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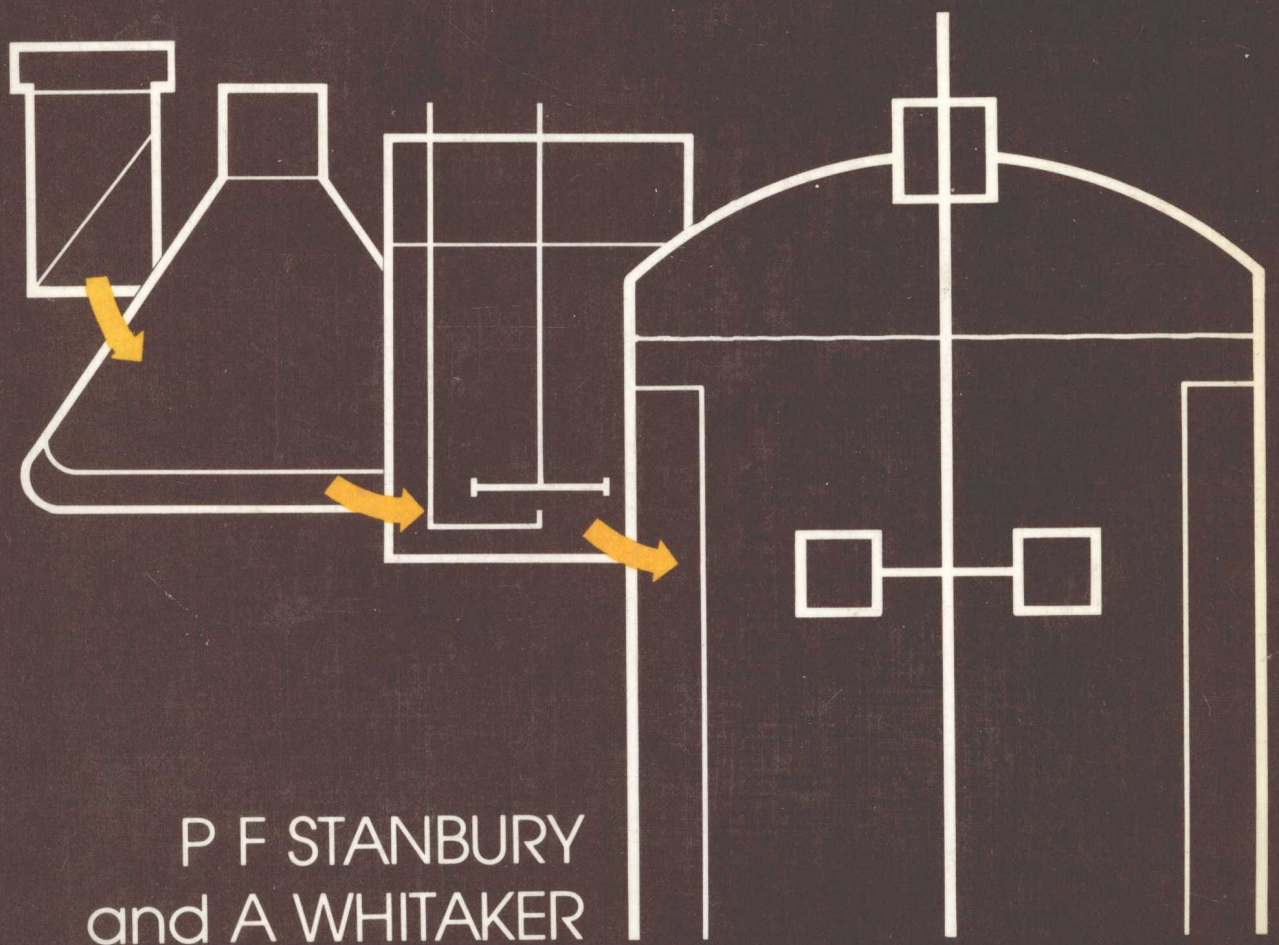
# Principles of Fermentation Technology



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# Principles of Fermentation Technology

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May, 1984

# Contents

# CHAPTER 1

# An Introduction to Fermentation Processes

THE term 'fermentation' is derived from the Latin verb, *fervere*, to boil, thus describing the appearance of the action of yeast on extracts of fruit or malted grain. The boiling appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugars present in the extract. However, fermentation has come to have different meanings to biochemists and to industrial microbiologists. Its biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology tends to be much broader.

The catabolism of sugars is an oxidative process which results in the production of reduced pyridine nucleotides which must be reoxidized for the process to continue. Under aerobic conditions, reoxidation of reduced pyridine nucleotide occurs by electron transfer, via the cytochrome system, with oxygen acting as the terminal electron acceptor. However, under anaerobic conditions, reduced pyridine nucleotide oxidation is coupled with the reduction of an organic compound, which is often a subsequent product of the catabolic pathway. In the case of the action of yeast on fruit or grain extracts, NADH is regenerated by the reduction of pyruvic acid to ethanol. Different microbial taxa are capable of reducing pyruvate to a wide range of end products, as illustrated in Fig. 1.1. Thus, the term fermentation has been used in a strict biochemical sense to mean an energy-generating process in which organic compounds act as both electron donors and terminal electron acceptors.

The production of alcohol by the action of yeast on malt or fruit extracts has been carried out on a large scale for very many years and was the first 'industrial' process for the production of a microbial metabolite. Thus, industrial microbiologists have extended the term fermentation to describe any process for the production of product by the mass culture of a micro-organism. Brewing and the production of organic solvents may be described as fermentations in both senses of the word but the description of an aerobic process as a fermentation is obviously using the term in the broader, microbiological, context and it is in this sense that the term is used in this book.

## THE RANGE OF FERMENTATION PROCESSES

There are four major groups of commercially important fermentations:

(i) Those that produce microbial cells (or biomass) as the product.
(ii) Those that produce microbial enzymes.
(iii) Those that produce microbial metabolites.
(iv) Those that modify a compound which is added to the fermentation—the transformation processes.

The historical development of these processes will be considered in a later section of this chapter, but it is first necessary to include a brief description of the four groups.

### Microbial biomass

The commercial production of microbial biomass may be subdivided into two major processes; the

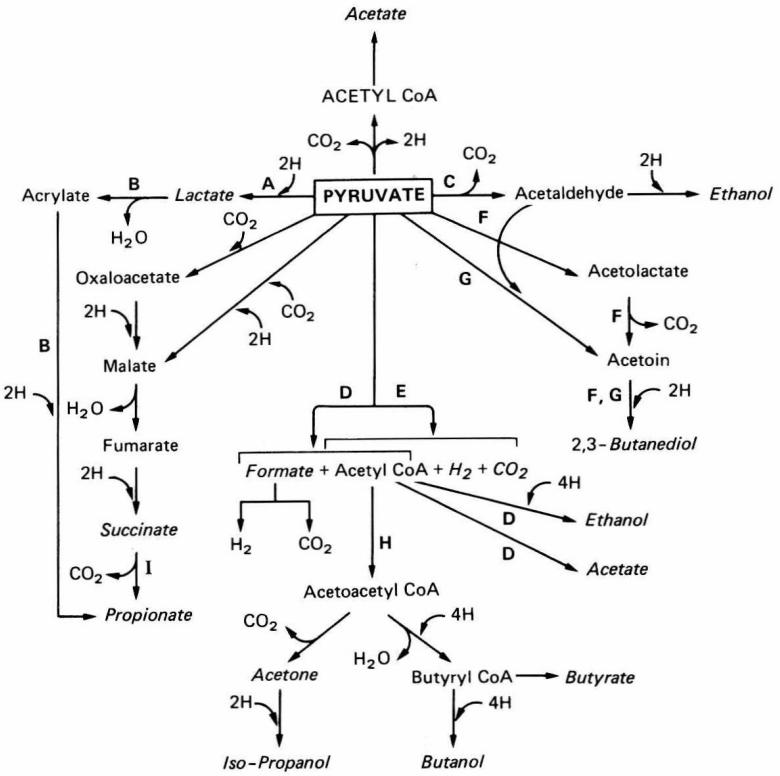


FIG. 1.1. Bacterial fermentation products of pyruvate. Pyruvate formed by the catabolism of glucose is further metabolized by pathways which are characteristic of particular organisms and which serve as a biochemical aid to identification. End products of fermentations are italicized (Dawes and Large, 1982).

A Lactic acid bacteria (*Streptococcus*, *Lactobacillus*)
B *Clostridium propionicum*
C Yeast, Acetobacter, Zymomonas, *Sarcina ventriculi*, *Erwinia amylovora*
D Enterobacteriaceae (coli-aerogenes)
E Clostridia
F Klebsiella
G Yeast
H Clostridia (butyric, butylic organisms)
I Propionic acid bacteria

production of yeast to be used in the baking industry and the production of microbial cells to be used as human or animal food (single-cell protein). Bakers' yeast has been produced on a large scale since the early 1900s and yeast was produced as human food in Germany during the First World War. However, it was not until the 1960s that the production of microbial biomass as a source of food protein was explored to any great depth. As a result of this work, reviewed briefly in Chapter 2, a few very large-scale continuous processes have been established in recent years using a variety of different carbon sources.

## Microbial enzymes

Enzymes have been produced commercially from plant, animal and microbial sources. However, microbial enzymes have the enormous advantage of being able to be produced in large quantities by established fermentation techniques. Also, it is infinitely easier to improve the productivity of a microbial system compared with a plant or animal one. The uses to which microbial enzymes have been put are summarized in Table 1.1, from which it may be seen that the majority of applications are in the food and related industries. Enzyme production is closely controlled in micro-organisms and in order to improve productivity these controls may have to be exploited or modified. Such control systems as induction may be exploited by including inducers in the medium, whereas feedback repression may be removed by mutation and selection techniques. These aspects are discussed in Chapters 4 and 3, respectively.

TABLE 1.1. *Commercial applications of enzymes* (Boing, 1982)

| Industry | Application | Enzyme | Source |
|---|---|---|---|
| Baking and milling | Reduction of dough viscosity, acceleration of fermentation process, increase in loaf volume, improvement of crumb score and softness, maintenance of freshness and softness | Amylase | Fungal |
| | Improvement of dough texture, reduction of mixing time, increase in loaf volume | Protease | Fungal/bacterial |
| Beer | Mashing | Amylase | Fungal/bacterial |
| | Chillproofing | Protease | Fungal/bacterial |
| | Improvement of fine filtration | $\beta$-Glucanase | Fungal/bacterial |
| Cereals | Precooked baby foods, breakfast foods | Amylase | Fungal |
| | Condiments | Protease | Fungal/bacterial |
| Chocolate, cocoa | Manufacture of syrups | Amylase | Fungal/bacterial |
| Coffee | Coffee bean fermentation | Pectinase | Fungal |
| | Preparation of coffee concentrates | Pectinase, hemicellulase | Fungal |
| Confectionery, candy | Manufacture of soft-centre candies and fondants | Invertase, pectinase | Fungal/bacterial |
| | Sugar recovery from scrap candy | Amylase | Fungal/bacterial |
| Corn syrup | Manufacture of high-maltose syrups | Amylase | Fungal |
| | Production of low D.E. syrups | Amylase | Bacterial |
| | Production of glucose from corn syrup | Amyloglucosidase | Fungal |
| | Converting corn syrup to a sweeter fructose-containing product | Glucose isomerase | Bacterial |
| Dairy | Residual $H_2O_2$ removal from milk (subsequent to sterilization by $H_2O_2$) | Catalase | Fungal |
| | Manufacture of protein hydrolysates | Protease | Fungal/bacterial |
| | Stabilization of evaporated milk | Protease | Fungal |
| | Production of whole milk concentrates, whey concentrates, and icecream and frozen desserts | Lactase | Yeast |
| | Curdling milk | Protease | Fungal/bacterial |
| Distilled beverages | Mashing | Amylase | Fungal/bacterial |
| Eggs, dried | Glucose removal | Glucose oxidase | Fungal |
| Feeds, animal | Pig starter rations | Amylase, protease | Fungal |
| Flavours | Clarification (starch removal) | Amylase | Fungal |
| | Oxygen removal | Glucose oxidase | Fungal |
| Fruit juices | Clarification, preventing gelling of concentrates, improvement of juice extraction yield | Pectinases | Fungal |
| | Oxygen removal | Glucose oxidase | Fungal |
| Laundry | Detergents | Protease | Bacterial |
| Leather | Dehairing, bating | Protease | Fungal/bacterial |
| Meat | Tenderization | Protease | Fungal |
| | Preparation of fish protein concentrates | Protease | Fungal/bacterial |
| Pharmaceutical and clinical | Digestive aids | Amylase, protease | Fungal |
| | Injection for bruises, inflammation, etc. | Streptokinase | Bacterial |
| | Various clinical tests | Numerous | Fungal/bacterial |
| Photography | Recovery of silver from spent film | Protease | Bacterial |
| Protein hydrolysates | Preparation of protein hydrolysates | Protease | Fungal/bacterial |
| Soft drinks | Stabilization of citrus terpenes from light-catalysed oxidation | Glucose oxidase and catalase | Fungal |
| Textiles | Desizing of fabrics | Amylase | Bacterial |
| Vegetables | Preparation of hydrolysates | Pectinase, cellulase | Fungal |
| | Liquefying purees and soups | Amylase | Fungal |
| Wine | Clarification of must | Pectinase | Fungal |

## Microbial metabolites

The growth of a microbial culture may be divided into a number of stages, as discussed in Chapter 2. After the inoculation of a culture into a nutrient medium there is a period during which growth does not appear to occur; this period is referred to as the lag phase and may be considered as a time of adaptation. Following a period during which the growth rate of the cells gradually increases the cells