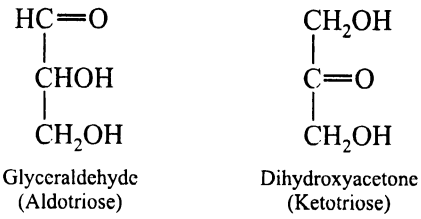

All the simple monosaccharides are white crystalline solids, sweet in taste and are freely soluble in water, but are insoluble in non-polar solvents. The carbon skeleton of the common monosaccharides is unbranched and each carbon atom except one, contains a hydroxyl group. The carbon atom has a carbonyl oxygen which is combined in an aldehyde or ketal linkage. If the carbonyl group is at the end of the chain, the monosaccharide is an aldehyde derivative and such monosaccharides are called aldoses. If it is at any other position, the monosaccharide is a ketone derivative and is called as ketoses. Among monosaccharides, the hexoses are abundant. Aldopentoses are important components of nucleic acids and various polysaccharides.

Disaccharides

Disaccharides are anhydrides of two monosaccharides, which include sucrose, maltose, lactose and cellobiose.

Sucrose

Sucrose occurs in sugarcane or sugar beets and it is disaccharide of glucose and fructose. Sucrose yields an equimolar mixture of glucose and fructose on hydrolysis. The hydrolysis of sucrose is called the inversion of sucrose because during the reactions, the sign of rotation changes from dextro to laevo. It is an enzymatic-catalysed reaction by invertase. The equimolar mixture of glucose and fructose is called 'invert sugar'.

It is sweeter than sucrose in taste. Hence hydrolysis of sucrose is carried out in confectionary industry. This is present naturally in honey.

Oligosaccharides

Oligosaccharides are anhydrides of several monosaccharide residues. Compounds containing ten or less monosaccharide units are called as oligosaccharides, while those containing more than ten are called polysaccharides.

Polysaccharides

Polysaccharides are also known as glycans. They are anhydrides of one or more monosaccharides in which a large number of units are combined. Many polysaccharides are composed of only one repeating monosaccharide and even where a large number exists, they appear to be arranged in a systematic fashion. Homopolysaccharides possess one repeating unit, while heteropolysaccharides contain more than one monosaccharide unit. D-Glucose is the most prevalent monosaccharide unit though polysaccharides of D-mannose, D-fructose, D- and L-galactose, D-xylose and D-arabinose are common. D-glucosamine, D-galactosamine, D-glucuronic acid, N-acetylmuramic acid and N-acetylneuraminic acid are the monosaccharide derivaties which are commonly found in natural polysaccharides.

Chapter 2

Fundamentals of Microbiology

INTRODUCTION

Small living creatures called micro-organisms interact in numerous ways with human activities. On the large scale of the biosphere, which consists of all regions of the earth containing life, micro-organisms play a primary role in the capture of energy from the sun. Their biological activities also complete critical segments of the cycles of carbon, oxygen, nitrogen and other elements essential for life. Microbes are also responsible for many human, animal and plant diseases.

In this text we concentrate primarily on mankind's use of microbes. These versatile biological catalysts have served mankind for millennia. Fermented foods such as cheese, bread, yoghurt and soya have long contributed to mankind's nutrition. Pasteur and Tyndall identified micro-organisms as the critical, active agents in prior fermentation practice and initiated the emergence of microbiology as a science. From these beginnings, further work by Buchner, Neuberg and Weizmann led to processes for production of ethanol, glycerol and other chemicals in the early 20th century.

In the 1940s complementary developments in biochemistry, microbial genetics and engineering ushered in the era of antibiotics with tremendous relief to mankind's suffering and mortality.

This period marks the birth of biochemical engineering, the engineering of processes using catalysts, feedstocks and/or sorbents of biological origin. Biotechnology began to change from empirical art to predictive, optimised design.

A later generation of fermentation processes produced steroids for birth control and for treatment of arthritis and inflammation. Methods for cultivation of plant and animal cells made possible mass production of vaccines and other useful biological agents. Clearly, mankind's successful harnessing and direction of cellular activities has had many health, social, environmental and economic impacts on past and contemporary human civilisation.

An interwoven fabric of research in molecular biology and microbial genetics has led to fundamental understanding of many of the controls and catalysts involved in complex biochemical syntheses conducted by living cells. On this foundation of basic knowledge, the methods of recombinant DNA technology have been erected. It is difficult to imagine the scope and magnitude of the eventual benefits of these marvelous tools. New vaccines and drugs have already been produced, but these are only the beginnings of revolutionary developments to come.

Our challenge in learning biochemical engineering is to understand and analyse the processes of biotechnology so that we can design and operate them in a rational way. To reach this goal, however, a basic working knowledge of cell growth and function is required. These factors and others peculiar to

is very similar to those involving moulds. One difference, however, is the susceptibility of actinomycetes to infection and disease by viruses which also can attack bacteria.

Algae and Protozoa

These relatively large eucaryotes have sophisticated and highly organised structures. For example, *Euglena* has flagella for locomotion, lacks a rigid wall and has an eyespot sensitive to light. The cell, guided by the eyespot, moves in response to stimulus by illumination—clearly a valuable asset since most algae require energy in the form of light. Many diatoms (another kind of algae) have exterior skeletons of complex architecture which are impregnated with silica. These skeletons are widely employed as filter aids in industry.

Considerable commercial interest in algae is concentrated on their possible exploitation as foodstuffs and food supplements. In Japan, several processes for algae food cultivation are in operation today. Also important in Asia is use of seaweed in the human diet. While not micro-organisms, many seaweeds are actually multicellular algae. Like the simpler blue-green algae, eucaryotic algae serve a vital function in the cycles or matter on earth.

Just as algae may be viewed as primitive plants, protozoa, which cannot exploit sunlight's energy, are in a sense primitive animals. The habitats, morphology and activities of protozoa span a broad spectrum. For example, some trypanosomes carry serious disease, including African sleeping sickness. The *Trichonympha* inhabit the intestines of termites and assist them in digesting wood. While the amoeba has a changing, amorphous shape, the heliozoa have an internal skeleton and definite form.

Although protozoa are not now employed for industrial manufacture of either cells or products, their activities are significant among the micro-organisms which participate in biological waste treatment. These processes, widely employed in urban communities and large industrial plants throughout the world, are suprisingly complicated from a microbiological viewpoint. Since a complex mixture of different nutrients and microbes are present in sewage or industrial wastes, a correspondingly large collection of different protists are present and indeed necessary in treatment operations. These diverse organisms compete for nutrients, devour each other and interact in numerous ways characteristic of a small-scale ecological system.

Animal and Plant Cells

Many vaccines and other useful biochemicals are produced by growth of animal cells in process reactors; i.e., by cell propagation outside of the whole animal. Improvements in cultivation techniques for these tissue-derived cells and emerging methods for genetic manipulation of animal and plant cells offer great potential for expanded commercial utilisation of these higher cells. The reactors in which 'tissue' cultures may be propagated may be quite similar to microbial reactors, admitting a unified treatment of cell kinetics and biochemical reactors.

When a piece of animal tissue, perhaps after disruption to break the cells apart, is placed in appropriate nutrient liquid, many cell types, such as blood cells, die within a few days, weeks or months. Other cells multiply and are called primary cell lines.

Often these cells can be 'passaged' by transfer to fresh medium after which further cell multiplication occurs giving a secondary cell line. Some secondary cells can be passaged apparently indefinitely; these cells are then dubbed an established, permanent or stable cell line. Many cell lines have been developed from the epithelial tissues (skin and tissues which cover organs and line body cavities), connective tissues and blood and lymph of several animals including man, hamster, monkey and mouse.

Inhibitors can interfere with catalysis as well as with substrate binding. In the simplest case, an inhibitory term affects the variable term in the denominator of the Michaelis-Menten equation, instead of the constant term:

$$v = \frac{k_0 e_0 a}{K_m + a(1 + i/K_{iu})} \quad \ldots (3.14)$$

This is called uncompetitive inhibition and the inhibition constant K_{iu} is the uncompetitive inhibition constant. This is important as a limiting case of inhibition, but in its pure form it is not at all common. Much more often one has mixed inhibition, when both competitive and uncompetitive effects occur simultaneously:

$$v = \frac{k_0 e_0 a}{K_m(1 + i/K_{ic}) + a(1 + i/K_{iu})} \quad \ldots (3.15)$$

There is no particular reason for the two inhibition constants K_{ic} and K_{iu} to be equal, and most of the mechanisms one might propose to account for mixed inhibition lead one to expect them to be different, yet the case where $K_{ic} = K_{iu}$ is often given an undeserved prominence in discussions of inhibition, largely because experiments done many years ago suggested that it was a more common phenomenon than it is. This is called noncompetitive inhibition and its rate equation is the same as equation 3.15, but with both K_{ic} and K_{iu} written simply as K_i.

All of these kinds of inhibition are conveniently discussed in terms of apparent Michaelis-Menten parameters. In the general case (equation 3.15), these are as follows:

$$k_0^{app} = \frac{k_0}{1 + i/K_{iu}}; \; k_A^{app} = \frac{kA}{1 + i/K_{ic}}; \; k_m^{app} = \frac{K_m(1 + i/K_{ic})}{1 + i/K_{iu}} \quad \ldots (3.16)$$

Note that the first two expressions have the same form and both simplify to independence of i in the event that one or other inhibition term is negligible. The expression for the apparent value of K_m is more complicated, especially when one considers how it varies with the different types of inhibition: It increases with the concentration of a competitive inhibitor, it decreases as the concentration of an uncompetitive inhibitor increases, it may change in either direction as the concentration of a mixed inhibitor increases, or it is independent of inhibitor concentration if the inhibition is noncompetitive. In general it is simplest to regard k_A as the parameter affected by competitive inhibition, negligibly so when the competitive component is negligible, k_0 as the parameter affected by uncompetitive inhibition, negligibly so when the uncompetitive component is negligible, and K_m just as the ratio of the two, so $K_m = k_0/k_A$.

The effects of the different kinds of inhibition on the common plots as illustrated in Figs. 3.2 through 3.4 follows naturally from equation 3.16. Any competitive effect affects the apparent value of k_A, hence, it increases the slope of the plot of $1/v$ against $1/a$ (Fig. 3.2), it increases the ordinate intercept of the plot of a/v against a (Fig. 3.3) and it decreases the abscissa intercept of the plot of v against v/a (Fig. 3.4). Conversely, any uncompetitive effect increases the ordinate intercept of the plot of $1/v$ against $1/a$, increases the slope of the plot of a/v against a, and decreases the ordinate intercept of the plot of 'v' against v/a. When both components of the inhibition are present, both kinds of effects occur. As an illustration we may consider just one example, the effect of competitive inhibition on the plot of $1/v$ against $1/a$: Plots made at various different inhibitor concentrations produce a family of straight lines intersecting on the ordinate axis, as shown in Fig. 3.5, the lack of effect on the ordinate intercept being a direct consequence of the lack of effect on the apparent value of V.