# Biochemical Engineering

R. STEEL

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*Unit Processes in Fermentation*

*Editor*

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# PREFACE

SEVENTY years ago, in 1887, George E. Davis gave a course of lectures on chemical engineering at the Manchester Technical School. It has been claimed, and the claim has been supported by no less a body than the Institution of Chemical Engineers, that these were the first lectures in the world on this subject, now recognized as a major technology. The lectures were expanded by Davis into a two-volume *Handbook of Chemical Engineering*, published in 1901, which is said to have been the first textbook of chemical engineering. These claims can, perhaps, be disputed in detail, but there is no disputing the fact that the Davis lectures and the handbook are important parts of the very foundations of chemical engineering.

The present book is an expanded version of a course of ten post-graduate lectures on biochemical engineering delivered by a group of experts at the Manchester College of Science and Technology, the successor to the old Manchester Technical School, in the spring of 1957. Sir Harold Hartley, who has done so much more than anyone else to draw attention to, and to foster, this new and important subject, has added a masterly and inimitable introduction. I am no biochemical engineer and my part in the whole enterprise has been simply that of a catalyst; proud though I am of having been asked to contribute this prefatory note, I can add nothing of real value to the book, but take this opportunity to express the hope that, in years to come, these lectures may prove to have a place in the history of biochemical engineering comparable to that occupied by the Davis lectures in the history of chemical engineering.

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# CONTENTS

# INTRODUCTION:

# THE PRINCIPLES OF BIOCHEMICAL ENGINEERING

Sir Harold Hartley, G.C.V.O., F.R.S.

# THE PRINCIPLES OF BIOCHEMICAL ENGINEERING

## 1. INTRODUCTION

I am very glad that the first course of lectures in Britain on Biochemical Engineering should be given in the Manchester College of Science and Technology since it was in its forerunner, the Manchester Technical School, that George Davis gave the first course of lectures on chemical engineering seventy years ago. It is a good omen for a subject that has a great future.

Some twenty years ago I was discussing with Karl Bosch, the head of the great German chemical combine, the I.G. Farbenindustrie, the prospects of tropical agriculture and the future competition between the natural and synthetic products. 'Let me tell you,' he said, 'what Steinmetz once said to me.' Steinmetz was the genius who contributed so much in its early days to the General Electric Company's research laboratories at Schenectady. 'Bosch,' he said, 'I know you can make indigo cheaper than God, someday you may make rubber cheaper than God, but you will never make cellulose cheaper than God.' That set me thinking and made me realize that there are limits, economic limits at any rate, to the man-made synthetics. We can synthesize comparatively simple molecules economically, and we can join them into regular polymers like Terylene, but we can never hope to make synthetically the complex proteins and polysaccharides on which Nature in the long course of evolution has decided that man must live. And yet living organisms make them so cleanly and efficiently by their template methods with the aid of enzymes, Nature's catalysts, whose accurate workmanship is such a frustrating contrast to the clumsiness of the man-made catalysts and chemical agents. For instance, if we break down a polypeptide chain by ordinary methods, we break it at a number of different points; an enzyme can neatly snip off a residue at each end and yield a definite product. Similarly in malting, the enzymes continuously remove two glucose residues from the ends of the complex amylose and amylopectin molecules to produce a molecule of maltose.

## 2. FERMENTATION AND THE MICRO-ORGANISM

Fermentation is one of the oldest industries. Pasteur first showed its dependence on micro-organisms and since his day biochemists have gradually unravelled the systems of enzymes involved in their metabolism. Also we have come to recognize the essential part that micro-organisms play in world ecology and our dependence on them. Without micro-organisms, higher life could not exist. The amount of nitrogen fixed by them in the soil is vastly greater than all that provided by nitrogenous fertilizers.

The earlier fermentation processes were used to make the simpler carbon compounds like alcohol, acetic acid and acetone. With the growth of the petrochemical industry and the use of synthesis gas from coal, these substances are being made more economically from these sources and we are looking more and more to micro-organisms for larger and more complex molecules. This, I think, is the field of the future for biochemical engineering.

I remember that when I first used the name ‘ biochemical engineering ’ I was severely criticized for suggesting that there was anything new in it. My critics said that it was just the application of living catalysts in place of, let us say, platinum, and that it needed no new techniques. My reasons for differing from this view are quite simple. During the lifetime of an inorganic catalyst it will continue to exert the same influence on any given chemical system with declining activity as it is gradually poisoned or rendered inert. The living cell is entirely different. It often passes through various metabolic changes in its lifetime when its growth phenomena and its excreta undergo radical alterations on which its industrial applications must be based.

Gowland Hopkins once described the living cell as ‘ above all things a heretic ’. It seldom conforms to standard laboratory practice. It does many unexpected things with an amazing range of versatility and adaptability to circumstances. As you know, the human organism is very choosy as regards compounds containing asymmetric carbon atoms. It will assimilate a dextro form and leave the laevo form untouched or vice versa. But without the aid of living organisms we can only produce mixtures of equal parts of the two asymmetric forms. Many micro-organisms have the power to produce one form without the other, a point of vital importance to us.

The living process depends fundamentally on two types of chemical reaction, the degradative and the synthetic. The oxidation of certain nutrients is necessary to supply the free energy needed by the organism for various purposes and particularly to supply the free energy required by the synthetic processes, the building up of fresh materials, on which growth and life depend. For nutrients, man is dependent on certain complex carbon compounds, fats, proteins and carbohydrates, and on the oxygen of the air. Micro-organisms are far more versatile. *Pseudomonas* species, for instance, can get their carbon from a large variety of compounds—over a hundred—ranging from benzoic acid or butylamine to glycerol. Anaerobic bacteria can use the oxygen from sulphates or carbonates.

Again, while the main products of the slow combustion of nutrients in the body are carbon dioxide, ammonia and water, different micro-organisms produce a wide range of products from the same substrate, e.g. from glucose one can get ethyl alcohol, lactic acid or acetone and many other compounds; and they can do amazing things like the chlorination and nitration of an organic compound in aqueous solutions at ordinary temperature, leading to chloramphenicol.

Then there are the chlorophyll-bearing organisms like the algae, which derive their free energy, like the green plants, from solar radiation, and can synthesize proteins, fats and carbohydrates.

And don't forget it was the living organism that first produced antibiotics with their complex structures and it is on living organisms that we still depend for most of them.

As Professor Kluyver said, ' As for the formula of streptomycin, it can most aptly be described as the product of an organic chemist just before his admittance to a lunatic asylum. The structures of each of the three moieties of the molecule are equally irrational and the mere idea that Nature has thought fit to combine these elements to form a molecule with remarkably specific action tends to depress the most modest amongst biologists.'

## 3. THE METABOLIC SYSTEM OF THE LIVING CELL

How do they do it? Not by any simple mechanism. As Hinshelwood has put it, ' A growing cell constitutes a manufactory with numerous departments, the conveyor belt leading from one to another being represented simply by the concentration gradients. Raw materials are broken down and built up into fresh forms in a series of stages.'

The key to the secret is of course the enzyme, Nature's catalyst, of which there are an infinite variety. These are used to break down any chemical change into a series of simple operations, like those in a production line. Each of these operations can be performed with accuracy with its own enzyme and by the interlocking of components they provide the free energy required in the synthetic operations from that derived from degradative changes.

The complexity of the mechanism of each cell is amazing. I remember once trying to devise a system of conveyor belts at Crewe Goods Shed to enable incoming and outgoing consignments of different origins and destinations to be transferred mechanically. The problem was too difficult to solve but it was as nothing compared to the complexity of the simplest cell. You can see the reasons underlying the methods which Nature has adopted in the long course of evolution. She has broken down each operation into stages, each of which involves a relatively small activation energy and therefore with a catalyst can proceed fast at ordinary temperatures. Equally, from a thermodynamic aspect, each involves only a conveniently small free energy change of the order of 10–15 kcal, which can be provided by her interlocking mechanisms, if my mechanical analogy is not interpreted too literally.

Since each stage is in principle (and usually in practice) reversible, a degradative process can, under suitable conditions, become a synthetic one, subject of course to overall thermodynamic limitations.

In some cases, the successive stages have the pattern of a chain, as in the Embden–Meyerhof–Parnas scheme of hexose breakdown in which carbohydrates are broken down in eleven successive stages to pyruvic acid, the fate of which varies in different species of microorganisms to yield a wide range of final products, e.g. alcohol, glycerol, acetone and lactic acid. It often happens, however, that cyclic processes are better suited to the economy of the microorganism, either for degradative or synthetic purposes. In these, carrier molecules pass through a cycle of changes involving enzyme catalysts, at some point picking up substrate molecules which are either degraded or built up into larger molecules by processes which would not be possible in isolation. For instance in the Calvin cycle of the photosynthesis of carbohydrates from carbon dioxide, the latter substance enters the cycle at one point, reduction, made possible by the radiant energy absorbed by the chlorophyll, takes place at another, and during a complicated series of rearrangements of sugar molecules, sucrose passes out of the cycle while the carrier molecules continue to circulate. The cycle has to be repeated six times for each hexose molecule that it produces. The necessary free energy is supplied at appropriate points by the transfer of rich energy bonds from adenosine triphosphate (ATP) to the circulating molecules, the ATP being reformed at the expense of degradative actions which are taking place simultaneously. This transfer of rich energy bonds from ATP is the key to the supply of the free energy required for the synthesis of the larger molecules produced in microbial metabolism.

## 4. THE PHENOMENA OF GROWTH

So far I have not emphasized sufficiently the dynamic character of microbial existence. The intricate pattern of chemical actions in the cell needs a continuous supply of nutrients, substrates as they are called, and also oxygen in one form or another. The substrates must consist of suitable compounds of carbon, nitrogen, phosphorus and any metals which the particular micro-organism may require. As the cell grows, nutrient materials diffuse in from the surrounding medium and waste products diffuse out. The total amount of reaction in the cell tends to increase in proportion to its volume, while the supply of raw materials increases only with the area of the surface. The chemical conditions in the cell must therefore change as the result of this scale effect and the conditions of growth must alter. Finally a critical stage is reached when the cells divide into two halves and the process of growth is repeated. If all the raw materials are supplied from a constant environment a steady state will be established at which all parts of the cell material expand at rates such that their related proportions remain unchanged. If, as often happens in practice, cells are inoculated into a given volume of medium, then they grow until an essential raw material has been exhausted or until the metabolic products which they themselves form inhibit further multiplication.

## 5. THE ADAPTABILITY OF MICRO-ORGANISMS

The stability of a cell depends on the dynamical relations between the various sections of its fabric of reactions, being, with certain limits, self-adjusting so that it can respond automatically to any outside disturbance. Thus the cell economy is not so rigid that it cannot adapt itself to changes in its environment and many micro-organisms show a remarkable degree of adaptability. Perhaps this is not surprising in view of their large ratio of area to volume and their accessibility to changes in outside conditions, with the consequent rapid exchange of materials between the cell and the medium in which it is growing.

Hinshelwood and other investigators have shown that in many instances a cell can be trained to change its habits by a gradual change of its environment. For example, bacteria which grow normally with substrate A (e.g. glucose) but not with substrate B (e.g. glycerol), can be trained through a series of subcultures in the presence of increasing amounts of substrate B to exist on this substrate alone, and after fifty subcultures the adapted strain is stable. Similarly bacteria which grow readily in asparagine may be trained to grow with ammonia as their source of nitrogen. Bacteria can also adapt themselves to resist the inhibiting effects of certain drugs if these are added gradually in successive subcultures.

There are, of course, two possible mechanisms which may account for these bacterial adaptations. In addition to the induced adaptation mechanism described above, there is the possibility of the selection of spontaneous mutants. Both types may operate in Nature: they are in no way mutually exclusive. But there is evidence in many cases that the induced adaptation is the controlling cause as all the cells appear to be affected.

The adaptation of the micro-organism to its environment obviously opens up the possibility of using it to good effect in fermentation processes and it is also possible to assist the productivity of a micro-organism by providing it with a material which otherwise it must synthesize for itself. Thus, there are infinite possibilities for the future of utilizing microbial metabolism in the production line.

## 6. GENETICS

Genetics is the study of heredity and variation, and micro-organisms like all other living things are subject to both. Until recently, genetics had been concerned mainly with the heredity of discontinuous variations among higher organisms with sexual reproduction, but now it has turned to the study of micro-organisms with important consequences in their practical application. Sexual processes have been discovered in a number of micro-organisms and techniques have become available which can be used to breed improved strains. In addition, there is the exciting discovery that there are processes other than sexual reproduction which give the same kind of results. Thus Pontecorvo's discovery of what he calls the 'parasexual mechanism' opens up new possibilities of breeding improved industrial strains of asexual organisms. These processes differ from species to species but the results are the same. They yield strains associating in new ways, properties which were previously found separately in different strains.

In addition to these means of breeding improved strains of micro-organisms by the mechanisms of sexual or asexual reproduction, there are two other ways of producing mutations which can be