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Biotechnology of Biomass Conversion

Morris Wayman · Sarad R. Parekh

In association with the Institute of Biology IOB

Biotechnology of Biomass Conversion

Fuels and Chemicals from Renewable Resources

Morris Wayman and
Sarad R. Parekh

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Biotechnology of Biomass Conversion

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Acknowledgements

While this book is the joint effort of its two authors, the Chapters which are primarily microbiology, Chapters 7–12, were written in the first instance by Dr Sarad Parekh, whose graduate training is in microbiology, and the other chapters by the senior author. Also, the senior author accepts sole responsibility for the CODA, which reflects both his interest in music and his optimistic vision of the future.

The substance of the book owes much to many people. For the procedures developed in this laboratory for the improvement of fermentation performance we are indebted to Dr Shiyuan Yu, now of Nanjing Forestry University. Our understanding of the manner in which yeasts respond to stress follows from his contributions. This is a relatively new and exciting field, which is not treated adequately in this book: it will have to await another.

Our knowledge of acetone-butanol fermentation was much enhanced by Dr Rannade Parekh, a skilled microbiologist, the wife of Sarad Parekh. She also developed useful procedures for saccharification of wheat chaff, a particularly refractory form of biomass.

The senior author is indebted to the many graduate students who laboured under his guidance, and particularly to those named in the references. Shu Chen, who at this time is a Ph.D. candidate, has taught us much about co-immobilization of yeast and the enzymes glucoamylase and cellulase. Dr T.N. Soundararajan, of New Delhi, obtained the very first molecular weight distributions of lignin in 1968–9, painful then, routine now. This work laid the procedural basis for the contributions of Dr Timothy I. Obiaga, of Lagos, including a much clearer concept of the architecture of the lignin molecule. Dr Miranda Chua Yap, of the National University of Singapore, and Dr Jairo Lora, of Repap Technologies, Valley Forge, left indelible marks on the chemistry of lignin. Dr Lora first suggested the procedure and the name of autohydrolysis.

Our industrial colleagues also added much to our knowledge. Brian Foody, of Iogen, operates a most interesting pilot plant which follows the most direct procedure for bioconversion of biomass: steam explosion, then enzymatic saccharification and fermentation of the resulting sugars. Dr Per Assarsson's continuous high shear plug flow reactor is being increasingly adopted for full scale starch saccharification, one installation being illustrated in the frontispiece. His excellent experience with HCl-catalysed saccharification at high temperature refutes some widespread notions about corrosion. Robert Clyde provided the stimulus and sold us our first piece of the remarkable equipment we now call a continuous dynamic immobilized biocatalyst bioreactor, which has led to much excitement and many publications. The senior author is indebted to Frank Lewarne, of Brascan, who introduced him to the dynamics of Brazil.

In government, three names stand out: the late Douglas Hayes, of Energy, Mines and Resources Canada, Dr Robert Greven, of the Ontario Ministry of Energy, and Dr Wayne Craig, of the Saskatchewan Research Council. Each of these has broadened the scope of our researches.

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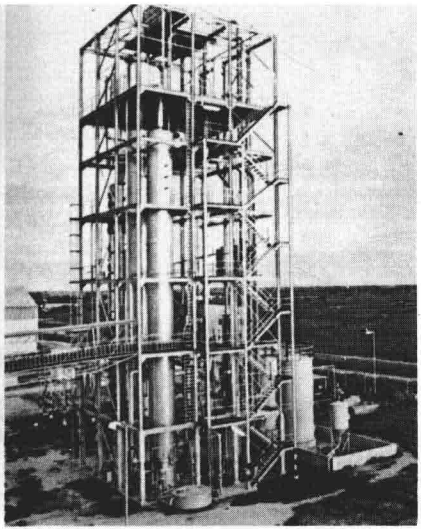
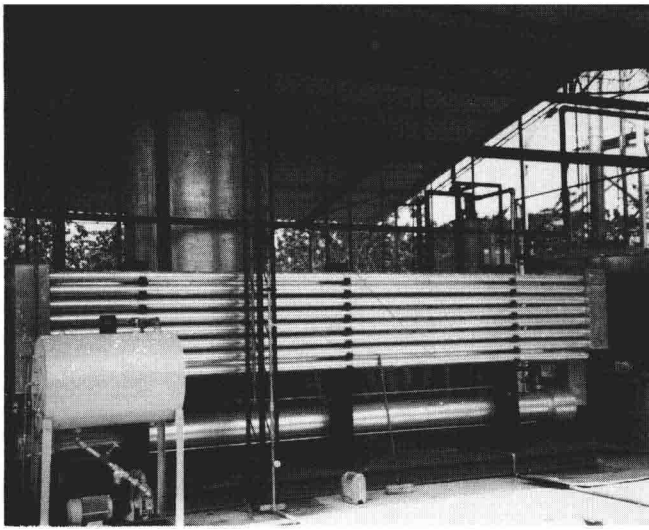
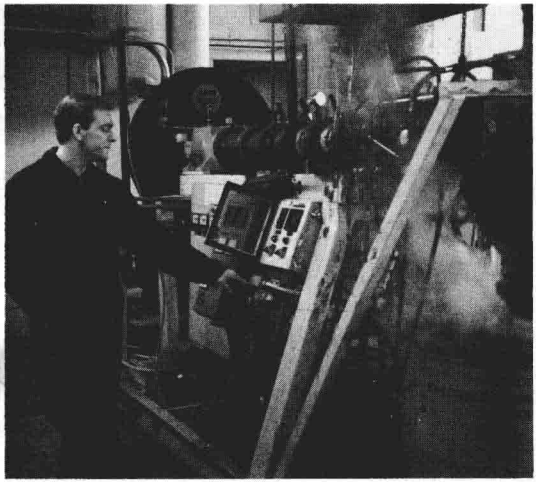
We are also grateful to the staff of Open University Press, who have been unfailingly helpful, and particularly to Richard Baggaley, who encouraged us to undertake this venture.

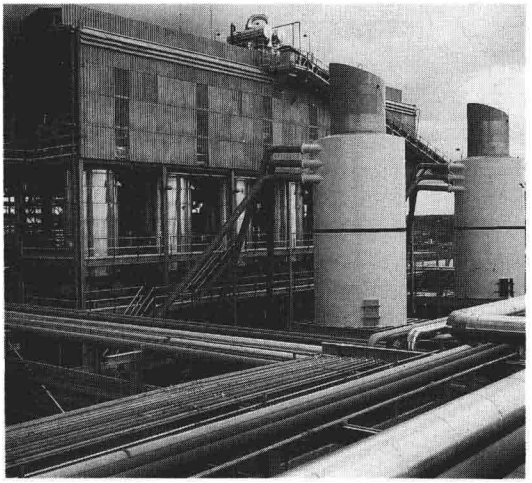
We invite our readers to write to us to draw our attention to any needed corrections, and with their comments and criticisms.

Morris Wayman
Sarad Parekh
University of Toronto









Frontispiece: *Upper left:* distillery near cane fields, Malawi. *Upper right:* continuous plug flow hydrolyser, Colombia. *Lower left:* cooker-extruder prehydrolyser, Canada. *Lower right:* wood hydrolysis ethanol plant, Brazil.

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SECTION I

Overview

Chapter 1

Why Biomass Conversion?

Will 'gasohol' from trees and from agricultural surpluses replace petrol as we now know it? Will the lignin byproduct from biomass conversion cure heart disease and cancer of the colon? What *is* 'biomass'? This brief introductory chapter will present the benefits which are expected to arise from the bioconversion of biomass to useful products, in particular to liquid motor fuels, and which provide the motivation for large-scale industrial development and research, particularly using the tools provided by biotechnology.

What is Biotechnology?

The term 'biotechnology' is applied to processes as ancient as fermentation and as modern as recombinant DNA genetic engineering. The making of bread, in which a living organism, yeast, provides the 'rise' and the texture we want, or the making of wine, in which a similar living organism provides its alcohol, or in making vinegar, in which process a bacterium produces the acetic acid which gives vinegar its tartness – all of these and similar processes may be encompassed in the term 'biotechnology'. However, just as these are all old processes, so this is a relatively simple and old-fashioned use of the term. Since the discovery of the double helix of DNA, the abbreviation for deoxyribonucleic acid, the carrier of the genetic information which enables living organisms to reproduce themselves thereby establishing that they are alive, the term has been applied more specifically to those manipulations of heritable characteristics which are called genetic engineering. Such genetic engineering may include the unravelling of the double helix, its decomposition into fragments of DNA and their recombination into new DNA. Between these extremes, simple fermentation and recombinant DNA genetic

engineering, lie a number of intermediate methods and procedures for changing, and hopefully improving, inherited and heritable characteristics of organisms, all of which may be included in the concept of biotechnology.

In a recent book *Biotechnology: The Biological Principles*, (Trevan *et al.*, 1987) biotechnology is defined as 'the application of biological organisms, systems or processes to manufacturing and service industries'. Figure 1.1 (taken from that book) illustrates the broad scope and application of biotechnology.

What is Bioconversion?

Bioconversion is the transformation of one substance to another by means of a living organism, or by enzymes or other products derived from a living organism. Yeast, for example, converts sugar to carbon dioxide and alcohol, and lactobacilli convert alcohol to acetic acid. The sweetener most used in soft drinks today is made by enzymatic conversion of the glucose of corn syrup to fructose, a process in which the enzyme glucose isomerase is first made by a mould or a bacterium and is then immobilized on a support where it catalyses the bioconversion. The bioconversion of the glucose of corn syrup or the sucrose of cane sugar to ethanol is the basis of the fuel alcohol industries of the United States and Brazil; the bioconversion of the cellulose and the hemicellulose of wood and agricultural crops and residues is an example of the contribution of biotechnology to the same industry.

Plant cell culture and mammalian cell culture are important processes for the production of specific chemicals, and therefore important aspects of biotechnology or, in the case where such culture is used for chemical transformation, of bioconversion. Plant cell culture has produced clones which have then in a few cases, such as carrots, resulted in whole plants. Oil-producing plants with a very high concentration of desired unsaturated oils such as oleic acid, or a very low concentration of toxic compounds such as erucic acids, have also been generated by such procedures. These are further examples of directed biotechnology.

What is Biomass?

The term biomass applies to the mass of substance generated by the growth of living organisms, be they micro-organisms, plants or animals. Sugar cane, grain, tubers and wood are the examples we shall deal with most extensively here. The term also includes byproducts of agriculture or processing of agricultural crops such as straw and sugar cane bagasse. In our context it may also include agricultural surpluses. Biomass may be generated deliberately as single cell protein, which refers to yeasts, moulds or bacteria grown primarily for their protein content which is used for animal feed or human food, or which may be used as a source of the various interesting pharmaceuticals with which micro-organisms are loaded.

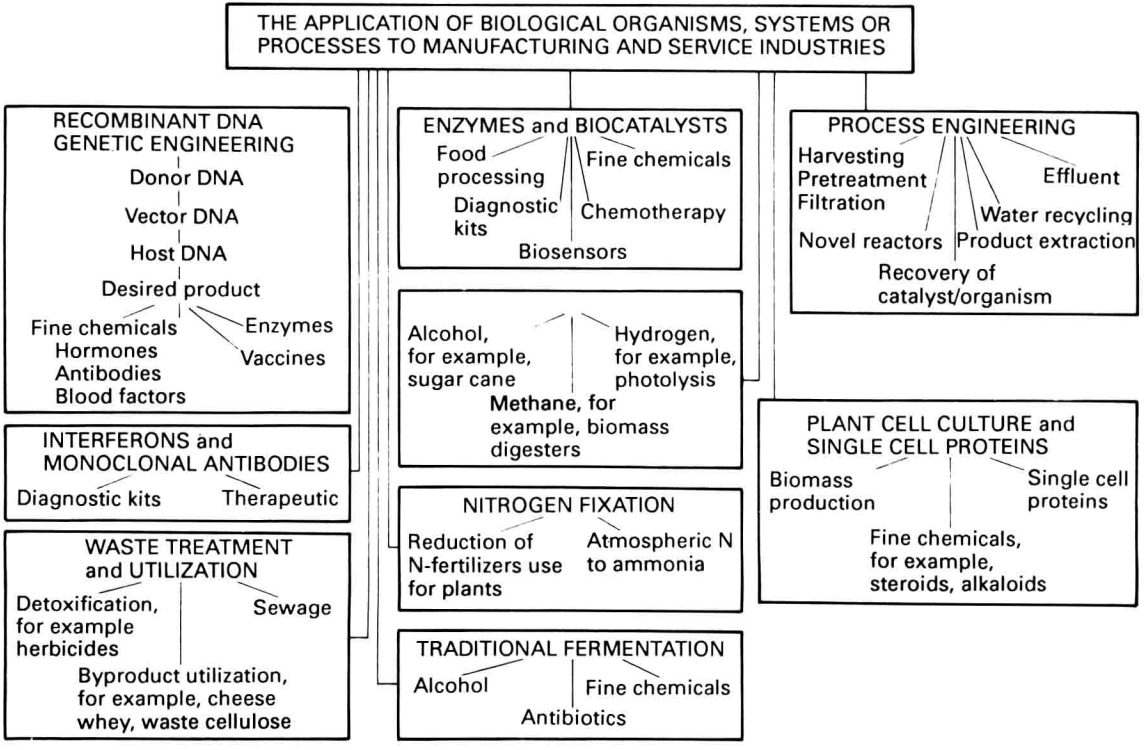

Fig. 1.1 The scope of biotechnology. (Reproduced with permission from Trevan *et al.*, 1987, Open University Press, Milton Keynes.)

Benefits from Biomass Conversion

An immediate and obvious benefit from bioconversion of biomass lies in its potential to supply liquid motor fuels, perhaps enough to offset shrinking world reserves of petroleum, and to avoid the environmental damage from burning petrol in piston engines. The motivation to proceed in this direction, in spite of resistance by the established motor fuel production and distribution industry, arises from:

(1) Environmental pressure to remove toxic lead from gasoline, and to use ethanol as an octane enhancer in its place.
(2) Gasoline is a major source of smog while fuel ethanol provides clean air.
(3) Pressure from the farm community for diversification of agriculture, particularly for the utilization of surplus crops – a major problem in Europe and North America.
(4) Long-term security of motor fuel supply since the sources of inexpensive petroleum will in due course dry up while biomass is an ever-present renewable resource.
(5) Export possibilities arising from the US shortfall in domestic petroleum supply.

In addition, the development of this major new industry would provide us with new byproducts with potential benefits of considerable magnitude, for example, the cholesterol-lowering action of dietary lignin, and the byproduct biomass as high protein cattle feed. In all western countries such a major new industry will provide great savings in foreign exchange and significant relief from the pressure of widespread unemployment and the drift from the countryside to the crowded cities. Furthermore, the absorption of atmospheric carbon dioxide by plantations of sugar cane, grain and trees dedicated to biofuel production will help offset the 'greenhouse effect'.

Renewable Sources of Biomass

Cellulose, hemicellulose and lignin are the organic compounds which make up the biomass of trees and agricultural crops, and are those produced in the largest quantity by natural processes such as photosynthesis. Cellulosic biomass, or lignocellulosic biomass, as a feedstock for bioconversion to renewable fuel, is now available in forests and fields, and can be grown as dedicated crops. Sources of renewable biomass for this purpose include forest industries' waste wood, agricultural crops and crop residues and dedicated energy plantations of fast growing wood species or other efficient photosynthetic converters of solar energy to biomass. While such energy plantations are few in number as yet, the potential for them is present in large areas of the western hemisphere, Asia and Africa presently lying fallow (Figure 1.2). In the United States corn is by far the largest

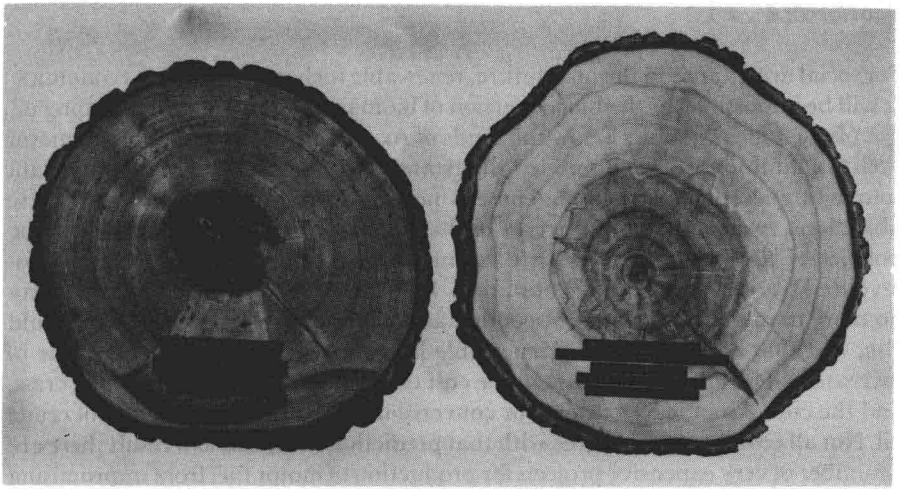

Fig. 1.2 Natural growth (left) and plantation growth (right) (Ontario Ministry of Natural Resources).

source of ethanol in motor fuel, whereas in Brazil most fuel ethanol is made from cane sugar.

Scale

The consumption of gasoline in North America is about 350 000 *million* litres per year, whereas the largest ethanol plants are 400 million litres per year. Is such large-scale ethanol production conceivable? Can the land provide so much biomass for bioconversion? Such questions need to be answered more in terms of when so much fuel would be required, and what the ultimate need might be. To supply 10% of present liquid fuel to US motors would require only 100 plants as large as the largest fermentation ethanol plants now in operation there. That is not too difficult to visualize. The supply of feedstock, however, may constitute the main limitation. One could confidently expect 3500 l/ha/year of ethanol. The supply of 10% of US motor fuel requirements would require almost 10 million ha or 100 000 km^2, about 1% of the total land area. While it would take a truly mega-effort to realize such an area devoted to a single purpose, it is not out of line with present areas under grain crops. We have the example of Brazil before us, with a total area of 8.5 million km^2 supplying about 30% of its motor fuel requirements from the products of the cane sugar industry without any apparent land pressure. Perhaps we shall need to turn to a more productive climate to find the land we need for this purpose, and much more efficient engines to be able to rely heavily upon renewable biomass as a source of most motor fuel worldwide.