ECONOMIC MICROBIOLOGY

Volume 4

MICROBIAL BIOMASS

edited by

A. H. ROSE

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PREFACE

Previous volumes in this series described production by microbiological processes of chemical compounds, including ethanol and associated flavour compounds in alcoholic beverages, that are of established commercial importance. This volume deals with commercial production of bulk quantities of micro-organisms, otherwise known as microbial biomass. Since by definition biomass is formed during exponential growth, it can be considered as a primary product of metabolism. Moreover, almost all of the commercially viable processes for production of microbial biomass have as their objective the availability of microbial protein, be it enzymically active or not. As such, the aim of these processes can be considered production of microbial protein.

The processes described in this volume fall into two classes. Firstly, there are those which entail production of viable, and therefore enzymically active, biomass. These processes are few in number, but range from the large and well established manufacture of baker's yeast to such small, and still to be fully exploited, processes as production of bacteria for inoculation into soil to promote nitrogen fixation and for use as insecticides.

The second class of process is equally diverse, although all of the processes have as their aim the manufacture of bulk quantities of micro-organism for use as food or fodder for animals, and conceivably in some instances for human beings. The *raison d'etre* for these processes is that it is now firmly established that the major nutritional deficiency in diets of man and animals is protein, and that the most rapid means of manufacturing large quantities of protein is by growing microbes in bulk. Human and animal diets have, for centuries, been supplemented with

valuable protein, usually inadvertently, in the form of traditional dishes whose preparation involves microbial growth. These dishes, particularly in the less well developed regions of the world, still make and will continue to make a valuable contribution to diets. At the other extreme, there are processes, mostly still at the developmental stage, which can produce large bulk quantities of micro-organism, with sophisticated technology, often from relatively expensive substrates. In many respects, this volume appears at a propitious time, for the rapid escalation in costs of energy world-wide has halted the development of most of these processes, and has forced operators to look in greater detail at the possibility of producing dietary microbial biomass from cheaper sources, including waste materials. The future of the more sophisticated process is very problematical indeed. Nevertheless, recent changes in the economic climate of the world have not altered the conviction of many nutritionists that microbial biomass can in the future make a valuable contribution to animal and human diets. We must wait and see.

August 1979 ANTHONY H. ROSE

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1. History and Scientific Basis of Large-Scale Production of Microbial Biomass

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I. HISTORY OF LARGE-SCALE MICROBE CULTIVATION

Plant and animal material which is acceptable and nutritionally valuable as a food for man is, by its very nature, susceptible to spoilage by micro-organisms. Where microbial spoilage of a foodstuff leads to the formation

of toxic substances in that food, Man has over the centuries learned to avoid eating the food, and more recently to prevent microbial spoilage of that type of food. On the other hand, microbial spoilage of a food may alter the appearance rather than the safety of a food. A case in point is spoilage of fermented beverages by bacteria and wild yeasts: a type of spoilage that is still encountered, but which must have been commonplace when these beverages were drunk from pewter vessels. Other types of microbial spoilage of foodstuffs are not only accepted but are actually encouraged. These include growth of moulds in cheeses and in the various tempe-type foods, in all of which microbial activity adds to the flavour and acceptability of the food. Encouraging growth of microbes in foods also adds to the nutritional value of the food, and it is interesting to note the comment made by Thaysen (1957) that an average daily diet of a fermented food could furnish between 6 and 10 g of microbial matter, and therefore 2–4 g of protein.

In a historical context, the development of processes for large-scale cultivation of micro-organisms is best considered under the following three headings.

A. Edible Fungi

When early man looked round for food to eat, he was quickly attracted by the fruiting bodies of several fungi—mushrooms—which have a pleasant and attractive taste. Early civilizations came to marvel at the ability of mushrooms to appear seemingly spontaneously overnight, and without seeds having to be sown, and as a consequence they acquired certain mystical qualities. Francis Bacon, in *Sylva Sylvarum* (1627), referred to mushrooms as a venerous feast. There is evidence, too, that they came to be associated with various forms of worship, and John Marco Allegro, in his book *The Sacred Mushroom and the Cross* (1970), has argued that the cult of the mushroom was a seminal influence in the rise of Christianity. Collecting mushrooms in the wild has continued over the centuries. It is today particularly popular in continental Europe where, sadly, several hundred deaths occur annually because some hunters are unable to recognize poisonous mushrooms.

The first organized efforts at cultivating mushrooms—species of *Agaricus*—apparently took place in France during the reign of Louis XIV (1639–1715). Underground caves in Paris provided a suitable

environment and indeed still do. It had been discovered that horse manure was a good medium on which to grow mushrooms, and this was stacked in rows on the floors of the caves. The earliest description of mushroom growing was by N. de Bonnefons in an article entitled *Le jardinier françois*, and published in 1650. This was translated into English eight years later by John Evelyn under the title *The French Gardiner*. But the first authoritative account of the new art came from the eminent French botanist Tournefort in 1707. Mushroom growing was soon taken up in England and, in 1779, Abercrombie published his treatise on the garden mushroom and its cultivation. Thereafter, mushroom growing gradually became a stable industry. Today, world-wide production is approaching half a million metric tonnes annually. More detailed accounts of its development can be found in Ramsbottom's (1953) book and in the chapter by Hayes and Nair (1975).

Regular attempts have been made over the years to cultivate other fungi, often without success. Particular attention has been given to truffles, *Tuber melanosporum*, which grow underground, and which are traditionally sniffed out using trained pigs or occasionally goats. They grow beneath oak trees, and have for centuries been prized for their culinary virtues. The *département* of Dordogne in France is a well known truffle region, and at the turn of the present century sold through its town markets well over 100 tons each year. Schemes have been started in that *département* to cultivate truffles in a more organized fashion, and more than a 100 hectares of new oaks have recently been planted and treated to induce truffle growth (Scargill, 1974).

B. Baker's Yeast

Bread, which has been prepared from time immemorial, consists basically of a slurry of ground cereal, known as dough, that has been baked. The Egyptians are credited with the discovery, undoubtedly made quite unwittingly that, if the dough was left to develop a microbial flora, gases produced by fermentation of carbohydrates by the micro-organisms gave rise to bubbles in the dough which, when baked, gave a lighter and much more acceptable bread. This is the process of leavening, one which figured prominently in the history of the Jewish race. The original leavening agents were probably a mixture of yeasts and lactobacilli, which was maintained by retaining a portion of each dough

before baking, and using it to start the next batch (Burrows, 1970). This practice continued for many centuries but, during the Middle Ages, use gradually was made of the surplus yeast produced during beer brewing and wine making; this was referred to as *barm*. However, using barm for leavening bread is fundamentally unsatisfactory, since the quality and activity of barm, even from a single brewery, are very variable.

Around the turn of the nineteenth century, increasing attention was given to processes that involved propagation of strains of *Saccharomyces cerevisiae* under conditions that gave a yeast with consistently good baking qualities. In the intervening years, the manufacture of baker's yeast has evolved into a highly specialized and sophisticated large-scale process (Frey, 1930). The first major advance came with the advent of the Vienna process around 1860 (Frey, 1930) which recognized that, when growing strains of *Sacch. cerevisiae* largely anaerobically in a grain mash, the yield of yeast was improved by passing a gentle stream of air through the mash. Then came Pasteur's researches, which stressed the need for a more intense aeration in order to develop high yields. A shortage of grain in most developed countries of the world during the 1914–1918 war led to molasses, a by-product in sugar manufacture, being substituted for grain. Finally, as a result of work patented almost simultaneously in Denmark (Sak, 1919) and Germany (Verein der Spiritusfabrikanten in Deutschland, 1919), and usually associated with the name of Soren Sak, there was introduced the fed batch or *Zulauf* process. In this, the carbohydrate supply (molasses solution) is fed incrementally during the growth phase, so that there is never an appreciable concentration of sugar in the culture in which, as a result, almost none of the sugar is converted into ethanol. Ethanol production represents a loss of carbohydrate which ideally should be converted into yeast cell material.

As a result of further development of the manufacturing process and genetic selection of strains of *Sacch. cerevisiae* with excellent leavening properties, many countries in the world now have highly efficient industries for manufacture of baker's yeast. Not enough credit is given to this industry which is able to produce, daily, huge quantities of a highly perishable commodity, namely pressed yeast, which must have a guaranteed fermenting capacity. Recent years have seen some attempts to foster production of active dried yeast, a form of baker's yeast which contains less water (about 8%, w/w) than pressed yeast (70%, w/w), and therefore has a much longer shelf life, and can safely be shipped to and used in hot climates.