply by dipping in alcohol and then flaming. The spatula should be allowed to cool before the sample is taken.

If biofouling is suspected in a process line, certain precautions are necessary in order to obtain a good sample for microbiological analysis. The line should be partially drained to flush out stagnant water, and then the liquid sample should be taken. An alternative approach, if the line is small, would be to remove the fouled section and then place it in a sealable plastic bag. The contaminated pipe and any relevant process information should be sent by surface courier to the laboratory for analysis.

SAMPLE STORAGE

If it is necessary to store samples, they should be stored at 4°C. Samples for microbiological analysis should not: (1) be allowed to dry out; or (2) be allowed to freeze. If samples are frozen to begin with, then they should remain frozen until they can be processed. If samples are suspected of containing aerobic bacteria, an air space should be left in the sample container. If samples are suspected of containing anaerobes, the sample bottle should be completely filled and oxygen should be excluded, if possible. Most samples for microbiological analyses will contain anaerobes and facultative anaerobes.

SHIPPING OF SAMPLES

Samples should be shipped by courier. The low temperatures and lack of pressurization in cargo aircraft will be detrimental to many microorganisms. Also, the less time that a sample is in shipment reduces the probability of contamination or death of organisms within the sample.

CHAPTER 7 - MICROBIAL APPLICATIONS AND PROBLEMS IN THE MINERAL INDUSTRY

Numerous CANMET reports and papers from the literature are provided to cover some of the topics presented in this section. Other topics, for which there are no relevant reports or publications, are presented below.

AUTOTROPHIC MINERAL LEACHING

Over the past three decades, copper has been recovered from waste rock and low-grade ores by heap leaching using indigenous *T. ferrooxidans*. Mineral leaching is accomplished via two mechanisms. The first mechanism is direct leaching, in which the thiobacilli attach to the mineral sulphide crystals within the ore matrix, solubilizing the metal by oxidation of the sulphide moiety to sulphuric acid and simultaneously oxidizing associated ferrous iron to ferric iron. The second mechanism is indirect leaching, wherein the organisms oxidize Fe^{+2} in solution to Fe^{3+}. The ferric ion, in turn, oxidizes the metal ion and is simultaneously reduced to ferrous ion. The bacteria then re-oxidize the ferrous ion back to ferric ion, and the cycle is reinitiated.

Many of the physical and chemical factors that affect bacterial leaching of metals are outlined in the enclosed articles.

HETEROTROPHIC MINERAL LEACHING

To date, only the thiobacilli and *Sulfolobus* spp. have been used for large-scale microbial leaching of metal ores for the recovery of base metals. In particular, *Thiobacillus ferrooxidans* has been used to leach low-grade copper and uranium ores. However, the thiobacilli are only effective for ores containing appreciable quantities of sulfide. Oxide, carbonate, and silicate ores are unsuitable for leaching by the chemolithotrophic bacteria. In particular, 80% of known nickel reserves are in lateritic deposits. Because of the low metal contents of these ores, extraction of nickel by conventional metallurgical techniques is very costly. In Canada, appreciable quantities of nickel occur in pentlandite deposits that are not fully exploited due to their refractory nature.

One of the major problems affecting the leaching of ore by *Thiobacillus ferrooxidans* is reduced permeability, which results in low leaching rates and insufficient oxygen transfer for microbial growth. Accumulations of elemental sulphur or iron salts, such as jarosite, are the principal causes of low permeability. Citric acid has been considered as a means of preventing the plugging of flow channels within leach dumps by chelation and solubilization of iron. The addition of organic acids or chelating agents to a solution of metal ions has been found to extend the pH range over which these metals are soluble.

Production of organic acids by heterotrophic organisms has been implicated in the weathering of rocks and minerals. It has been postulated that organic acids weather aluminosilicate minerals by acid hydrolysis, as well as by

chelation of the cations contained within these minerals. Nickel can be extracted from lateritic ores by organic acids, but limonite is completely resistant to organic acids. Citric acid has been found to be the most effective metal extractant but lactic, oxalic, glycolic, and aspartic acids have also been shown to be effective. A 0.5 M citric acid solution solubilized 70% of the nickel in a lateritic ore after 15 days. Similar results were obtained with a citric acid-producing culture of *Penicillium simplicissium*. However, citric acid also extracts large quantities of iron and may not be the most suitable compound for the extraction of nickel. Potentially, other organic acids or amino acids could be more selective for the extraction of the metal of interest.

BIOADSORBANTS

Certain species of microorganisms have been observed to accumulate large quantities of metals of interest. These metals of interest include metals that are toxic to humans (e.g., cadmium) and metals of economic value (e.g., copper, silver). Some organisms have been shown to accumulate metal contents as high as 40% of the dry weight of the biomass (Table 7.1).

The ability to accumulate a particular metal varies among the different microbial species; some metals are accumulated to a greater degree than others. Metal accumulation has been observed to occur in association with extracellular products (e.g., polymers), at or within the cell membrane, and intracellularly. Biosorption is a physico-chemical reaction between the dissolved metal and the cellular components, in many respects similar to ion exchange. Insoluble metal species may be trapped in extracellular polymers produced by the microbes. Specialized cell functions, such as ion transport, metabolically mediate metal uptake. Moreover, metals may also accumulate within microbial cells by undefined non-metabolically mediated processes.

Physiological conditions of the cells, the chemical state of the reactive sites on the cells or cell products, and the chemical state of the metals are factors that influence the uptake of metal values.

EXTRACELLULAR ACCUMULATION

The removal of metals from biological waste systems has been widely reported. Organisms such as *Zoogloea* produce microbial polymers that are capable of physical entrapment of the precipitated metals and the complexation of soluble metal species by charged entities of the polymers. Although most polymers are neutral polysaccharides, others often contain compounds such as hexosamines, uronic acids, and phosphates in organic matrices that are capable of forming complexes with metal ions. The complexation of metal ions by charged organic groups on cell walls is similar to ion-exchange-type reactions and hence susceptible to the chemical environment and the presence of other metal ions.

The formation of extracellular polymers and the wall composition of microorganisms are not only species-dependent, but also subject to the conditions under which the organisms are grown. For example, McMurrough and Rose (1) have shown that the protein and phosphate contents of yeast cell walls are higher for organisms cultured at a slow growth rate.

INTRACELLULAR METAL UPTAKE

Microorganisms are exposed to both essential (nutritionally speaking) and toxic metals in the environment. Hence, the organisms have mechanisms whereby they control their intracellular metal concentrations. The organisms have transport mechanisms for essential metal ions. It is postulated that intracellular accumulation of other non-essential metals is a result of the lack of specificity in the metal transport mechanisms. Although a number of metals such as Ag, As, Hg, Zn, Pb, and Cd are toxic, certain microorganisms are resistant to them. The specific mechanisms by which these metals are taken up by the cells have not been established. Intracellular metal deposition can also occur due to non-metabolically mediated processes. It is postulated that certain organisms are able to produce specific metal-binding proteins and that the production of these proteins can be induced. Metal-binding protein, metallothionein, has been identified in several genera of microorganisms.

Commercial utilization of biomass for the removal of metals from process or waste streams has not been reported. There have been a number of proposals for utilizing algae and photosynthetic organisms to recover arsenic and lead, respectively, from mining and milling effluents.

EFFLUENT WASTE TREATMENT

CYANIDE

Cyanidation followed by zinc precipitation is the major process used in Canadian gold mills. The gold concentrate is treated with lime and sodium (or calcium) cyanide, and the mixture is then contacted in a series of countercurrent decantation cells. The mixture and the supernatant are treated with zinc to precipitate the gold. Approximately 50% of the gold-free or barren solution is discharged from the mill to prevent the build-up of zinc and other metals in the circuit. The tailings slurry also contains appreciable concentrations of cyanide.

In gold mill effluents, cyanide can exist as free cyanide (CN^-, HCN) soluble salts [$Ca(CN)_2$, $Hg(CN)_2$, KCN, NaCN]; relatively insoluble compounds [$Zn(CN)_2$, $Cd(CN)_2$, CaCN, $Ni(CN)_2$, AgCN]; weak complexes [$Zn(CN)_4^{-2}$, $Cd(CN)_3^{-1}$, $Cd(CN)_4^{-2}$]; moderately strong complexes [$Cu(CN)_2^{-1}$, $Cu(CN)_3^{-2}$, $Ni(CN)_4^{-2}$, $Ag(CN)_2^{-1}$]; and strong complexes [$Fe(CN)_6^{-4}$, $Co(CN)_6^{-4}$]. Barren solutions contain total cyanide levels of 40-750 ppm and tailings repulp streams contain 13-280 ppm of cyanide.

Both chemical and biological techniques have been used for the treatment of gold mill effluents. The best chemical technique for the treatment of cyanide-containing wastes is alkaline chlorination. The destruction of cyanide by alkaline chlorination may be accomplished by chlorine gas, calcium hypochlorite, or sodium hypochlorite. Cyanide can be oxidized by either ozone or hydrogen peroxide. A new process has been developed by Inco that involves the addition of SO_2 and air at pH 9.0 in the presence of a copper catalyst. Cyanide is destroyed by the following reaction:

$$CN^- + O_2 + SO_2 + H_2O \text{ -------- } CNO^- + H_2SO_4$$

Cyanide effluents can also be acidified to allow volatilization of HCN, followed by reneutralization. Various adsorption procedures such as ion exchange, ion floatation, and activated carbon have been considered. Electrolytic decomposition and the conversion of cyanide to less toxic forms have also been investigated as alternate methods of treatment. Most of the chemical techniques have a number of problems: (1) complex cyanides are not degraded; (2) residual cyanide levels can still be too high; and (3) other toxic reaction products can be formed.

NITRITE

Biological treatment of cyanide-containing wastes has been considered as an alternative to chemical treatments. Laboratory and pilot-plant studies have been carried out with trickling filters, activated sludge digesters, extended aeration, fluidized-bed reactors, rotary biological contactors, and immobilized enzymes. At present, Homestake Mining Company is using rotating biological contactors for the detoxification of gold mill effluent. In their process, the first reactor degrades cyanides and thiocyanates to ammonia, and in the subsequent stage ammonia is biologically oxidized to nitrate. The process removes free and complexed cyanides, as well as toxic metals, and reduces the effluent cyanide concentration to very low levels. The main disadvantages of this process are: (1) high capital cost; and (2) cyanide concentrations above 200 ppm that are inhibitory to the process.

Immobilized fungal cells have also been considered for the degradation of cyanide in gold mill effluents, and ICI Ltd. is currently producing immobilized cells for this purpose. *Stemphylium loti* produces an enzyme (cyanide hydratase) that hydrolyzes cyanide to formamide:

$$CN + H_2 \text{ -------- } HCONH_2 \text{ (formamide)}$$

The advantages of this system are: (1) low capital cost; (2) initial cyanide concentrations of up to 8000 ppm can be detoxified; and (3) no toxic reaction products are formed. The disadvantages of this system are: (1) the immobilized cells have limited stability; (2) the enzyme will not hydrolyze metal-cyanide complexes; and (3) the technology has not been proven beyond laboratory-scale experiments.

Biological removal of nitrogen from various waste streams is carried out sequentially using nitrification and denitrification. Municipal waste contains organic nitrogen, ammonium, and nitrate. Nitrates and nitrogenous compounds in mining and mineral-processing wastewaters originate from the use of nitrate-based explosives, and the use of nitrates in processing, such as the elution of ion exchange resins. During the initial step of nitrification, bacteria convert ammonium to the nitrate form so it can subsequently be denitrified. The denitrifying bacteria reduce various oxides of nitrogen to nitrogen gas by the following scheme:

$$NO_3^- \text{----- } NO_2^- \text{----- } N_2O \text{ ----- } N_2$$

Both nitrous oxide and nitrogen are lost as gases. Bacteria vary in how many of these reduction steps they can carry out. Wastewater treatment systems usually contain a mixed culture of denitrifying bacteria. The major denitrifying bacteria are heterotrophs, and are members of the genera *Pseudomonas* and *Alcaligenes*. A few autotrophic denitrifying bacteria have been identified, such as *Thiobacillus denitrificans*.

A readily utilizable carbon source is necessary for denitrification to occur. Because of its low cost, methanol is commonly used. Other inexpensive carbon sources for denitrification that appear promising are brewery waste, chemical process waste, cornstarch waste, molasses, whey, sulphite liquor, and winery residues. The optimum pH is 7.8 and the temperature around 40°C. The maximum oxygen concentration that can be tolerated by suspended cultures is 0.5 mg/L but oxygen concentrations up to 2.0 mg/L can be tolerated by attached cultures. Denitrifying bacteria can be inhibited by sulphate, sulphide, acetylene, cyanide, and heavy metals.

A number of systems can be used for biological denitrification: suspended growth reactors, rotating biological contactors, packed-bed reactors, and fluidized-bed reactors. There are no known cases where denitrification has been used in the mining industry. However, denitrification has been used to treat other effluents similar to mining industry wastes.

BIOFOULING

Because of their ability to attach to solid surfaces and obtain nutrients from solutions passing over their surfaces, bacteria often cause plugging or constriction within waterlines or pipelines. Such occurrences increase industrial operating costs and, in some cases, can alter the quality of the product or decrease its per cent recovery.

Once the organisms have established themselves on a surface, they may form mucoidal capsular material that may trap particulates; be sloughed off, plugging fine orifices or valves; or may secrete enzymes that damage gaskets, valve seats, etc.

One example of a mining operation biofouling problem is presented in the enclosed handouts.

CARBOGEL

Carbogel is the trademark name for a 70% suspension of pulverized coal in water. A patented stabilizer is added during Carbogel preparation to form a coal in water emulsion. Cape Breton Development Corporation, in cooperation with the National Research Council of Canada, is assessing the use of Carbogel for power generation. Test burns are being conducted in Chatham, New Brunswick, to test burner nozzle designs and Carbogel burning efficiency.

Because Carbogel is prepared in a plant in Sydney, Nova Scotia, the material was shipped in tank cars to Chatham, New Brunswick. Early in these studies, one tank car of Carbogel arrived in Chatham as a solid mass of coal overlain with water. The emulsion had cracked.

Investigation showed the emulsifying agent had been degraded, and high bacterial counts (10^6/mL) were observed in the preparation.

Subsequent studies showed that the washed coal used in preparing Carbogel contained 10^4-10^5 bacteria/g of coal. Since the wash water was partially recycled within the Victoria Junction Wash Plant, high bacterial numbers were observed. Furthermore, once the coal had been cleaned, it was stockpiled adjacent to the Wash Plant, becoming contaminated with soil organisms due to dust and atmospheric washout during precipitation events.

To add to the problems, the well water utilized for Carbogel preparation has been contaminated with surface soil, resulting in bacterial counts of 10^2-10^3/mL.

An investigation was initiated to determine the best biocide to be added to the formulation of Carbogel. Since Carbogel is used as a liquid fuel, the biocide could not contain Cl^- or nitrogen. During combustion, Cl^- ions would result in HCL, a corrosive; nitrogenous compounds would result in nitrous oxides, causing atmospheric pollution.

The soil organisms were identified and laboratory studies showed that they were all sensitive to formaldehyde. Further studies showed that the Carbogel could be sterilized, and remain sterile for 14 days, by adding 5 ppm of formaldehyde.

MICROBIAL CORROSION

Initial research on microbial corrosion of metal concentrated on the role of the anaerobic sulphate-reducing organisms. However, recent studies have indicated that all groups of microorganisms - bacteria, yeast, and fungi - can cause metal corrosion. Furthermore, the major group of organisms responsible for corrosion appears to be those involved in the microbial sulphur cycle (Fig. 7.1).

Recent research indicates that the partially oxidized sulphur intermediates (SO_3, S_2O_3, S_3O_6, S_xO_y), in combination with chloride ions, are extremely aggressive toward metals including aluminum, stainless steel, pipeline steel alloys, etc. Several articles are enclosed covering various aspects of corrosion by a variety of microorganisms.

REFERENCE

1. McMurrough, I., and Rose, A.H. *Biochem J* 105:189-203; 1967.

TABLE

Table 7.1 - Levels of metal accumulation by microorganisms

Metal	Organism	Metal uptake (g metal per g cells dry weight)
Ag	Mixed Culture	0.32
	T. ferrooxidans	0.25
Co	*Zoogloea* sp.	0.25
Cu	*Zoogloea* sp.	0.34
Ni	*Zoogloea* sp.	0.13
Pb	*Citrobacter* sp.	0.35
U	*Rhizopus arrhius*	0.18
Th	*Rhizopus arrhius*	0.17

FIGURE

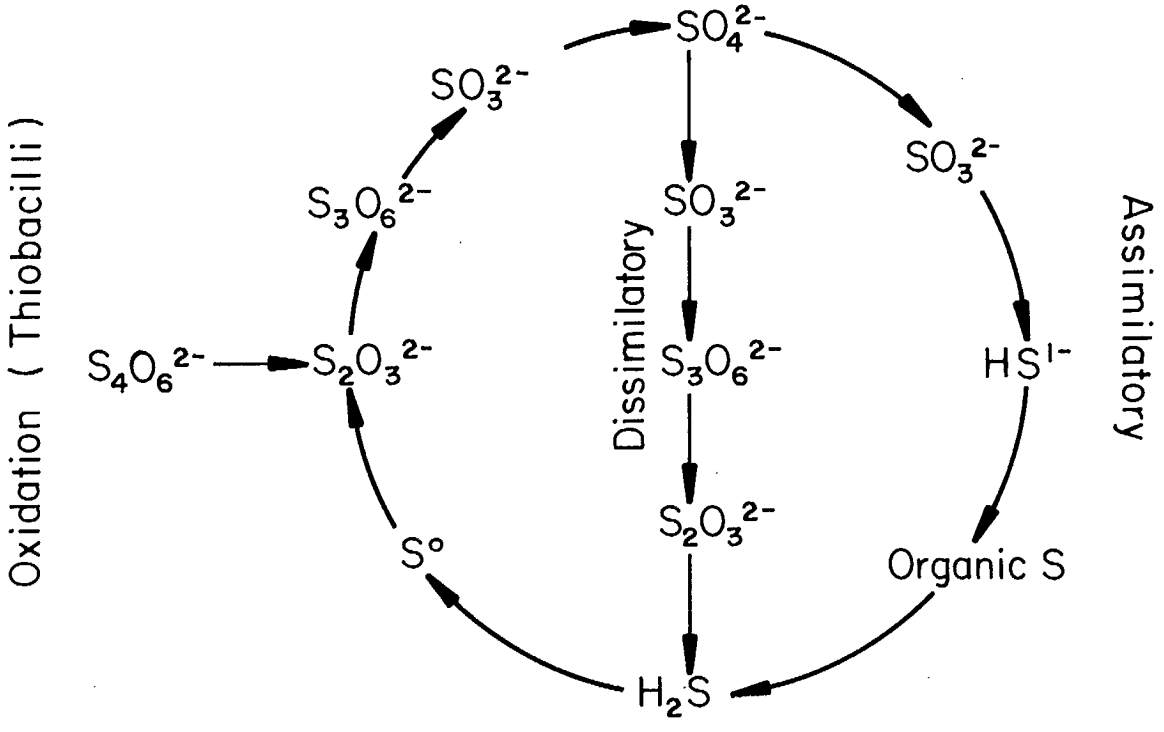


Fig. 7.1 - Sulphur cycle

CHAPTER 8 - BIOENGINEERING IN THE MINERAL INDUSTRY

FERMENTATION

Biochemical processes are involved with "fermentation". From the bioengineering point of view, fermentation refers to chemical changes produced in organic compounds (substrates) through the activity of living microorganisms. Because all biological reactions are catalyzed by enzymes, fermentation in a broad sense is the process in which chemical changes are brought about in a substrate by the action of enzymes, whether the enzymes occur within the cell (*in vivo*) or are in the cell-free form (*in vitro*). A reaction vessel in which fermentation is carried out is called a fermenter. The flow pattern of the liquid in a vessel is dependent on the geometric arrangement of the vessel components; these also contribute to the performance of a fermenter in terms of yield and production rate.

Various fermenter types are available depending on the mode of operation (batch or continuous), as well as the arrangement for aeration and mixing, etc. Microbiological processes carried out on a batch basis involve a number of steps associated with the development of the culture, followed by feed and production stages. The time required for a batch fermentation varies from hours to weeks depending upon the organism, conditions used for fermentation, and the biochemical conversion desired.

The fundamental difference between batch and continuous fermenters is that in the latter case the contents of the vessel are at *steady-state*, i.e., they do not vary with time. The concentrations of the components of the medium (substrate and products) and the microorganisms are all in equilibrium. The steady-state condition can be achieved in two ways: "chemostatic" or "turbidostatic" operating conditions. Chemostatic operation involves adjusting the flow rate to the fermenter at a chosen constant value and allowing the concentrations of microorganism, substrate, and product to attain equilibrium values. Turbidostatic operation is when the fermenter operates at a constant turbidity value. Turbidity is an indirect measure of the concentration of the microorganisms. Chemostatic operation is simple and hence is the process used in large-scale operations.

THE BATCH FERMENTER

Figure 8.1 shows a completely mixed fermentation vessel, in which the liquid motion is induced by a mechanical stirring device. A logical arrangement for mixing the medium is to use the evolved gas of the reaction or to bubble air through the reactor. The latter system also provides the oxygen required for aerobic organisms. The fermenter and the substrate are sterilized and an inoculum of the desired microorganism is added. Since the density of the microorganism is only slightly greater than water, mixing of the fluid surrounding them will keep the microorganisms suspended. The concentration of the substrate decreases, while the concentrations of the biomass and the product increase with time. The reaction is allowed to proceed until the

required conversion is achieved. The resultant mixture is then discharged for further processing. This is a dynamic system in which the conditions in the fermenter change with time.

CONTINUOUS STIRRED TANK REACTOR (CSTR)

The continuous stirred tank reactor is essentially a cylindrical vessel in which the contents are well mixed. Basically, it is a batch reactor with provision for continuous feed and removal of product. If the contents are not well mixed there could be 'channeling' or 'dead space' (Fig. 8.2). The effect of good mixing is that all elements of the fluid in the vessel have virtually the same composition. The main advantage of the CSTR, apart from simplicity of construction, is the ease with which the temperature and pH can be controlled. The open construction of the CSTR makes it easy to clean the internal surfaces, which is an important factor in maintaining aseptic conditions in large-scale operations. Since biochemical reactions cannot take place in the absence of microorganisms, it follows that microorganisms suspended in a continuous fermenter can be 'washed-out' at high flow rates. Continuous stirred-tank fermenters are limited in throughput as a result of this phenomenon.

A train of continuous stirred-tank fermenters, consisting of several well-stirred fermenters in series, offers an advantage in that any shock load imposed on the system is easily absorbed.

In the mineral industry, the CSTR could be used for bacterial leaching of concentrates.

AIR-LIFT FERMENTER

Air is used to circulate the contents of the fermenter, either through external tubes or a draft tube used internally. Figure 8.3 illustrates the main features of the air-lift fermenter. It consists of a column with an internal draft-tube. Compressed air, sparged at the base, causes the air-entrapped liquid to rise in the draft-tube and return through the annular space on the sides. Oxygen diffusion into the fermentation broth takes place in the draft-tube as well as in the annulus.

Usually air-lift fermenters are used in a batch mode. A disadvantage of the activated sludge sewage treatment process is the low efficiency of the oxygen transfer and the large area required for installation. The diffusion of oxygen can be increased by the use of air-lift fermenters, which would increase the efficiency of treatment of the waste waters.

ROTATING BIOLOGICAL CONTACTOR (RBC)

The RBC provides an effective means of alternately submerging a film of microorganisms in a substrate medium and then exposing the film to air for oxidation. A very large surface area is provided in a small volume of substrate,

hence permitting excellent transfer of nutrients and oxygen necessary for bacterial metabolism. RBCs are widely used in municipal and industrial wastewater treatment where a high BOD or NH_3 removal is required.

The RBC offers several significant advantages:

- low energy requirements
- low space requirements
- simplicity of operation
- low maintenance requirements
- high treatment efficiency
- resistance to shock loads.

Various configurations are possible for RBC systems. Figure 8.4 shows a conventional arrangement of the media (discs), the rotating shaft, the tubular body, and the end plates. The shaft can be rotated by coupling it to a motor or by an air-drive. The rising air bubbles exert sufficient torque to rotate the discs, if the bubbles are captured efficiently. The rate of rotation can be controlled by regulating the amount of air sparged into each reactor.

The Homestake Mining Company uses 48 RBCs followed by clarification and pressure filtration to oxidize thiocyanates, complexed cyanides, and ammonia. The system also removes metals from the mine and tailings decant water of about 21 000 cubic metres per day (3800 USgpm).

IMMOBILIZED CELLS AND ENZYMES

A large number of organic materials can be synthesized and are produced on a large scale using microorganisms. Although there are advantages to the production of complex biochemicals in this manner, there are a number of inherent disadvantages:

1. A significant portion of the substrates is utilized for the growth of microorganisms. This results in the wasteful build-up of biomass.
2. Since microorganisms have a number of enzymes and since many metabolic pathways co-exist, a mixture of products is formed. The products have to be separated and some of the components may have no economic value.
3. Generally, complex mixtures of nutrients are required to maintain the growth of the organisms.
4. Some of the strains of organisms used are difficult to maintain and the development of suitable strains is costly.
5. The operation of continuous processes is made very difficult due to the continuous accumulation of biomass and due to the tendency of organisms to mutate.
6. High capital and operating costs are involved in the construction and operation of fermenters under aseptic conditions.
7. The separation of biomass from the product is difficult.

Most of these disadvantages have been overcome by the use of immobilized cells and enzymes.

The production and exploitation of cells in an immobilized form leads to a number of technological advantages:

1. The cells occupy a fraction of the reactor volume, thus enabling the product to be obtained at higher concentrations, which facilitate downstream processing.
2. Change of substrates or washes can be very short. This can be critical where promoters and inhibitors have to be used in a critical time sequence.
3. The cells are not exposed to physically demanding environments.
4. Adherence of cells to measurement probes can be eliminated.
5. Separation of the cells from the product is easy.
6. Immobilized cells can be used as flow-through biocatalysts.

Immobilized cells are of two kinds: (1) cells grown on a solid (or relatively solid gel) surface; or (2) cells that can be restrained within the matrix of a particle, gel, mesh, or sponge.

Enzymes are proteins possessing catalytic functions, with molecular weights ranging from about 15 000 to over a million. A small number of enzymes consist of single-folded polypeptide chains and are called monomeric enzymes. Most enzymes are oligomers, that is, they consist of two or more polypeptide chains combined to form catalytically active proteins.

One important characteristic of an enzyme is its substrate specificity. Enzymes are given names according to the reaction they catalyze. The immobilized enzyme, like the immobilized cell, is used in a suitable biochemical reactor, into which the raw material is fed and from which the product is removed. Since most reactions are carried out in an aqueous phase, in which the enzyme is soluble, it is imperative that the method used for immobilization of the enzyme is such that it would retain the enzyme without, at the same time, preventing diffusion of substrate or product to, or away from, the enzyme. Immobilized enzymes are prepared by mechanical retention, chemical bonding, or physical adsorption. Immobilized systems suffer from the following:

1. At normal temperatures, the stability of some enzymes is low.
2. Microorganisms have the ability to sythesize enzymes and hence replenish any losses.
3. The effective functioning of a system of enzymes may be achieved only when their active centres are in the appropriate position relative to each other.

Most of the reactors that could be used with microorganisms can be used, with suitable modifications, for immobilized cells and enzymes. Currently, immobilized systems are used mainly in the pharmaceutical industry. It would appear that the mining industry has to wait patiently for developments in genetic engineering.

TRICKLE FILTERS

The trickling filter (Fig. 8.5) consists of a bed of stones or sand, and the wastewater is allowed to trickle down over the medium that supports the biomass. The organic matter contained in the waste material flowing over the bed is oxidized by the organisms. The trickling filter has the advantage that the throughput is not restricted by wash-out. Since wash-out does not occur, it is highly suitable for wastewater treatment. However, it has a number of disadvantages:

1. The biomass cannot be recovered from the bed.
2. Not all microorganisms adhere to surfaces. Organisms that do not adhere cannot be used in a trickle filter.
3. The thickness of growth on the support particles must be controlled, otherwise the accumulation will block the bed.
4. Aseptic operation is difficult, if not impossible.

The large-scale applications of the reactor have so far been limited to production of vinegar and wastewater treatment. In the mining industry it could be used for effluent treatment.

FIGURES

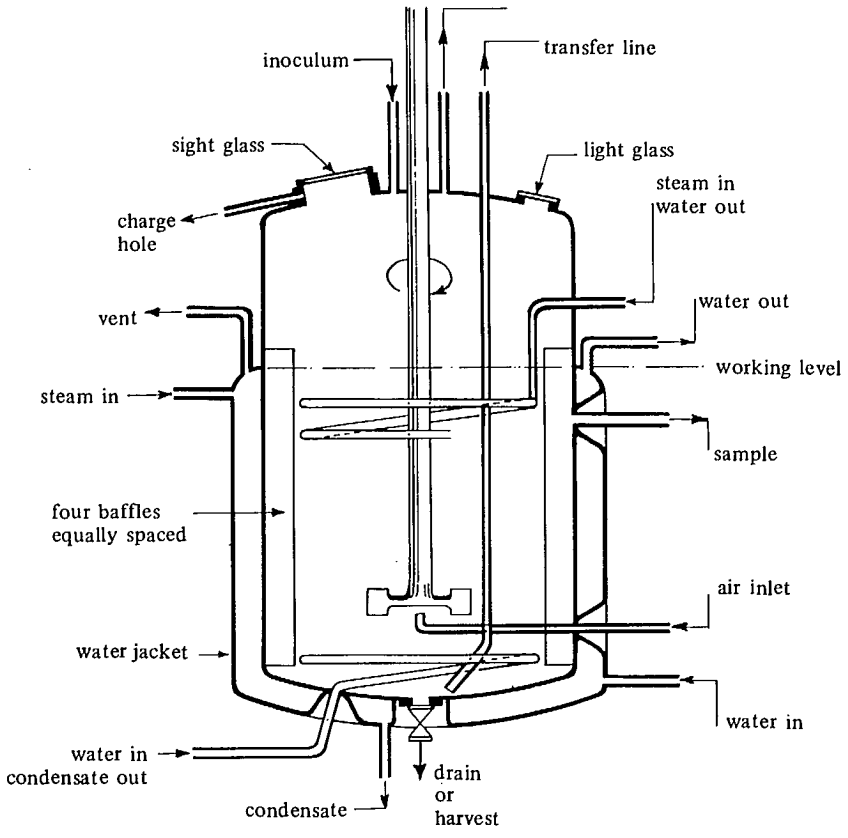


Fig. 8.1 - Fermentation vessel

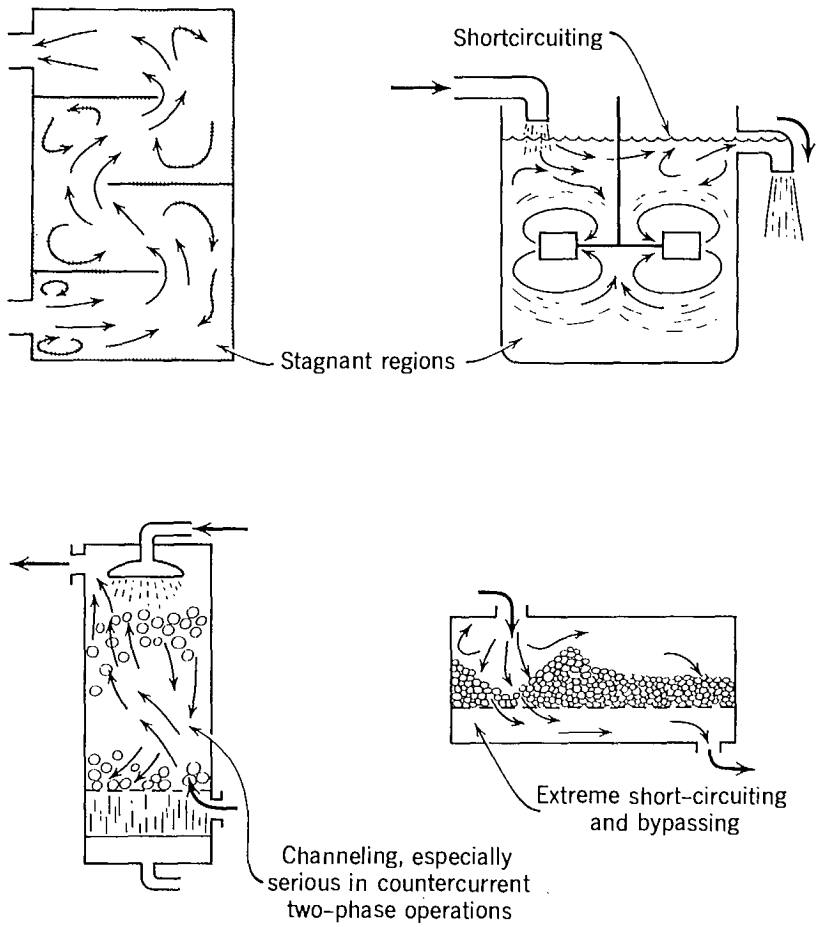


Fig. 8.2 - Nonideal flow patterns that may exist in process equipment

Airlift dimensions

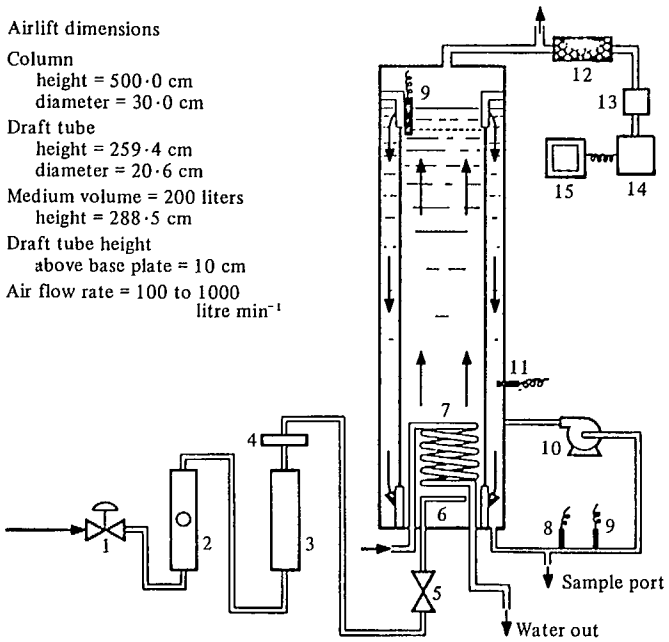
Column
height = 500·0 cm
diameter = 30·0 cm

Draft tube
height = 259·4 cm
diameter = 20·6 cm

Medium volume = 200 liters
height = 288·5 cm

Draft tube height
above base plate = 10 cm

Air flow rate = 100 to 1000 litre min^{-1}



1 Air-pressure regulator
2 Rotameter
3 Glass-wool air filter
4 Millipore air filter
5 Check valve
6 Air sparger
7 Heating and cooling coil
8 pH electrode
9 Oxygen probe
10 Centrifugal pump
11 Thermistor probe
12 Silica-gel bed
13 Diaphragm pump
14 Paramagnetic oxygen analyser
15 Recorder

Fig. 8.3 - Air-lift fermenter

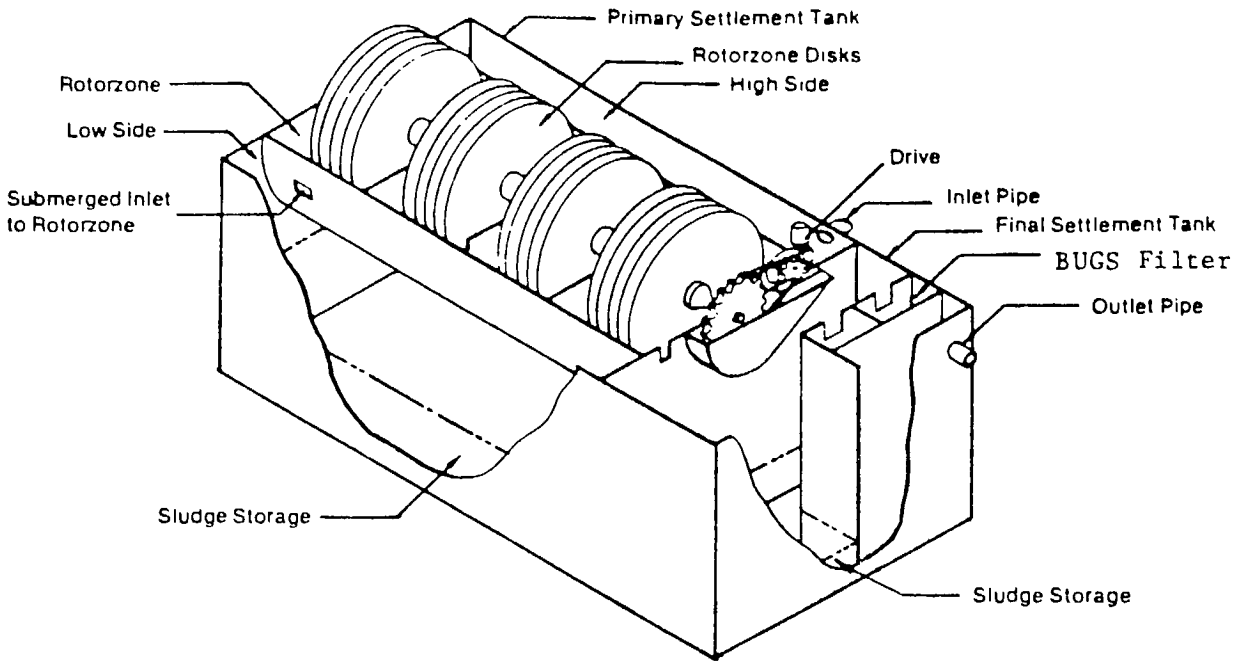


Fig. 8.4 – Rotating biological contactor

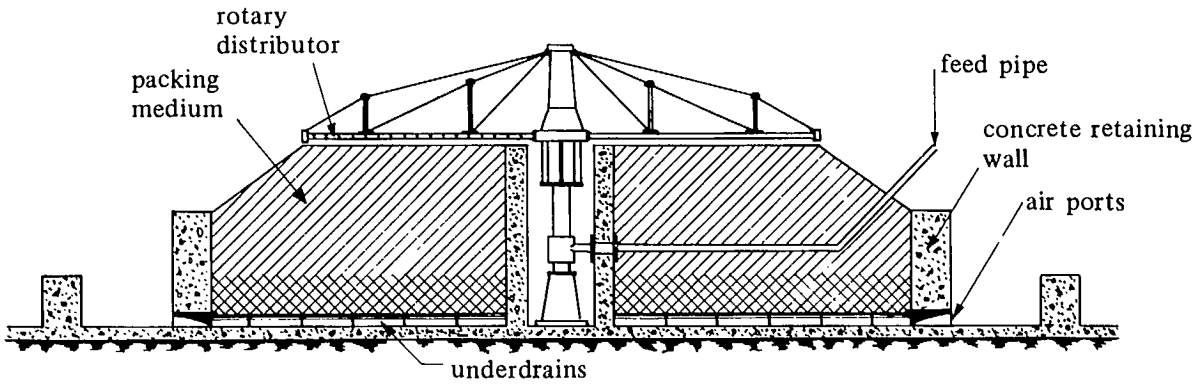


Fig. 8.5 - Trickling filter

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GLOSSARY

actinomycete. A member of the bacterial order Actinomycetales.

activated-sludge process. The use of biologically active sewage sludge to hasten the breakdown of organic matter in raw sewage during secondary treatment.

adaptation. Ability to exist in a changed environment.

adenine. A purine component of nucleosides, nucleotides, and nucleic acids.

adenosine. A mononucleoside consisting of adenine and D-ribose, produced by the hydrolysis of adenosine monophosphate.

adenosine triphosphate. A compound of one molecule each of adenine and D-ribose, and three molecules of phosphoric acid, which plays an important role in energy transformations in metabolism. Abbreviation: **ATP.**

aerobe. Any oxygen-requiring organism. Compare **anaerobe.**

agar-agar. A dried polysaccharide extract of red algae (Rhodophyceae) used as a solidifying agent in microbiological media. Commonly referred to as agar.

amino acid. An organic compound containing both amino ($-NH_2$) and carboxyl ($-COOH$) groups.

ammonification. Decomposition of organic nitrogen compounds, e.g., proteins, by microorganisms with the release of ammonia.

amphitrichous. Having a flagellum at each end of a cell.

amylase. An enzyme that hydrolyzes starch.

anaerobe. An organism that grows in the absence of molecular oxygen. Compare **aerobe.**

antibiosis. Antagonistic association between two organisms in which one is adversely affected.

antibiotic. A substance of microbial origin that has antimicrobial activity in very small amounts.

antimicrobial agent. Any chemical or biologic agent that either destroys or inhibits growth of microorganisms.

antiseptic. Acting against or opposing sepsis, putrefaction, or decay by either preventing or arresting the growth of microorganisms.

antitoxin. An antibody capable of uniting with and neutralizing a specific toxin.

ascomycetes. A class of fungi distinguished by the ascus.

ascospore. A sexual spore characteristic of the Ascomycetes, produced in a saclike structure (an ascus) after the union of the two nuclei.

ascus. A saclike structure, characteristic of the Ascomycetes, in which ascospores are produced.

asepsis. A condition in which harmful microorganisms are absent. Adjective: **aseptic**

aseptic technique. Precautionary measures taken to prevent contamination.

assimilation. Conversion of nutritive material into protoplasm.

autoclave. An apparatus using steam under pressure for sterilization.

autolysis. Disintegration of cells by the action of their own enzymes.

autotroph. A microorganism that uses inorganic materials as a source of nutrients; carbon dioxide is the sole source of carbon. Compare **heterotroph.**

axenic culture. An organism of a single species, e.g., a bacterium, fungus, alga, or protozoan, growing in a medium free of other living organisms.

bacillus. Any rod-shaped bacterium.

bacterial filter. A special type of filter through which bacterial cells cannot pass.

bactericide. An agent that destroys bacteria.

bacteriolysin. An agent or substance that causes disintegration of bacteria.

bacteriophage. A virus that infects bacteria and causes lysis of bacterial cells.

bacteriostasis. Inhibition of growth and reproduction of bacteria without killing them.

bacterium, pl. bacteria. Any of a group of diverse and ubiquitous procaryotic single-celled microorganisms.

basidiomycetes. A class of fungi that form basidiospores.

basidiospore. A sexual spore produced following the union of two nuclei on a specialized clublike structure known as a basidium.

basidium. A club-shaped specialized structure of the Basidiomycetes on which are borne the exogenous basidiospores.

beta hemolysis. A colourless, clear, sharply defined zone of hemolysis surrounding certain bacterial colonies growing on blood agar.

biochemical oxygen demand. A measure of the amount of oxygen consumed in biological processes that break down organic matter in water; a measure of the organic pollutant load. Abbreviation: **BOD**

biodegradable. Capable of being broken down by microorganisms.

biomass. The mass of living matter present in a specified area.

BOD. See **biochemical oxygen demand.**

botulism. Food poisoning due to the toxin of *Clostridium botulinum*.

budding. A form of asexual reproduction typical of yeast, in which a new cell is formed as an outgrowth from the parent cell.

buffer. Any substance in a fluid that tends to resist the change in pH when acid or alkali is added.

capsule. An envelope or slime layer surrounding the cell wall of certain microorganisms.

catabolism. The dissimilation, or breakdown, of complex organic molecules releasing energy. A part of the total process of metabolism. Compare **anabolism.**

catalase. An enzyme that converts hydrogen peroxide to water and oxygen.

catalyst. Any substance that accelerates a chemical reaction but remains unaltered thereby in form and amount.

cell. The microscopic, functionally and structurally basic unit of all living organisms.

cellulase. An extracellular enzyme that yields cellobiose on hydrolysis of cellulose.

cell wall. A rigid external covering of the cytoplasmic membrane.

chemoautotroph. An organism that obtains energy by oxidizing inorganic chemical compounds. Carbon dioxide is the sole source of carbon. Also called **chemolithotroph.**

chemolithotroph. See **chemoautotroph.**

chemostat. A device for maintaining a bacterial culture in the exponential, or log, phase of growth.

chemotroph. An organism that obtains its energy from the oxidation of chemical compounds.

chlamydospore. A thick-walled, resistant spore formed by the direct differentiation of the cells of the mycelium.

chloroplast. A cell plastid (specialized organelle) in plants and algae that contains chlorophyll pigments and functions in photosynthesis.

chromosome. A gene-containing filamentous structure in a cell nucleus; the number of chromosomes per cell nucleus is constant for each species.

cilium. A hairlike appendage on certain cells.

coagulase. An enzyme, produced by pathogenic staphylococci, that causes coagulation of blood plasma.

coccus. A spherical bacterium.

coenzyme. The non-protein portion of an enzyme.

colony. A macroscopically visible growth of microorganisms on a solid culture medium.

commensalism. A relationship between members of different species living in proximity (the same cultural environment), in which one organism benefits from the association but the other is not affected.

competitive inhibition. Inhibition of the action of an enzyme by a nonsubstrate molecule's occupation of the site on the enzyme that would otherwise be occupied by the substrate.

conidiophore. A branch of mycelium that bears conidia.

conidiospore. See **conidium.**

conidium. An asexual spore that may be one-celled or many-celled and of many sizes and shapes. Also called **conidiospore.**

constitutive enzyme. An enzyme whose formation is not dependent upon the presence of a specific substrate.

contamination. Entry of undesirable organisms into some material or object.

culture. A population of microorganisms cultivated in a medium.

cytochrome. One of a group of reversible oxidation-reduction carriers in respiration.

cytoplasm. The living matter of a cell between cell membrane and nucleus.

cytoplasmic membrane. A membrane surrounding the cytoplasm and its contents.

DAP. Diaminopimelic acid, a component of cell-wall mucopeptide in some bacteria.

deamination. Removal of an aminogroup, especially from an amino acid.

decarboxylation. Removal of a carboxyl group, -COOH.

decimal reduction time. The amount of time at a particular temperature sufficient to reduce a viable microbial population by 90%.

dehydration. The removal of water.

dehydrogenation. A reaction involving an enzyme that causes oxidation of substrate by removing hydrogen from it.

denature. To modify, by physical or chemical action, the structure of an organic substance, especially a protein, in order to alter some properties of the substance, such as solubility.

denitrification. Reduction of nitrates to free nitrogen.

deoxyribonucleic acid. The carrier of genetic information; a type of nucleic acid occurring in cells, containing phosphoric acid, D-2-deoxyribose, adenine, guanine, cytosine, and thymine. Abbreviation: **DNA.**

deoxyribose. A five-carbon sugar having one oxygen atom less than the parent sugar, ribose; a component of DNA.

dextran. A polysaccharide (glucose polymer) produced by a wide range of microorganisms, sometimes in large amounts.

differential stain. A procedure using a series of dye solutions, or dye solutions or staining reagents, to bring out differences in microbial cells.

diplobacilli. Bacilli occurring in pairs.

diplococci. Cocci occurring in pairs.

disaccharide. A sugar composed of two mono-saccharides.

disease. A state of impaired body function occurring as a response to infection, stress, or other conditions.

disinfectant. An agent that frees from infection by killing the vegetative cells of microorganisms.

dissimilation. Chemical reactions that release energy by the breakdown of nutrients.

DNA. See **Deoxyribonucleic acid.**

effluent. The liquid waste of sewage and industrial processing.

endoenzyme. An enzyme formed within the cell and not excreted into the medium. Also called **intracellular enzyme.**

endospore. A thick-walled spore formed in the bacterial cell.

endotoxin. A toxin produced in an organism and liberated only when the organism disintegrates.

enzyme. An organic catalyst produced by an organism. See also **adaptive enzyme, constitutive enzyme, endoenzyme, exoenzyme.**

eucaryote. A cell that possesses a definitive or true nucleus. Compare **procaryote.**

exoenzyme. An enzyme excreted by a microorganism into the environment. Also called **extracellular enzyme.**

exospore. A spore external to the vegetative cell.

exotoxin. A toxin excreted by a microorganism into the surrounding medium.

exponential phase. The period of culture growth when cells divide steadily at a constant rate. Also called **logarithmic phase.**

extracellular enzyme. See **exoenzyme.**

facultative anaerobe. A bacterium that grows under either aerobic or anaerobic conditions.

fastidious organism. An organism that is difficult to isolate or cultivate on ordinary culture media because of its need for special nutritional factors.

fermentation. Anaerobic oxidation of compounds by enzyme action of microorganisms; gaseous oxygen is not involved in this energy-yielding process. An organic compound is the electron acceptor.

fibrinolysin. A substance, produced by hemolytic streptococci, that can liquefy clotted blood plasma or fibrin clots. Also called **streptokinase.**

filamentous. Characterized by threadlike structures.

fimbriae, sing. **fimbria.** Surface appendages of certain gram-negative bacteria composed of protein subunits. They are shorter and thinner than flagella. Also called **pili.**

fixed nitrogen. Nitrogen in a compound.

flagellates. One of the subphyla of the Phylum Protozoa.

flagellum, pl. **flagella.** A flexible, whiplike appendage on cells, used as an organ of locomotion.

fluidized bed. Bed of small particles freely suspended in upward flow of liquid or combined liquid and air flow.

formalin. A 37 to 40% aqueous solution of formaldehyde.

fractional sterilization. Sterilization of material by heating it to 100°C (212°F) on three successive days with incubation periods in between.

free nitrogen. Atmospheric nitrogen.

fruiting body. A specialized, spore-producing organ.

fungicide. An agent that kills or destroys fungi.

fungus, pl. **fungi.** A microorganism that lacks chlorophyll and is usually filamentous in structure; a mold or yeast.

gelatinase. An exoenzyme that degrades gelatin.

generation time. The time interval necessary for a cell to divide.

genome. A complete set of genetic material, i.e., a complete set of genes.

genus, pl. **genera.** A group of very closely related species.

glucose. A carbohydrate classified as a monosaccharide and hexose, used as an energy source by many microorganisms. Also called "dextrose" or "grape sugar".

glycogen. A carbohydrate of the polysaccharide group stored by animals. It yields glucose on hydrolysis.

gram-negative bacteria. Bacteria that appear red after being subjected to the Gram stain.

gram-positive bacteria. Bacteria that appear blue or violet after being subjected to the Gram stain.

gram stain. A differential stain by which bacteria are classed as gram-positive or gram-negative depending upon whether they retain or lose the primary stain (crystal violet) when subjected to treatment with a decolourizing agent.

growth. In microorganisms, an increase in the total mass or number of cells (e.g., in a culture) rather than in the size or complexity of any individual organism.

growth curve. Graphic representation of the growth (population changes) of bacteria in phases in a culture medium.

halophile. A microorganism whose growth is accelerated by, or dependent on, high salt concentrations.

hanging-drop technique. A technique in which microorganisms are observed suspended in a drop of fluid.

hemoglobin. The constituent of red blood cells that gives them their colour and carries oxygen.

hemolysin. A substance that lyses (dissolves) red blood cells, liberating hemoglobin.

hemolysis. The process of dissolving red blood cells.

heterotroph. A microorganism that is unable to use carbon dioxide as its sole source of carbon and requires one or more organic compounds. Compare **autotroph.**

hydrolysis. The process by which a substrate is split to form products through the intervention of a molecule of water.

imperfect fungi. Fungi that do not have a sexual cycle.

inactivate. To destroy the activity of a substance, e.g., to heat blood serum to 56°C for 30 min to destroy complement.

incubation. In microbiology, the subjecting of cultures of microorganisms to conditions (especially temperatures) favourable to their growth.

induced mutation. Mutation produced by use of a mutagen.

infection. A pathological condition due to the growth of microorganisms in a host.

infectious. Capable of producing disease in a susceptible host.

inhibition. In microbiology, prevention of growth or multiplication.

inoculation. The artificial introduction of microorganisms or substances into the body or into a culture medium.

inoculum. The substance, containing microorganisms or other material, that is introduced in inoculation.

in situ. In the original or natural location.

intracellular. Within a cell.

intracellular enzyme. See **endoenzyme.**

in vitro. Literally, "in glass". Pertaining to biologic experiments performed in test tubes or other laboratory vessels. Compare **in vivo.**

in vivo. Within the living organism; pertaining to laboratory testing of agents within living organisms. Compare **in vitro.**

lactose. A carbohydrate (disaccharide) that is split into glucose and galactose on hydrolysis. Also called "milk sugar". Abbreviation: **lac.**

lag phase. The period of slow, orderly growth when a medium is first inoculated with a culture.

lipase. A fat-splitting enzyme.

lipid. A fat or fatlike substance.

lipolytic enzyme. An enzyme that hydrolyzes lipids.

liquefaction. Transformation of a gas or solid (e.g., gel) to a liquid.

litre. A metric unit of volume containing 1000 millilitres (mL), or 1000 cubic centimetres.

lithotroph. See **autotroph.**

litmus. A plant extract used as an indicator for pH and oxidation or reduction.

logarithmic phase. Commonly called "log phase". See **Exponential phase.**

lophotrichous. Having a polar tuft of flagella.

lyophilization. Preservation of biological specimens by rapid freezing and rapid dehydration in a high vacuum.

lysin. An enzyme, antibody, or other substance capable of disrupting or disintegrating cells (lysis).

lysis. The disruption or disintegration of such cells as bacteria orerythrocytes, e.g., by the action of specific antibodies plus complement.

lysozyme. An enzyme capable of digesting the cell wall of certain bacteria.

maltase. An enzyme that hydrolyzes maltose, yielding glucose.

maltose. A carbohydrate (disaccharide) produced by the enzymatic hydrolysis of starch by diastase.

marine. Of or relating to oceanic and estuarine environments.

medium. A substance used to provide nutrients for the growth and multiplication of microorganisms.

membrane filter. A filter made from such polymeric materials as cellulose, polyethylene, or tetrafluoroethylene.

mesophile. A bacterium growing best at the moderate temperature range 25 to 40°C.

messenger RNA. The intermediary substance that passes information from the DNA in the nuclear region to the ribosomes in the cytoplasm. Abbreviation: **mRNA.**

metabolic pathway. A series of steps in the chemical transformation of organic molecules.

metabolism. The system of chemical changes by which the nutritional and functional activities of an organism are maintained.

metabolite. Any chemical participating in metabolism; a nutrient.

metachromatic granule. An intracellular body found in some bacteria and yeasts that becomes stained with a colour that is different from the colour of the dye used to stain.

microaerophile. Any microorganism that grows best in the presence of small amounts of atmospheric oxygen.

microbe. Any microscopic organism; a microorganism. Adjective: **Microbial.**

microbial cytology. The study of the structures and function of microbial cells.

microbial ecology. The study of microorganisms in their natural environments.

microbial film. Adherent aggregate of microorganisms attached to a supporting surface.

microbial floc. Adherent aggregate of microorganisms in suspension.

microbiology. The study of organisms of microscopic size (microorganisms), including their culture, economic importance, pathogenicity, etc.

microcysts. Vegetative cells transformed to resting cells during the fruiting process.

micrometre. A unit of measurement: one-thousandth of a millimetre. Abbreviation: **µm.**

microorganism. Any organism of microscopic dimensions.

mitochondrion. A cytoplasmic organelle in eucaryotic cells, the site of cell respiration.

mold. A fungus characterized by a filamentous structure.

mononucleotide. The basic building block of nucleic acids (DNA and RNA).

monosaccharide. A simple sugar, such as a five-carbon or six-carbon sugar.

monotrichous. Having a single flagellum.

mordant. A substance that fixes dyes.

morphology. The branch of biological science that deals with the study of the structure and form of living organisms.

mRNA. See **messenger RNA.**

mutagen. A substance that causes the occurrence of mutation.

mutant. An organism with a changed or new gene.

mutation. A stable change of a gene, such that the changed condition is inherited by offspring cells.

mycelium. A mass of threadlike filaments, branched or composing a network, that constitutes the vegetative structure of a fungus.

mycology. The study of fungi.

nanometre. A unit of length equal to one-billionth of a metre or 10^{-9} metre; one millimicrometre. Abbreviation: **nm.**

neurotoxin. Any nerve poison, such as that produced by certain marine algae.

nitrate reduction. The reduction of nitrates to nitrites or ammonia.

nitrification. The transformation of ammonia nitrogen to nitrates.

nitrogen fixation. The formation of nitrogen compounds from free atmospheric nitrogen.

nitrogenous. Relating to or containing nitrogen.

nomenclature. Any system of scientific names, such as those employed in biological classification.

nuclear material. Strands or threadlike pieces of nuclear substance, which is DNA.

nucleic acid. One of a class of molecules composed of joined nucleotide complexes; the types are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

nucleolus, pl. **nucleoli.** A small body in a cell nucleus.

nucleoprotein. A molecular complex composed of nucleic acid and protein.

nucleotide. A compound formed from one molecule each of a sugar (pentose), phosphoric acid, and a purine or pyrimidine base.

nucleus. The structure in a cell that contains the chromosomes.

nutrient. Substance used as a food.

obligate. Necessary or required.

ocular micrometer. A glass disk etched with equidistant lines that fits into the eyepiece of a microscope.

organism. Living biological specimen.

organotroph. An organism that obtains nourishment from the ingestion and breakdown of organic matter.

osmosis. The passage of a fluid through a semipermeable membrane due to osmotic pressure.

osmotic pressure. The force or tension built up when water diffuses through a membrane.

osmotic shock. Any disturbance in a cell when it is transferred to a hypertonic or hypotonic medium.

oxidation. 1. The process of combining with oxygen. 2. The loss of electrons or hydrogen.

penicillin. The generic name for a large group of antibiotic substances derived from several species of the mold *Penicillium*.

pentose. A sugar with five carbon atoms, e.g., ribose.

peptide. A compound consisting of two or several amino acids.

peptidoglycan. A large polymer that provides the rigid structure of the procaryotic cell wall, composed of three kinds of building blocks: (1) acetylglucosamine, (2) acetylmuramic acid, (3) a peptide consisting of four or five amino acids.

peptone. A partially hydrolyzed protein.

perfect fungi. Fungi with both an asexual and a sexual life cycle.

periplast. A surface cell membrane or pellicle of certain algae and bacteria.

peritrichous. Having flagella around the entire surface of the cell.

permeability. The extent to which molecules of various kinds can pass through cellular membranes.

permease. Any of a group of enzymes that mediate the phenomenon of membrane transport.

pH. A symbol for the degree of acidity or alkalinity of a solution; pH = log $[1/(H^-)]$ where (H^-) represents the hydrogen ion concentration.

phenol. A compound that is microbicidal or microbistatic, depending on concentration and temperature.

phenol coefficient. The ratio between the greatest dilution of a test germicide capable of killing a test organism in 10 min but not in 5 min and the greatest dilution of phenol giving the same result.

photoautotroph. An organism that derives energy from light and uses CO_2 as its sole carbon source.

photoreactivation. The restoration to full viability, by immediate exposure to visible light, of cells damaged by exposure to lethal doses of ultraviolet light.

photosynthesis. The process in which chlorophyll and the energy of light are used by plants and some microorganisms to synthesize carbohydrates from carbon dioxide and water.

photosynthetic autotroph. An organism that requires CO_2 as a carbon source and that must have a source of light for energy.

phototroph. A bacterium capable of utilizing light energy for metabolism.

physiology. The study of the life process of living things.

pili, sing. pilus. See **fimbriae.**

plasma. See **blood plasma.**

point mutation. A change in a single base as a result of the substitution of one nucleotide for another in DNA (RNA in some viruses).

polypeptide. A molecule consisting of many joined amino acids.

polypeptide chain. A chain formed of a large number of amino acids joined together by peptide bonds.

pour-plate method. An agar-plate technique used to culture colonies of bacteria.

procaryote. A type of cell in which the nuclear substance is not enclosed within a membrane, e.g., a bacterium or cyanobacterium. Compare **eucaryote.**

protein. One of a class of complex organic nitrogenous compounds composed of an extremely large number of amino acids joined by peptide bonds.

proteinase. An enzyme that hydrolyzes proteins to polypeptides.

protein biosynthesis. The synthesis of new proteins by organisms.

protoplasm. The living matter, living material, or living substance of a cell. The term usually refers to the substance within the cytoplasmic membrane.

protoplast. An active metabolizing cell without its cell-wall structure.

psychrophile. A "cold-living" microorganism, capable of growing at 0°C.

pure culture. A culture containing only one species of organism.

quaternary. An antimicrobial cationic detergent.

reduction. A chemical process involving the removal of oxygen, the addition of hydrogen, or the gain of electrons.

ribonucleic acid. A nucleic acid occurring in cytoplasm and the nucleolus, containing phosphoric acid, D-ribose, adenine, guanine, cytosine, and uracil. Abbreviation: **RNA.**

RNA. See **ribonucleic acid.**

RNA polymerase. An enzyme that synthesizes mRNA on a DNA template.

saccharolytic. Capable of splitting or degrading sugar compounds.

septum. A crosswall in a hyphal filament.

serial dilution. Dilution of a specimen in successive stages. Thus, a 1:100 dilution is achieved by combining one part of a 1:10 dilution (one part of specimen plus nine parts diluent, such as sterile water) with nine parts diluent.

sewage. Liquid or solid refuse (domestic and industrial wastes) carried off in sewers.

sexual reproduction. Reproduction in which two cells (gametes) fuse into one fertilized cell.

simple stain. The colouration of bacteria or other organisms by applying a single solution of a stain to a fixed film or smear.

slime layer. A gelatinous covering of the cell wall. The term is sometimes used as a synonym of **capsule.**

sludge. The semisolid part of sewage that has been sedimented or acted upon by bacteria.

smear. A thin layer of material, e.g., bacterial culture spread on a glass slide for microscopic examination. Also called a "film".

species. A single kind of microorganism; a subdivision of a genus.

spheroplast. A gram-negative bacterial cell with peptidoglycan and other cell-wall components removed, leaving it devoid of rigidity.

spirillum. A spiral or corkscrew-shaped bacterium.

spore. A resistant body formed by certain microorganisms; a resistant resting cell; a primitive unicellular dormant body.

sporulation. The process of spore formation.

stage micrometer. An instrument that functions as a ruler for measurement of microorganisms under the microscope.

staphylococci. Spherical bacteria (cocci) occurring in irregular, grapelike clusters.

starter culture. A known culture of microorganisms used to inoculate milk, pickles, and other food to produce the desired fermentation.

sterile. Free of living organisms.

sterilization. The process of making sterile; the killing of all forms of life.

stock cultures. Known species of microorganisms maintained in the laboratory for various tests and studies.

strain. A pure culture of microorganisms composed of the descendants of a single isolation.

streaked-plate method. A procedure for separating cells on a sterile agar surface so that individual cells will grow into distinct, separate colonies.

streptobacilli. Bacilli in chains.

streptococci. Cocci that divide in such a way that chains of cells are formed.

substrate. The substance acted upon by an enzyme.

symbiosis. The living together of two or more organisms; microbial association.

taxonomy. The science of classification of organisms, based as far as possible on natural relationships.

teichoic acid. A cell-wall constituent unique to procaryotes.

tetanus. Lockjaw; a disease caused by *clostridium tetani*.

thermal death point. The lowest temperature at which microorganisms are killed in a given time.

thermoduric. Capable of surviving exposure to high temperature.

thermolabile. Destroyed by heat at temperatures below 100°C (212°F).

thermophile. An organism that grows best at temperatures of 50°C (122°F) or higher.

toxin. A poisonous substance elaborated by an organism, such as bacterial toxin.

trickling filter. A secondary treatment process in which sewage is trickled over a bed of rocks so that bacteria can break down organic wastes.

type culture. A particular species considered representative of the characteristics of the species and used as a reference culture.

ultrasonic waves. Sound waves of high intensity (beyond the audible range), used for the destruction of microbes or the cleaning of materials.

ultraviolet rays. Radiations in the part of the spectrum occupied by wavelengths from about 3900 to about 2000 angstroms.

vegetative stage. The stage of active growth, as opposed to the resting or spore stages.

viable. Capable of living, growing, and developing; alive.

vibrio. A slightly curved bacterium resembling a comma.

viricide. An agent that kills virus.

virus. An obligate intracellular parasitic microorganism that is smaller than bacteria. Most viruses can pass through filters that will retain bacteria.

yeast. A kind of fungus that is unicellular and not characterized by typical mycelia.