

BIOTECHNOLOGY

**INTERNATIONAL
TRENDS AND PERSPECTIVES**

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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

The Organisation for Economic Co-operation and Development (OECD) was set up under a Convention signed in Paris on 14th December 1960, which provides that the OECD shall promote policies designed:

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One of the important tasks of the OECD Committee for Scientific and Technological Policy is to follow the emergence of major fields of technology, to debate the various policy issues arising from it, and to help solve those which fall within its Mandate.

Thus, I am pleased that this report to OECD on Biotechnology is being made available to the public at large. This work is but the beginning of a more continuous interest of the Committee in a technology that is likely to modify the lives of most people in the OECD Member countries and beyond, through impacts on health, nutrition, energy and the environment.

Three factors come together — in my view — to explain the interest which this report has generated in the Committee. First, it is an up-to-date, comprehensive review of prospects in an area of science and technology of major interest to Member countries. A second factor is the conviction of the authors of the report that the successful development of biotechnology depends upon conditions and directions which the Committee has attempted to foster in other sectors of the research system and in other areas of science policy. They are: the need for increased emphasis on inter- and multi-disciplinary research, the close interaction between fundamental research, applied research and engineering and the corresponding need for balanced support of all components of the R&D spectrum, the importance of assessing future opportunities in science and technology and their societal impacts, and the need for integration of science, economic and other policies (such as education and regulation policies).

A third feature of this report is its timeliness. Several OECD countries have launched biotechnology plans or policies during the last few years, and they have now to concern themselves increasingly with the international implications of their projects. Other countries are in the process of drafting their first plans.

Accordingly, the Committee has agreed to undertake further work on biotechnology. This work will focus on four issues:

- Patent Protection in Biotechnology, which among others, will enable individual OECD countries to compare their legal situation to that of others.
- Safety and Regulations, which will investigate among other aspects, the problems that might arise in industrial mass production.
- Government Policies and Priorities in Biotechnology R&D, which will compare past and present R&D priorities related to biotechnology, and review the national debates and mechanisms that have helped to set these priorities.
- Economic Impacts of Biotechnology, an important project that might begin when the other projects are nearing completion.

I can only express my hope that the next steps in the Committee's work on biotechnology will meet with as much interest and success as this first step.

Prof. Dr. A.A.Th.M. Van Trier
Chairman of the Committee for
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FOREWORD

The report presented by Professors Bull, Holt and Lilly is a most valuable summary of current knowledge in the new field of biotechnology; it sets out, fully and precisely, the state of the art from the science, technology and economics standpoints. Its many predecessors have dealt with biotechnology in given countries or groups of countries, or else in a particular discipline or field of industrial application. The merit of the OECD report lies in considering the issues on a comprehensive international scale, and covering the whole field of biotechnology. Earlier work, notably the reports by specialist private firms, also lacked sufficient detachment and tended to present enthusiastic conclusions that mirrored the views of their sponsors.

Before the report was submitted to the Governments of the OECD Member countries it was examined by a Group of experts¹ of which I had the privilege of being Chairman. The experts unanimously considered the report to be a major new contribution of interest to governments, the scientific community and industry alike. The Group concentrated largely on drawing conclusions and recommendations, primarily intended for governments, and these are shown on page 10. Here I should like to stress and enlarge upon certain of the conclusions and recommendations which I believe to be especially significant.

One essential point is how to define and delimit the field of biotechnology. The definition is needed at international level for scientific, technological and economic reasons. Common definitions are important when establishing statistics, gauging results and comparing budgets.

The authors and the Group of experts have both sounded warnings of the danger of definitions that are too narrow or too broad. A narrow definition, often found in the press, reduces biotechnology simply to genetic engineering. At the other, equally dangerous, extreme, the definition would have biotechnology include all activities in which live materials are used, in particular all agrofood activities. The definition given in the report describes biotechnology as the use of biological functions as a technological tool, together with the activities that derive from this use, such as purification and recovery.

The point is made in the report and the discussions of the Group of experts that the enthusiasm biotechnology has aroused is excessive and that a return to reality is imperative. The authors demonstrate the potential of biotechnology and the prospects that it opens up, but point also to the constraining factors which are far from negligible. Their effect will be considerable, and while they need not rule out rapid development in biotechnology, they have to be taken into account from the outset in defining policy.

One instance of over-enthusiasm is the tendency in the press and among certain people involved in the field to confuse the formulation of projects and forecasts with their materialisation, which is liable in fact to be lengthy and expensive in many cases.

The recommendations of the Group of experts do not necessarily endorse the line widely taken in other reports and in the press. Rather, while acknowledging the importance of conventional molecular biology and genetic engineering, we assert that it would be dangerous to consider only that aspect of biotechnology and stress that many other fields of value for agricultural, industrial and medical applications receive far too little attention at present.

The experts considered four fields of R&D to merit broad priority.

1. The members of the Group are listed on pp. 15–17.

First, there is a need to increase our knowledge in the field of molecular plant biology. What we know about plants has historically lagged behind our knowledge of microorganisms and animals.

Second, it is important to improve our knowledge of microbial physiology, especially for industrial applications. This discipline is far advanced in Japan, but it is backward or virtually non-existent in every other country.

A further important recommendation for industry is the use of microorganisms other than those invariably studied in laboratories throughout the world. That applies particularly to *E. coli*, which unfortunately is not always of greatest interest in industrial terms. Microbiological research should accordingly focus on other microorganisms.

The fourth field that is felt to have priority is biochemical engineering, which includes fermentation technologies, the development of biological catalysts through enzyme engineering, and problems of molecule recovery and purification. Recovery and purification methods, for instance, are absolutely essential in ensuring that advanced techniques in genetic engineering or enzyme engineering do not simply remain forecasts, projects and newspaper articles, but become established processes capable of stimulating the industrial and economic development of our countries.

In addition to research, the report deals thoroughly with the problems of training in biotechnology. Training questions are important for the development of bioindustry, since that calls for people trained in both science and technology. In the present circumstances it is difficult to speak of a single training route for “biotechnologists”, and a multidisciplinary approach would be of far greater use. While people in science and industry need to be broadly and properly familiar with the overall problems, what is particularly important is that each specialist in science, technology and industry should be highly skilled in a given field — microbiology, genetics, molecular biology, chemical engineering or biochemical engineering.

One general problem, especially relevant to biotechnology at present, has to do with relations between universities, or academic institutions in the broad sense, and industry. A transfer of knowledge between universities and industry is essential. It must be promoted as effectively as possible through the dissemination of information, through personnel mobility, and through co-operation between university research, which is usually public, and industrial research and production, which are usually private.

All the same, important though these transfers to industry may be, governments should not overlook the fact that it is essential to maintain a high standard of fundamental research in all the life sciences, particularly those which directly concern biotechnology. It would be wrong to think that biotechnology can be developed simply by improving its technological and applied aspects. The areas to be studied may vary from one country to another, depending particularly upon the resources available, but the vital point is that each country should have a fundamental research activity through which it can acquire knowledge that can be transferred to industry.

Another problem of concern to the experts in biotechnology is a tendency for fundamental knowledge to remain in private hands. Many university institutions are building up special exclusive relationships with industrial corporations and we fear that a lack of dissemination of fundamental knowledge or restrictions on such dissemination may to some degree impede the development of the pool of basic scientific knowledge at the international level.

On the economic problems that the development of biotechnology may involve, one merit of the present report is that it is virtually the first not to overlook the question of raw materials. That question does arise, and the answer is by no means simple.

Very substantial quantities of raw materials will be needed in order to establish biotechnology industry on a large scale, and the sources will have to be diversified. The raw materials of the biotechnology industry are principally sources of carbon (starch, sugars and in coming years possibly cellulose). The problem is especially important because these carbon-containing substances are obtained from agricultural activity. Being at the interface between agricultural activities and non-food industries utilising agricultural products will not always be easy. The sectors concerned will be the chemical and pharmaceutical industries, and possibly the energy sectors. The movement of carbon-containing

substances from the agricultural sector into industry poses many thorny problems of regulation, taxation and financing, for which there is no ideal solution.

Another problem in biotechnology is that of patents. It is more difficult to apply clear and strict rules to define the patentability of life-based products or processes than to define the patentability of more conventional technological inventions. The patenting problems which arise in the area of genetic engineering, microorganisms and the use of biocatalysts need to be discussed at international level, the OECD being a particularly appropriate forum.

Safety is another matter to which the experts wish to draw attention. The problem is not confined to genetic engineering and microbiology; it also and particularly applies to industrial processes utilising very large quantities of microorganisms. Some leading experts are sure that, above a certain scale, it will be practically impossible to guarantee absolutely no leakages of microorganisms during production itself or during the essential stages of molecule purification and recovery.

Safety matters need to be examined at international level, since otherwise they could lead to differences in legislation and practice and accordingly generate economic and industrial tension between countries.

Biotechnology and the biotechnology industry open up new horizons for human activity, and especially for industry, in the near future. Starting from science, and from a range of technologies, biotechnology does not apply to one area of industrial activity alone but to a whole range: the agrofood industry, pharmaceuticals and chemicals, the energy sector, and some aspects of agriculture as well.

Governments need to be alive to what is happening in this field and they will have to take initiatives and define appropriate policy, even though the private sector will frequently be the driving force in industrial development.

At all events, the time has come to leave the stage where biotechnology was the subject of an uncontrolled publicity cult. Biotechnology has a great future, but it will come up against many constraints. It will solve certain problems but it will not be a universal panacea, and the projects in hand are liable to prove expensive and in many cases will not come swiftly to fruition.

Prof. Daniel Thomas
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**CONCLUSIONS AND RECOMMENDATIONS
OF THE EXPERTS WHO MET AT THE OECD
ON 17th AND 18th MARCH 1982**

1. *The Need for a Common Definition and for Comparable Statistics*

One fundamental recommendation addressed to governments is to try to find a common definition of biotechnology, difficult though this may be, to allow for the collection of internationally comparable data on R&D, production, manpower, etc.

It was suggested that countries should be asked already to list their research centres or faculties which do R&D in biotechnology, indicating the type of research carried out as well as the numbers of personnel and funds involved. It is equally important to collect data on government expenditures for biotechnology R&D.

2. *R&D Priorities*

Although specific priorities have to be set by each country individually, a number of general priorities apply to almost all. Four to five priority areas of comparable importance have been defined:

a) *Basic Plant Science*

Plant science is much less advanced than microbiology. Strong support will be necessary to increase basic knowledge of plant physiology and plant genetics if governments want the expected agricultural impacts of biotechnology to materialise.

b) *Microbial Physiology*

With the exception of Japan, microbial physiology, i.e. the study of the relationship between the metabolic capability of microorganisms and their environment, is almost a universal bottleneck. Industry does little or no fundamental research in this discipline which thus needs public support.

It is also most important to devote much more study to mixed microbial cultures because of the advantages these can offer compared to monocultures.

c) *Study of New, Atypical Organisms*

Exclusive concentration on a few organisms has led to the neglect of large sectors of microbial life. Much is known about *E. coli* which is not very useful in industry and difficult in fermentation when genetically engineered, but very little is known about other more useful organisms. It is therefore urgent to do R&D on anaerobic, photosynthetic and thermophilic bacteria, filamentous fungi, as well as yeasts that have been ignored because they are more difficult to study, or for other opportunistic reasons.

d) *Biochemical Engineering*

R&D on product recovery and purification may be less spectacular than the “glamour” areas of biotechnology and therefore governments do not take them sufficiently into account. However, if there is not considerably more research on, and progress in, biochemical engineering, advances in other areas might have little or no practical results.

Down-stream processing and recovery could now be improved by the introduction of new methods resulting from the use of genetic concepts (e.g. monoclonal antibodies).

e) *Other*

Other R&D subjects have been proposed which should be priority areas in some, if not many, countries. The transformation of xenobiotics in order to promote, inter alia, new waste treatment systems certainly needs more research, and so do the physiology and genetics of pathogenic bacteria. In the field of genetic engineering, host systems with a high degree of universality, safety and optimal process properties are an important area for further study.

3. *Training*

There is at present a considerable need to increase the awareness of biotechnology at the higher education level. However, the experts agreed that there was no need for a new university discipline and that no one should be trained just as a “biotechnologist”; specific skills should be developed in an interdisciplinary context. In any event, no biotechnology specialisation should be envisaged at the undergraduate level.

Although the situation varies from country to country there is a wide-spread shortage of microbial physiologists and of biochemical engineers capable of exploiting the results of genetic engineering. Furthermore, computer science is a general, essential tool that every biotechnologist should be able to use.

4. *Industry–University Links*

Experts spoke of the danger that excessive business orientation of university researchers could result in a reduction of fundamental research, or that certain types of industry–university links could lead to a loss of knowledge due to trade secrecy.

Ways must be found to avoid these risks, even if the reduction of government funds for R&D is making increased industrial financing inevitable.

5. *The Need for Better Culture Collections and for Data Banks*

By funding and improving microbial culture collections, governments could make a vital contribution to the progress of biotechnology with comparatively modest financing.

Keeping microorganisms is a very difficult task, particularly as it is now necessary to differentiate between microorganisms, plasmids and viruses. Each country seems to follow a different policy on this, and there are wide-spread complaints about bad performance of most, if not all, existing culture collections. Thus, it might be necessary to reduce their numbers, and to improve the quality of their work, but also to give them the necessary financial backing which they often have great difficulty in finding.

Of similar importance is the creation and financing of national and international data banks on several areas of biotechnology, including nucleotide sequencing, enzyme information and microbiology, to facilitate access to important research material.

6. *Economic Conditions of Biotechnology: Raw Materials and Competitiveness*

The future of biotechnology depends to a considerable degree upon the availability of raw materials. No government should launch biotechnology plans without careful study of their implications with regard to renewable and other raw material resources. In some OECD countries, this applies also to water resources.

A comparative examination of the raw material basis for biotechnology in several countries might also lead to a somewhat modified picture of relative strength or weakness in this sector. In Japan for example, almost all of the raw materials for the fermentation industry have to be imported from abroad; in the United States, almost none.

In addition to raw materials, the competitiveness of biotechnologies compared to other technologies must be studied. There are many promising developments in other R&D areas as well and it would therefore be misleading to carry out economic studies on biotechnology in isolation from other technologies, on the presumption that biotechnology will be a good solution under all circumstances, as has been done in the past. This warning is especially true, among others, for the energy sector where biotechnologies are often expected to provide major new supplies, regardless of other evolving energy technologies.

7. *Economic Impacts of Biotechnology*

It is now becoming increasingly critical to leave dreams and fashions behind and to begin more serious, long-term impact studies.

a) *Industrial and Service Sectors*

It is widely agreed that the largest, short-term impact of biotechnology will be felt in the fine chemicals sector, followed by the sewage disposal and pollution control sector (present sewage treatment is partly outdated). However, biotechnology will not replace basic chemicals.

More precise studies should investigate whether and where biotechnology might supplant traditional technologies or sectors, and where it might expand beyond them and create new opportunities. The possible substitution of agricultural animal stock feed by biotechnology-produced stock feed must be investigated.

Linked to industrial and other economic consequences of biotechnology are employment effects which need careful study.

b) *Trade Effects*

Biotechnology will have considerable trade effects between agricultural or raw material producing and industrialised countries, both within the OECD area and between OECD and developing countries. These effects must be studied.

c) *The Eco-System*

Biotechnology will affect the ecosystem in many ways, just as hydrocarbon technologies have done and are still doing. However, this time, an assessment of ecological consequences should precede large-scale biotechnology developments. Thus, the mistake of basing our entire economy on oil and coal without investigation of larger ecological implications must not be repeated.

8) *Patents*

The experts pointed to the need for an improved patent system suited to new developments in biotechnology. They acknowledged the importance of ongoing OECD work in this respect.

9. *Safety and Regulations*

The experts agreed on the necessity to study and implement new and pragmatic safety measures. The safety of genetic engineering research has ceased to be a major reason of concern. Although one cannot assume that genetic engineering will not present dangers in the future, the existing regulatory frameworks seem to be sufficient to cope with them.

This does not apply to industrial biotechnology. It is true that even without special regulations the biotechnology industry has not experienced a biological accident for decades. However, large scale industrial applications of new biotechnologies based on genetically engineered microorganisms could create problems which have received very little attention compared to that devoted to possible risks at laboratory or pilot plant scale. It is impossible to avoid completely leakages in large-scale biotechnology production and in recovery and down-stream operation. Thus, it is essential to make sure that microorganisms are safe before mass-production starts.

Serious study of these new issues, and concern for human safety should not lead to over-emphasis of the risks, as has occasionally happened in the past. It is important not to discourage innovation but to maintain a favourable climate in this sector.

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In recent years many articles and reports have been written on biotechnology. When invited to prepare a state of the art report for OECD we were hesitant to add yet another document to the pile. However, on reflection, we felt there were certain aspects of biotechnology that had not been dealt with in a sufficiently balanced and critical manner. We have endeavoured to underline the scientific basis of biotechnology but have not attempted to provide a comprehensive review. It has been our intention to emphasize what we consider to be universally important trends and issues.

In this report we have given what we hope will become a widely adopted working definition of biotechnology. No short definition can adequately describe this diffuse field and it has been necessary to provide guidance on the interpretation of our definition (Introduction). Also to avoid the confusion which often exists in discussions of biotechnology, we wish to stress two points: first, biotechnology is not a discipline but a field of activity; second, genetic engineering *per se* is not biotechnology but an exciting development which will have an enormous impact on biotechnology. We have focused on the potential of contributing sciences to future developments in biotechnology (Chapter I). At the same time we draw attention to possible scientific, technological and resources constraints and indicate how some of these may be overcome (Chapter II). We also raise a number of important issues for further debate which are linked, directly or indirectly, with government policies (Chapter III), many of which are highlighted in the Conclusion.

We hope that this report will stimulate both scientists and non-scientists alike. To help the latter, we have provided a short glossary of commonly used scientific terms.

We wish to acknowledge the useful comments and discussions which we have had with colleagues throughout OECD Member countries and to thank the OECD Secretariat, especially Miss Bruna Teso and Dr. Salomon Wald, for their valuable assistance.

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INTRODUCTION

1. *Definition of Biotechnology*

As a result of the increasing interest by Governments in the rapidly developing field, referred to as biotechnology, many organisations and working parties have published reports which include definitions of biotechnology (Appendix I). There is considerable diversity of definition (and not infrequently confusion) depending on the interests and prejudices of those involved.

For the purpose of this report, it was essential to have a working definition, which we give below and hope that it will find general acceptance. Without a common definition, governments risk speaking at cross-purposes when they discuss biotechnology in international contexts, as has occurred occasionally in the past, and international statistical comparisons of biotechnological research and production, which are among the prerequisites of rational policy-making, will remain difficult and unreliable.

In proposing a common definition for OECD Member countries, it is important to distinguish between biotechnology itself and those activities upon which it has an impact. Technology, according to the Oxford Dictionary, is the “scientific study of the practical or industrial arts” or the “terminology of a particular art or subject”. It is not an industry but a scientific activity. Thus it seems reasonable to define biotechnology as:

“the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.”

In this definition we refer to “scientific and engineering principles”. These cover a wide range of disciplines but rely heavily on microbiology, biochemistry, genetics, biochemical and chemical engineering. Therefore, it is unlikely that a biotechnologist will be well versed in all of the disciplines underpinning biotechnology but, following his/her initial training in a particular discipline, will have gained a broader understanding of biotechnology by applying his/her skills to practical problems.

In this definition “biological agents” refers to a wide range of biological catalysts but particularly to microorganisms, enzymes and animal and plant cells. Similarly our concept of “materials” is all-embracing of organic and inorganic materials. In our definition we include not only the actual process in which the biological agent is used but also those processes concerned with its preparation and with the processing of biological materials resulting from its action.

Our definition refers to the provision of “goods and services”. The former include the products of industries concerned with food, beverages, pharmaceuticals, biochemicals and the winning of metals; the latter is largely concerned with water purification, industrial and domestic waste management.

With regard to the health field, biotechnology is restricted to the production of useful medicines, such as antibiotics, vaccines and antibodies, and does not include their use in medical treatment. Also, it does not cover those areas of medical engineering and technology, often referred to as biomedical engineering (or sometimes bioengineering). We agree with the statement (*Biotechnology: A Dutch Perspective*, p. 2) that agriculture and traditional crop and animal breeding are not generally regarded as biotechnology [8]. However, aspects of these activities must be considered since plants provide the raw materials for most biotechnological processes. Also, biotechnology, through the production of microbial pesticides and the use of modern genetic manipulation techniques for the development *in vitro* of animal

and crop varieties and improved nitrogen-fixing capabilities, will have a profound impact upon agriculture in the future.

2. *The Importance of Biotechnology and the Need for Comparative Statistics in OECD Member countries*

The present public interest in biotechnology, particularly in recent developments in genetic engineering, tends to emphasize the future value of biotechnology to society. However, the existing importance of biotechnology to existing industries should not be underestimated.

Waste management (including sewage treatment) in tonnage terms is by far the largest application of biotechnology; society, both in the home and factory, is dependent on the supply of good quality water and disposal of wastes.

Many biological products are made in very large tonnages per annum. Production of processed food often exceeds that of major chemicals. For instance, Dunnill in his review indicates that world productions of milk and sugar exceed that of naphtha and frozen foods that of aluminium [16]. Some indication of the importance of the biological industries in OECD Member countries is illustrated in Appendix II. The magnitude of food production is shown in Table 1 where it is compared with the production of chemical products. Except for the major industrialised countries, food production exceeds that of chemical products. The data in Table 1 exclude beverages, which for most OECD countries is 10-40 per cent of the value of food production. Food and beverages are also an important component of the external trade of OECD countries (Table 2). In the UK, the fermentation industry sales in 1979 represented about 20 per cent of total food and drink sales. In the Netherlands the value was about 15 per cent in 1978.

The financial value of drug and medicines production is much less than that of foods and beverages (Table 1) but their contribution to external trade is significant (Table 3). The importance of these health products to the community's welfare is belied by their commercial value.

As has been pointed out by Dunnill, government statistics for the industries based on biotechnology lack the coherence accorded to the chemical industry [16]. For instance, it is difficult to distinguish the production of antibiotics by fermentation from the overall statistics of the pharmaceutical industry. We urge that in future more detailed comparative data on the biological industries should be more readily available for all countries.

3. *Present Activities and Future Impacts of Biotechnology*

The existing state of biotechnology-based industries and the range of activities which these encompass have been documented in a plethora of government, nongovernment and UN reports, proprietary information surveys and reviews in scientific, trade and financial journals in the past few years. It is not our intention to reiterate this information in any detail: Appendix III summarises the major fields of biotechnological innovation, classified according to industrial sector, volume-value basis or technological level. Several of the reports and reviews include statistics relating to specific OECD Member countries. What follows is a brief appraisal of trends within the relevant sectors during the recent past and some comment on middle and longer term predictions.

Analysis of world biotechnology-based industries shows that those associated with water treatment and purification (predominantly domestic sewage) comprise the largest single sector in terms of volumetric capacity; beer and spirits, cheese (and other dairy products), baker's yeast, organic acids (chiefly citric) and antibiotics follow in that order, both in terms of tonnage and value (see also above). In the fine chemicals or high value added sector (Appendix III B) antibiotics currently have the dominant position with penicillins, cephalosporins and tetracyclines being the major products. In the USA, for example, the fine chemicals-via-biotechnology market value is of the order of \$8 000 million, of which antibiotics comprise greater than 50 per cent.

Some indication of trends in biotechnology can be judged from the patent literature. It must be pointed out that analysis of patent applications gives only a partial picture of activity because of the difficulty of accurately identifying all biotechnology-based products and processes in computer searches and the different property protection strategies employed by different industries and by industries for different products/processes. Despite these limitations, analysis of the type recently made by Marstrand [18] are valuable. In terms of products, the number of patents relating to antibiotics, enzymes and coenzymes, pharmaceuticals, fine chemicals, biomass, amino acids, polymers, organic acids, food additives and steroids have risen sharply over the past two decades with major upswings in those concerning antibiotics, enzymes, pharmaceuticals and fine chemicals occurring about 1974–1975. At this time also, patenting in the biomass, amino acids, microbial pesticides, growth stimulants, oils/fats and polymers fields first became noticeable and has continued to develop. The Noyes Data Corporation review of relevant US patents (1975–1979) [15] revealed that activity was especially high in amino acids, peptides and proteins, carbohydrates and organic acids in the late 1970s. When biotechnology patents are viewed in the context of national activities, Japan's position appears outstanding. Out of nearly 2 400 patents issued between 1977 and 1981, and analyzed by Marstrand, 60 per cent were issued to Japanese applicants, 10 per cent to the USA, 5 per cent to the USSR and between 4 and 2 per cent to countries such as Poland, Czechoslovakia, German Democratic Republic, Federal Republic of Germany, United Kingdom and France. Over 80 per cent of these patents were to applicants in OECD Member countries and 16 per cent to countries having centrally planned economies.

The prediction of future trends and market sizes for biotechnology-based industries is a somewhat hazardous exercise. Among recent reports that have attempted such predictions are the US Congress OTA Report "Impacts of Applied Genetics" [7]. This Report contains market analysis data assembled by Genex Corp., a resumé of which is given in Appendix IV together with predictions of when recombinant DNA technology (pp. 27-29) will have impact on the pharmaceuticals, chemicals, food, agriculture and energy sectors.

Most commentators agree that biotechnological developments, certainly over the next decade, will be dominated by high value added products, especially those for use in the medical fields. However, in the longer term, applications in agriculture are likely to have a similar or even greater impact. Trade in agriculture and other organic commodities is particularly susceptible to changes initiated by biotechnology. Hence biotechnology will affect trading relationships between OECD Member countries, and between OECD Member countries and developing countries.

4. International Organizations Involved in Biotechnology and the Needs of Developing Countries

In addition to the several countries which have surveyed the potential of biotechnology and drawn up strategic plans to exploit it for national needs, a large number of international agencies have or are in the process of addressing biotechnology. Appendix V lists a selection of organizations involved in such work, together with an indication of their particular concerns. Collaborative activities within the framework of the European Community are being undertaken through various programmes including Biomolecular Engineering, Environment and Energy. Thus, the energy from biomass (anaerobic digestion of algae) project under the aegis of the CEC Solar Energy Programme has been developed on the basis of Belgian–German–Italian collaboration. The programmes promulgated by the international agencies have been concerned mostly with research, development and demonstration, training and the financing of specific projects. It will be apparent from the information (which is not comprehensive) contained in Appendix V that there is a pressing need for coordination of activities on the international scene: it would be pointless, even counterproductive, to establish additional structures.

Much of the international activity has focused, and rightly so in our opinion, on the development of appropriate biotechnology for developing countries. It should be pointed out that research and

development in industrialised countries of the North can be applied *mutatis mutandis* in developing countries to confront the major strategic problems of energy, food, fertilizer and health.

With the predicted population explosion by the end of the century, and expanding industrialisation in developing countries, the demand for energy will become a critical constraint on growth in Third World countries. Developing countries without fossil fuel resources have suffered from soaring oil prices. The present imbalance in energy consumption between North and South has to be overcome and biotechnological answers for deriving energy from indigenous biomass must be sought (see pp. 35-39).

While countries fortunate enough to have oil or wanted materials have been able to meet their requirements for food, many developing countries are increasingly unable to feed themselves. Forty years ago Asia, Africa and Latin America were net food exporters; today, these continents are all food importers. World food security in the long term requires greater agricultural diversity in crop plants related to rural developments in locally specific conditions which can be achieved by biotechnological advances. The loss of crops in the field and postharvest can be significantly reduced through biotechnology, by pesticides (chemical or biological), and better food processing and storage technologies. Correction of dietary inadequacies and improvement in the health and productivity of animals are additional benefits arising from biotechnology.

One of the major limiting factors to increasing plant production on available land is the supply of nitrogen. Considering the increase in world population it can be estimated that the nitrogen fertilizer supplies will require many hundred new ammonia plants requiring several hundred million tons of oil equivalent per annum as fuel stock. The prices of fertilizer, therefore, to developing countries are clearly prohibitive. However, the additional nitrogen requirement of the world could be met by increasing the levels of biological nitrogen fixation and different biotechnological ways of achieving this are actively under investigation (see pp. 27-29).

Human and animal health in developing countries are often considerably below the standard enjoyed in developed parts of the world. Here, too, biotechnology has a central role in providing the means of controlling or eliminating microbial and protozoal diseases that plague poorer countries.

We believe it is vitally important that developing countries should have their own pool of scientists and engineers working in biotechnology, that is, people fully conversant with the problems of their own country or region and who are skilled in using their own domestic resources. Facilities and mechanisms for training such persons have been a prime concern of the below-mentioned networks and of a few international centres such as the International Centre for Cooperative Research and Training in Microbial Engineering (Osaka) and the Institute of Biotechnological Studies recently established in the UK by the authors of this Report.

The most effective means of promoting international cooperation is via networks. Conspicuous among international networks devoted to applied microbiology/biotechnology are the Regional Microbiology Network for S.E. Asia (major support from the Government of Japan and UNESCO) and the network of Microbiological Resources Centers (MIRCENs) which is supported by UNEP, UNESCO and ICRO and to which a Panel on Microbiology serves in an advisory capacity. The former has the active participation of groups in Australia, Hong Kong, Indonesia, Japan, Korea, Malaysia, New Zealand, Philippines, Singapore and Thailand; the latter has MIRCENs located in Nairobi and Porto Alegre (nitrogen fixation, *Rhizobium* inoculants); Cairo and Guatemala (biotechnology); and Bangkok (fermentation, food and waste recycling). In addition, the two MIRCENs at Brisbane and Stockholm provide support and technical assistance for microbial culture collections and simple diagnostic systems respectively. The activities of both networks have a clear regional focus which starts by identifying those problems which are relevant to applied microbiology and those resources which may be so utilized.

POTENTIAL OF CONTRIBUTING SCIENCES AND TECHNOLOGIES TO BIOTECHNOLOGY

The ultimate success of biotechnology is dependent upon advances in and support for the fundamental sciences which underpin it. Short cuts, empiricism and superficial attention to basic scientific principles are likely to lead at best to poor process performance and at worst to expensive failures. In this section are highlighted some features of microbiology, biochemistry, genetics and engineering which we believe have a significant bearing on the development of biotechnology.

A. Microbiology and Biochemistry

In discussing microbiological and biochemical inputs and opportunities, it is useful to recall the uniqueness of biotechnology, that is, the application of biocatalysts as agents of chemical transformation; the most significant of these catalysts at present are microorganisms and enzymes while cells and tissues of higher organisms, in most cases, are at early stages of development. When developing a process it is necessary to select an appropriate biocatalyst, optimize its structure, its properties, and the environment in which it will be required to function.

1. Organisms for Biotechnology

Compared with the number of known species of microorganisms, the number which has been considered for industrial exploitation is extremely small. The reasons for this situation are several-fold:

- traditional use and accumulation of “know-how” on a small number of organisms such as yeasts and a few microfungi;
- concentration of microbial biochemists and geneticists on five or six species (the “*Escherichia coli syndrome*”);
- opportunism in general has led to comparative neglect of anaerobes, autotrophic microbes, slow growing organisms, nutritionally fastidious species, and organisms (like filamentous fungi) that present rheological problems.

Fortunately, this position is changing as the demand grows for a wide spectrum of organisms having particular biocatalytic properties. In the 1960s and 1970s, for example, much effort was expended in searching for microorganisms that would grow and produce amino acids, proteins, citric acid and other materials from the then cheap feedstock, petroleum. Currently, the desideratum is for organisms which are more efficient and have a broader substrate range than yeast in the production of ethanol; hence the growing interest in *Zymomonas* and thermophilic *Clostridia*, for example. It will be clear that the impetus to search for new microorganisms and to screen these, and those which are already known, for required properties stems from all facets of biotechnology. Reference to one area — environmental biotechnology — will serve to illustrate the point. The need to increase crop yields and to exploit marginal land and, even, deserts is a pressing one. Thus, the development of *Rhizobium* and mycorrhizal inoculants, microbial pesticides and processes utilizing halophilic microorganisms is part of the response to such needs. The matter of microbial insecticides is especially interesting because although some effective commercial products have been developed (*Bacillus thuringiensis*, viruses) the range of entomogenous microorganisms

is large and new species are being reported that, for example, could be exploited for the specific control of aquatic pests. In a similar fashion, microorganisms which can be applied to the environment for the clean up of specific pollutants, *in situ* metal leaching, bioaccumulation and crop residue silaging are, or have been, developed. In short, it would be extremely short-sighted to neglect studies of microbial taxonomy and descriptive ecology: the bottle-neck which can arise here is the lack of awareness by the applied scientist and technologist of the richness of microbial types and activities.

Isolation, screening and selection of organisms account for much of the effort in industrial microbiology and innovation in each of these areas is very desirable. Thus, with regard to isolation, continuous-flow enrichment procedures provide unlimited scope for obtaining organisms with required attributes in a rational and reproducible way. Similarly, the development of screening procedures for antimicrobial and pharmacologically active compounds has been fruitful and argues strongly for further investment. The most important innovation in recent years has been the screening for enzyme inhibitors of microbial origin introduced by Umezawa (see Schindler) [26]. A large number (more than 50) of novel compounds has been identified by this means and among those that are in clinical use or trials are ones that may be used in the treatment of hypertension, thromboses, obesity, cancers, hyperlipidaemia and stomach ulcers. The search for inhibitors which inactivate enzymes that degrade antibiotics promises to be a decisive step in developing effective chemotherapy and to date the success achieved with β -lactamase inhibitors, such as clavulanic acid, thienamycins and olivanic acids, is of great significance. The introduction of novel screening methods is vital in the antibiotics field now that traditional procedures are failing to reveal new compounds. What is required now is a refinement of the questions being posed in chemotherapy and pharmacology so that appropriate screening systems can be devised. Finally, it must be recalled, also, that the critical observation and interpretation of natural phenomena remains an important ingredient in exploratory research programmes. Once again, therefore, the provision of competent general microbiologists for biotechnological projects is underlined: serendipity is favoured strongly in trained minds.

The process of organism isolation, screening and selection traditionally has been a labour-intensive operation and the introduction of automated procedures becomes essential in modern industrial operations. Considerable technical advances have been made so that now it is possible not only to plate out very large numbers (say 1.5 million) of isolates or mutants and expose them to a myriad of environments but to record responses automatically and over any time period (e.g. television monitoring plus computer control) and to recover — also automatically — organisms from all or selected colonies which register the required response. The capacity of microorganisms to synthesize an enormous range of novel chemicals is well known. A major limitation to the exploitation of this capacity is the availability of suitable screening systems which will reveal the properties of such chemicals, particularly in the presence of culture medium constituents.

Under this general heading of organisms for biotechnology, we would draw attention also to the following issues:

- i) Conservation of microbial gene pools via the proper maintenance and extension of culture collections and consolidation of the world network of collections which is coordinated by the World Data Centre at the University of Queensland in Australia (p. 21 and pp. 47-48). Similar provision is necessary for conserving animal and plant genetic material.
- ii) Risk assessment associated with the deployment of plant pathogenic microorganisms for large-scale processing. Many species of phytopathogenic bacteria and fungi are being used or are expected to be used in biotechnology and whereas the reasonably large-scale handling of animal pathogens for vaccine production has been achieved routinely and safely, comparable experience of phytopathogens is much less. In view of the fact that certain phytopathogens may be cultured to produce commodities (e.g. single cell proteins, polymers) and because of the genetic vulnerability of crops (see p. 38) the recent report by Evans *et al.* is reassuring [24].

- iii) Mixed culture fermentations. It is becoming increasingly clear that the concurrent growth of two or more microbial species can confer advantages and properties not characteristic of monocultures of the constituent organisms. Applications of mixed culture technology can be found in traditional and novel food/feed production, metabolite synthesis, metal recovery and detoxification. In general terms the advantages of mixed cultures are: increased growth yield and specific growth rate (and hence productivity); increased culture stability; increased efficiency of mixed substrate utilization and enhanced resistance to contamination.
- iv) Many newly isolated wild strains of microorganisms (particularly bacteria) cannot be classified or are very difficult to classify within the existing taxonomic systems. Consequently, the opportunities for erecting new strains and species could have significant repercussions in patenting strategies. Similarly, a strain which has been subject to intensive genetic manipulation also may not be classified to the original parent strain.

2. *Physiology*

Microbial physiology is the study of the relationship between metabolic capability and the environment in which the organism exists, either in a growing or non-growing state. In microorganisms the ability to modulate cell structure, chemistry and function has evolved most fully and in a biotechnological context such “phenotypic variability” is both exploitable and a source of difficulties. Thus, on the one hand, fine tuning of environmental conditions (process optimization) can produce cultures possessing precisely those properties which the microbiologist is anxious to define. If, on the other hand, environmental conditions are not closely reproduced during the scale-up of laboratory experiments to pilot and production scale, this variability may lead to the desired activity or product being diminished or even lost. It is known, for example, that culture conditions may have a profound effect on the chemistry of microbial walls and membranes and thence on their physical properties. A systematic understanding of these effects would have considerable bearing on fermentation design and enable a rational approach to cell recovery from broths and metabolite overproduction and, where required, to cell lysis. Continuous culture is unquestionably the method of choice for obtaining such information. Metabolite overproduction, which is sought by the fermentation technologist, is an abnormal condition in microorganisms because of the tight control of metabolism mediated by feedback regulation. Overproduction can be achieved by altering the permeability of the cell membrane thereby allowing the metabolite to leak out of the cell and thus circumvent feedback regulation. Glutamate overproduction (ca 100 g per litre), for example, is realised by causing growth of the producer organism, *Corynebacterium glutamicum*, to be limited by biotin, oleate or glycerol or by adding penicillin to the culture medium: the common effect of all these treatments is to alter the chemistry of the cell membrane such that glutamic acid excretion is favoured. Continuous processes also will be essential, because of their higher productivity over batch systems, for the synthesis of high tonnage materials, e.g. single cell proteins, chemical feedstocks, biofuels. It is ironic, therefore, that the comparative neglect of microbial physiology in recent years — particularly at the expense of microbial biochemistry and microbial genetics — has led to a global shortage of well-trained microbial physiologists which, in time, seems likely to create a significant bottleneck in the development of biotechnology. There is at least one major exception to this statement. In Japan there is not a shortage of well-trained microbial physiologists, indeed the major proportion of biotechnologists in Japan have their background in microbial physiology and the most acute shortage of expertise is in genetic engineering.

3. *Biochemistry*

There already exists a profound understanding of primary metabolism in a wide range of organisms, and as our knowledge of secondary metabolite synthesis and plant biochemistry increases, we

can expect to see more rational exploitation of product formation. Similarly, the development of effective treatment processes for industrial wastes and effluents will become possible as knowledge of the catabolism of xenobiotic chemicals grows. In addition, biochemistry has an important role in providing the fundamental understanding of enzyme structure and function that is needed to achieve fully the potential of these catalysts.

In comparison with other catalysts used in organic synthesis, enzymes are exceptional for several reasons, — a wide spectrum of reactions can be catalysed; they are usually very selective in terms of the type of reaction and structure of the substrate (reactant) and product; reaction rates can be very fast. Nevertheless, a much greater insight into the structural properties of enzymes, especially those associated with cellular membranes, would assist in the devising of methods for increasing the stabilities of these catalysts (pp. 48-50), particularly on immobilization (see pp. 31-32).

Knowledge of the mechanisms of enzyme action may allow us to change their catalytic properties. In addition to chemical modification, altered enzymes may also be obtained in some cases by selection of mutants capable of growing on compounds structurally related to normal substrates of the wild-type organism. For instance, a change in a single amino acid in the enzyme acetamidase led to a mutant able to grow on butyramide. In the future, when we appreciate completely the relationship between amino acid sequence, enzyme activity and substrate specificity, the techniques of site-specific mutagenesis will allow tailor-made synthesis and modification of enzymes and other molecules (see pp. 27-29).

Another important area of biochemical research is focused on the development of high-affinity systems for recognition, isolation and purification of biological molecules. This is crucial for products obtained in low concentration as, for example, with mammalian proteins synthesized in prokaryotes.

B. Genetic Manipulations

Genetics is not new to industry and agriculture. Broadly speaking, in the former, strain development to produce say a microorganism with a high antibiotic yield has been the result of mutagenesis followed by strain selection whereas in the latter case, natural breeding systems have been harnessed to improve the stock. Both means of genetic manipulation have been applied empirically but with remarkable success. However, Pontecorvo has pointed out that industry often has adopted a “prehistoric” strategy by using exclusively mutagenesis and that lessons should be learned from evolution where for “improvement of living organisms, mutation and selection has been supplemented with a wonderful variety of mechanisms for the transfer of genetic information” [31]. In some cases the criticism is justified but one of the reasons, in addition to its obvious success, that an empirical approach was adopted was that very little fundamental work has been undertaken on commercially important organisms. For the applied scientists working on specific organisms it was not easy to see how techniques and methods developed in, for example, the prokaryote *Escherichia coli* were immediately applicable to fungi, plants and animals, particularly since with eukaryotic organisms the “species barrier” prevented outcrossing of characteristics from widely divergent organisms.

The spectacular advances in genetics in the last 10–20 years have indeed resulted from studies of academic scientists working with organisms not of obvious “applied” interest and it has been this technology more than any other development which has led to the upsurge of biotechnology.

Two major discoveries, cell fusion and *in vitro* recombinant DNA methods are taking the experiments out of the laboratory and into the market place. Both techniques allow the “species barrier” to be overcome and for new combinations of genes to be produced.

1. Cell Fusion

a) Animals

The spontaneous fusion of two different types of somatic cells to form a heterokaryon (two or more different nuclei with a single cytoplasm) was first reported in 1960 by Barski and colleagues in France, although the observation that mammalian tissue infected with certain viruses often showed polynucleate cells had been made previously. Inactivated viruses such as *Sendai* and some chemicals, including polyethyleneglycol, can be used to induce cell fusion and one of the first deliberate attempts to induce heterokaryons was successfully achieved with cells from mouse and man by Harris and Watkins in 1965. It is possible in such heterokaryons to have expression of genes from both parents. In 1975 Kohler and Milstein exploited this in their now famous production of monoclonal antibodies obtained by fusing antibody producing lymphocytes (from the spleen of mice immunised with a particular antigen) with malignant, rapidly proliferating myeloma cells. These hybrid–myeloma cells or “hybridoma” expressed both the lymphocytes’ specific antibody production and the myeloma property of continuous proliferation.

As a result of their high specificity and purity, monoclonal antibodies have enormous potential (Appendix VI). Until this discovery antibodies have never been considered as products to be administered instead of antibiotics and drugs in medicine. They were thought to be too complex to be synthesized chemically and there seemed to be no practical way of harvesting small amounts from the body. Therefore, the ability to manufacture large quantities of antibodies and interferons (see genetic engineering below) is likely to revolutionize prophylaxis and the treatment of disease.

b) *Plants and Microorganisms*

Work on the fusion of protoplasts (cells deprived of their cell wall) and regeneration of the fusion product into a cell–wall bearing strain has expanded rapidly. Using conditions known from careful analysis of various parameters to be optimal for the fusion of human cells, fusions have been achieved similarly in a wide range of plants and microorganisms. As with mammalian cells, polyethyleneglycol has proved effective for inducing somatic fusion. In both plants and fungi, cell fusion has been used to bypass incompatibilities to give hybrids between species that are impossible to cross conventionally.

In fusion experiments involving protoplasts from two different species the cytoplasmic mix obtained from the heterokaryotic fusions (fusion products of protoplasts belonging to two different species) is novel but the heterokaryon is usually unstable and segregation occurs during regeneration and subsequent growth. Nevertheless the process has given rise to strains showing properties not expressed by either parent or recombination of characteristics from the two parents. For example, fusion is so efficient in antibiotic–producing *Streptomyces* that two species can be hybridized to give rise to a population in which one cell in five has a new combination of genes. Thus the potential of interspecific fusion to generate new antibiotic structures as well as increasing the pool of yield–enhancing genes can be envisaged in the *Streptomyces* and elsewhere.

This technique may also prove to be useful to bring about the transfer of nitrogen fixing, nodule specific genes from legumes to non–legumes. Certainly it can be expected that during the next few decades, many interspecific hybridizations in agriculture and horticulture will lead to improvements in forage legumes, vegetables, fruit and flowers. Techniques have been devised also for transferring only part of the genome of one donor strain to a different recipient strain and it is interesting to note that irradiation of the donor cells prior to fusion has successfully made the process unidirectional in animals, plants, fungi and bacteria.

Protoplast fusion then is likely in itself to lead to new species having novel or other desirable properties and the ability of protoplasts to regenerate proves to be a useful adjunct to recombinant DNA techniques in plants and microorganisms (pp. 43-45).

2. *In vitro Recombinant DNA Methods*

In contrast to protoplast fusion which is particularly useful for combining large parts of the genome in a mixed cytoplasm, especially where the characteristics of interest are controlled in a complex

manner by a large number of genes, the power of recombinant DNA technology is greatest when small numbers of individual genes controlling known gene products are involved.

With comparatively simple laboratory techniques involving enzymes (restriction enzymes) obtained from microorganisms to cut DNA molecules into a number of short fragments and others (ligases) to splice or rejoin different fragments, recombinant DNA can be obtained which, given a suitable method of introducing it into a cell or protoplast (usually by means of a “vector” or carrier DNA molecule), represents a remarkable new capability, perhaps best emphasized by the example of human growth hormone (HGH). The Swedish company Kabi Vitrum is the major world producer of HGH made from cadaver pituitaries. The hormone is used to treat HGH deficient children reckoned to be about 10 per million of population. The nature of the source limits production and, therefore, in September 1978 Kabi made an agreement with the American venture capital company Genentech to produce HGH by cloning in *E. coli*, based on a 28 month project. The appropriate strain was developed in the USA by Genentech within seven months! Subsequently the engineered organism was grown in 450 litre fermenters and HGH harvested from a few batches was enough to supply, for example, the UK demand previously met by pituitaries from some 60 000 cadavers. Application to run a 1 500 litre fermenter for HGH production in the south of Stockholm has been made by Kabigen and is likely to proceed.

A wide range of therapeutic proteins such as other growth hormones and insulin, and the antiviral agent interferon have been produced and others, such as vaccines, are likely to follow (see Appendix VI). The case of insulin demonstrates another possibility in that here the donor DNA was a chemically synthesized gene fragment. This is appropriate for those cases where all or part of the sequence of the desired gene has been established already. The chemistry of nucleotide synthesis is an expanding field. So far the longest reported gene to be synthesized is 514 nucleotides long and represents a fragment of double stranded DNA coding for a human interferon (α_1). Insulin is also a good example of competing technologies achieving the same result. The table below (*SCRIP*, November 23, 1981, p. 8) postulates the following dates for the widespread use of genetically–engineered pharmaceutical products:

1985	Human insulin
1987	Interferon for cancer treatment
1988	Interferon as an antiviral
1989	Interferon for inflammatory disease
1990	Hepatitis B vaccine
1990	Human growth hormone

However, work carried out by Novo Industri to convert chemically porcine insulin to human insulin has been so successful that this product is likely to find more immediate use.

There are now on the market a number of “gene machines” able to synthesize specified short sequences of single strand DNA automatically and very quickly under the control of a microprocessor. Although difficulties have been encountered with these machines, the second generation systems about to be launched show that these technical problems have been overcome. It should be noted that in the early days of this work, one of the bottlenecks for its development was the scarcity of good synthetic nucleotide chemists required to construct even the most simple of nucleotides. The advent of reliable gene machines which are relatively easy to use will change this and the chemists available in adequate supply will be able to devote themselves to more sophisticated reactions and syntheses. Although in principle a gene of any size could be made chemically, it will often be easier to isolate natural genes and then, after full sequencing, to “edit” the message by constructing nucleotides with specific changes (mutations).

However, perhaps another major impact of *in vitro* recombinant DNA technology will be an understanding of the basic causes of disease. Already studies on cancer viruses are beginning to illuminate the fundamental mechanism of oncogenesis and, regardless of whether or not interferon proves to be a panacea for cancer and viral diseases, studies with it will help to elucidate the mechanisms of natural resistance to viruses.

Another advantage of the technique is that it is possible to produce complete gene libraries by “shot-gun” cloning in, for example, *E. coli* from any organism including man. A genomic library contains all of the sequences in the genome and if the number of fragments in the library is large enough for complete sequence representation, then, in principle, any gene can be isolated provided that a specific probe for detecting the gene is available. If the average size of the cloned sequence is 20 kilobases, approximately 700 000 individual clones would be required for a complete library of the human genome. It is possible now to clone a library corresponding to the genome of an individual from 20 ml of blood in a week, thus providing an unlimited supply of an individual’s genetic material in order to elucidate gene function and to allow characterisation of possible disease-causing defects. The correction of that defect is a problem for the future, but a number of ways for inserting genetic information into the early embryo of a plant or animal can be suggested and, therefore, the prospect of combining recombinant DNA technology with fertilization of human eggs *in vitro* is real as experiments with injecting the human insulin gene into mouse embryo show. However, gene therapy will require proper expression of the gene and accurate transmission in heredity.

The situation of expressing heterologous DNA (DNA from a different species) in *E. coli* has been discussed and it is clear that the well investigated genetic system of *E. coli* has enabled much valuable information to be gathered. However, it may not prove to be the ideal organism in every case. For example, proteins and other interesting metabolites are not normally readily exported from the cell as they are in some other bacteria such as *Bacillus subtilis* and *Streptomyces* spp. which also, unlike *E. coli*, are not pathogenic to man. Secondly, the fidelity of expression of eukaryotic DNA may be better in a eukaryote and here the yeast, *Saccharomyces cerevisiae*, is under investigation and, already, Genentech has succeeded in expressing human serum albumin in yeast. Albumin is presently prepared from blood and is used clinically to replace blood loss.

Although the medical applications have been the most immediate, perhaps the greatest long term potential for recombinant DNA techniques is in agriculture. The agronomy and technology of producing food vary from primitive to being highly sophisticated. One of the major limiting factors to increasing plant production on available land is the supply of nitrogen. It is interesting to reflect that although research in plant breeding has led to dramatic increases in yields of cereals such as wheat, rice and maize, many of the genetic changes have been towards a more efficient use of nitrogen in the soil. As already mentioned on p. 21), the estimated nitrogen fertilizer supplies (at present 40 Mte/year) will require many hundred new ammonia plants requiring several hundreds million tons of oil equivalent per annum as a fuel stock. This additional nitrogen requirement could be met, however, by increasing the levels of biological nitrogen fixation (at present 122 Mte/year principally from the *Rhizobium*/legume association) and it is not surprising, therefore, that much work has been done in this area.

For example, the possibility of introducing the nitrogen fixation genes from bacteria into important crop plants lacking the nitrogen-assimilating symbiotic relationship of the legumes has been studied. The genes involved in nitrogen fixation (*nif*) have been investigated largely in the free-living bacterium, *Klebsiella pneumoniae*. A detailed genetic map of the *nif* region is available and the *nif* gene cluster has been cloned, amplified and transferred to other organisms such as *Azotobacter vinelandii*, *Rhizobium* spp, *E. coli*, *Salmonella* and, even, into yeast. In the last case, although transfer was successful, expression did not occur and so far no successful expression of *nif* genes has been reported in a higher plant. However, it should be remembered that even if it becomes possible to transfer these genes to crop plants, yields may be reduced as energy will be drawn off for nitrogen fixation. Nevertheless, experimentation aimed at overcoming these problems is continuing.

An alternative and more propitious strategy is to transfer structural genes of legumes (nodule-specific genes) involved in the symbiotic nitrogen-fixing association with *Rhizobium* to non-legumes and one such gene, that coding for leghaemoglobin, has been cloned already. In addition to genes for nitrogen fixation, other characteristics likely to be controlled by single or a few clustered genes are under investigation, e.g. disease resistance, improving key enzymes in photosynthesis and plant

storage proteins which could be enhanced for essential amino acids. In this latter connection the report that the gene for the major storage protein (phaseolin) in seeds of the French bean *Phaseolus vulgaris* has been cloned using a viral vector is promising.

Of course, for many of the present industrially important strains, growth, production and extraction conditions and facilities will already be established. The major benefits of recombinant DNA technology will arise most likely from the use of host-vector systems endogenous to the groups of organisms used in the process and a successful outcome will involve the introduction of characteristics controlled by one or a few genes. This point is taken up on pp. 43-45. A good example of the technique comes from work carried out by ICI where a gene for an energy conserving nitrogen pathway taken from *E. coli* was transferred into their SCP (single-cell protein,) organism *Methylophilus methylotrophus* strain AS1 where it substituted for the similar but energy consuming pathway possessed by the strain. In laboratory experiments this led to a significant improvement in the conversion of methanol.

In any programme of strain improvement based on successive mutation and selection, there are many examples of deleterious mutations being accumulated along with the desired properties. Only a process of recombination, either using classical or recombinant DNA methods, offers the possibility of removing these. It worth remembering, also, that it is not necessarily yield which is important but the total cost of the process so that the ability of a strain to utilize, say a cheaper carbon source by gaining the appropriate gene(s) from another organism using recombinant DNA technology can improve profitability.

With many industrial microorganisms where a multistep synthesis is required, manipulation is likely to be more difficult. However, strategies have been devised, for the use of cloned genomic libraries from microorganisms to produce novel metabolites such as antibiotics. Here it should be emphasized that new combinations of genes could be selected giving a product which under normal environmental circumstances would adversely affect the organism and hence would not be retained in evolution. Also, the cloning of DNA permits base changes to be made at specific pre-determined points in the DNA. Modern developments in mutagenesis, including this site directed approach, will be increasingly important for improving commercially important organisms and will rationalize the often hitherto used "hit and miss" mutation and selection procedures. Site-directed mutagenesis is most powerfully employed when key genes and their products are known. A good example is afforded by penicillin G acylase, an important industrial enzyme from *E. coli* which converts penicillin G to 6-aminopenicillanic acid, the latter being the starting material for semisynthetic penicillins. The gene coding for this has been cloned and experiments have focused on changing individual nucleotides in the gene in order to improve efficiency and yield. In addition, by cloning the gene on a multicopy plasmid, increases in cellular yield of the enzyme have been obtained.

In summary, genetics has made substantial contributions to existing biotechnology and the methods for producing cell fusion and *in vitro* recombinant DNA add a new dimension to this genetic capability. In future, any biotechnological process in any area such as health, agriculture, energy and chemical feedstocks, and waste treatment is likely to involve a geneticist working closely with colleagues in other disciplines in an attempt to produce the "ideal" cell or organism to carry out the desired process efficiently. Some applications of this genetic engineering are listed in Appendix VI.

C. Engineering

Biological discoveries, including those of genetic engineering, which open up new horizons in biotechnology have rightly attracted much attention. Unfortunately the great emphasis on biological aspects has led to a distorted view of biotechnology and it is worth stating the obvious fact that unless biological innovations can be translated into working processes, they are of little value to the community.

There are important differences between the introduction of a new process in the biological industry, especially if fermentation based, and the chemical industry. In the latter it is quite usual for a company to specify its requirements and then to get an engineering contracting company to undertake the detailed design, construction and start-up of the new plant. In contrast, pharmaceutical companies often

begin to design and even construct plants before all the operating conditions are known. There is often a long period of modification and improvement to the process. There are also similar difficulties in the construction of food processing plants because it is not possible to specify exactly the product as subjective characteristics such as flavour and texture may be crucial to product acceptability.

Despite its vital contribution to biotechnology, engineering has received less attention than the biological sciences. There are several reasons:

- in contrast to the rapid progress being made in genetic manipulation and other relevant areas of biology, engineering advances are inevitably much slower;
- the cost of engineering research and development with biological materials and systems is high, often requiring the production of large batches of material to test the performance of a single unit operation. In the past, industry frequently has not made extensive biochemical engineering studies but has relied on a rather empirical approach;
- major breakthroughs are infrequent.

In engineering there are few operations that cannot be done somehow, i.e. there are no absolute constraints. However, improvements in performance or cost reduction may make a major difference to the economics of the process. Engineers have been reasonably successful in developing economic biological processes despite many problems (for instance pp. 41-43). Future advances in engineering will have important repercussions on biological processing. Some areas where progress is expected or further research and development is needed are covered in the following sections.

1. *Aseptic Operation*

The biological industries in general have a good record in terms of safe operation. In the food processing industry, techniques for cleaning equipment in place, that is without dismantling, are being used more. Further improvements in such operations are likely.

In the fermentation industry, infection of batch cultures does occur and on a few occasions is serious. The antibiotics industry has been disinclined to use fermenters larger than 200 m³ because of the substantial financial loss when contamination occurs. Most industrial fermentations are batch operations which allow all the equipment to be sterilised between production runs, so that any contaminating microorganisms have only a limited period in which to proliferate. As continuous operation becomes more widely used, the fermenters will have to operate without interruption for long periods. This will mean an even more rigorous (and expensive) approach to sterile operation.

As a result of the concern expressed about the safety of genetically–engineered microorganisms, some companies have produced laboratory and pilot–scale fermenters with additional safeguards such as modified seals, valves and sterile connections but at considerable extra cost.

Although only a few bacteria are grown on a large–scale, some problems with phage infection have been experienced. As genetically–engineered bacteria are introduced into pilot and larger scale vessels, problems with phage infection may increase. Sterilisation procedures will have to ensure either phage inactivation or exclusion. It is also possible to make phage–resistant strains but these are not necessarily as good as the parent strain as the ability to make the desired product may be impaired.

2. *Reactor Design*

a) *Fermenters*

Apart from the need to ensure sterile operation, three requirements dominate fermenter design: good mixing, adequate oxygen transfer and heat removal. Although one of the arguments for biocatalysis is the low temperatures of operation compared to chemical catalysis, the normal temperatures at which fermentations are run (20–40°C) make it necessary to provide large surface areas for the removal of the

heat that is produced. One of the attractions of thermophilic microorganisms is the ability to operate fermentations at elevated temperatures, thereby easing the heat removal problems and at the same time decreasing the risk of contamination from mesophilic microorganisms.

Most fermentations to produce useful products are done in aerated stirred tanks. During the last decade a variety of other fermenter designs have been investigated at laboratory and pilot-scale, but in only a few cases have commercial systems been built. At present there is dispute about the relative merits of stirred agitated vessels and non-agitated sparged vessels.

There is still much to be done to improve fermenter designs to minimise operating costs. Some companies have modified the agitators fitted to their fermenters so that they draw less power. Though product titres are reduced, the energy saving is decisive in reducing product cost. Cost reduction also may be achieved by using larger vessels. ICI has built a 1500 m³ pressure recycle vessel for production of a single cell protein which required some engineering novel to biological processing.

The increasing interest in non-agitated sparged vessels, such as the ICI vessel, has emphasized the non-homogeneity of the conditions to which microorganisms are subjected in a large fermenter. There is a lack of knowledge of the effect of such environmental fluctuations on microbial product formation. Similarly, there is insufficient understanding of the interactions between shear forces in fermenters and the behaviour and morphology of fungal cultures. This is an even more serious problem for the growth of animal and plant cells and a new range of culture systems is being developed for growth of such cells, either in suspension or on wetted surfaces. Many of these systems are still small in size and much work needs to be done on scale-up.

Aerobic oxidative systems of sewage treatment, such as biological filtration and activated sludge, have reached a degree of efficiency in terms of BOD removal such that only marginal improvements in respect of exploitation of the kinetics of BOD removal can be expected. There is still room for improvement in the settling characteristics of sludge used in the activated sludge system and, for example, for producing sludge with particular characteristics, e.g. rich in phosphates. Biological fluidised beds are being examined at pilot-scale and may soon be used for large-scale treatment of sewage and effluents. The very high biomass concentrations achieved (10–40 kg dry biomass m⁻³) compared to activated sludge-plants lead to a much higher rate of reaction per unit volume and therefore smaller plants.

In contrast to aerobic processes for sewage and effluent treatment, anaerobic processes, which have been applied mostly in the past to the processing of sludges to make them less offensive and reduce their bulk, offer much greater promise for further development. They do not require oxygen and they yield methane, but suffer from the disadvantage that the outflow does not give a high-quality effluent.

b) *Immobilized Biocatalyst Reactors*

The use of degradative enzymes, such as amylases and proteases, in food processes is well established. These and some other enzymes secreted by microorganisms may be produced at a price which allows them to be used once in a process. The cost of most enzymes is such that they only become economic if they can be reused. This has led to the development of immobilization techniques, which involve converting the water-soluble enzyme protein into a solid form of catalyst, either by entrapping the enzyme within a cross-linked matrix of a water-insoluble polymer or by binding the enzyme tonically or covalently to the surface of a solid supporting material. It is also possible to immobilise microbial cells by similar techniques. These immobilized biocatalysts can be made in the form of particles, cylinders, sheets and membranes and have been used in a variety of reactors, including packed bed, fluidised bed, tubular and well-mixed reactors (see pp. 48-50).

It must be emphasized that existing immobilized biocatalyst processes are relatively simple one-step conversions. There is still an enormous untapped potential when one considers the amazing versatility of microorganisms. In order to allow this potential to be tapped, several problems must be overcome. Many of these are biological and will be dealt with on pp. 48-50. Engineering problems are of several kinds. Nearly all the existing processes use packed beds, where catalyst abrasion is minimal. Many biochemical reactions result in the consumption or production of acid and others involve the

participation of a gaseous reactant, such as oxygen. For these types of reaction, it is necessary to ensure an adequate control of the pH by acid or base addition or an adequate supply of oxygen. Packed beds are not suitable for these purposes and some kind of mixed reactor is required. Apart from the deacylation of benzyl-penicillin, there is little experience of operating large-scale mixed reactors for immobilized biocatalytic conversions.

Many reactants of industrial interest, such as steroids, alkanes and aromatic derivatives, are either water-insoluble or water-immiscible. In some cases the water-insoluble reactants may be solubilized in a non-aqueous solvent. The design of immobilised biocatalysts and reactors for these reactants will require a much greater understanding of such multi-liquid phase systems, especially the rates of transfer of reactants and products and the effects of the reactants and solvents, when used, on the activity and stability of the biocatalyst.

3. *Product Recovery*

In the past, product outputs from fermenters have increased, particularly in the antibiotics industry, due to strain improvements and optimization of fermentation conditions. It is to be expected that outputs will continue to rise. Even in those fermentations where good yields are already possible, higher product concentrations may be reached. As outputs increase, the proportion of the total costs incurred during product recovery will rise, making efficient downstream processing even more important.

As described in the previous section, one of the advantages of stirred tanks for fermentation is their versatility, allowing a wide range of different fermentations to be done in reasonably similar vessels. In contrast, the recovery processes for fermentation products tend to be specific for each product. In the future it may be necessary to design downstream processes such that they can be used for more than one product.

Genetic engineering is likely to have a major impact on downstream processing. First, there is likely to be a greater emphasis on specific protein products. Secondly, the recovery engineer may have some choice about the microorganisms used. As there are large differences in the ease of handling and processing of microorganisms, this ability to select a suitable organism may be a most important step forward (see pp. 22-24).

There is a great need for good biochemical engineering studies on product recovery. Research on the processing of fermenter effluents is an area which chemical engineers have tended to avoid because of its difficulty. It is not yet clear whether specific proteins produced by genetically-engineered microorganisms will have to be recovered from the liquor or from inside the microbial cells. This depends not only on whether secretion can be achieved, but also on the views of the recovery engineer. He is faced with the choice of a product extracellular and in dilute solution or intracellular in high concentration but mixed with many other cell constituents. During the last decade, large-scale techniques for extraction of intracellular microbial protein have been developed but, at present, only a few intracellular microbial enzymes, e.g. glucose isomerase and penicillin acylase, are isolated on a large-scale. As indicated on p. 42, there are serious problems with solid/liquid separation. Studies on the control and stability of biological precipitates and flocs should enhance the performances of existing equipment. However, such studies require expensive pilot-plant equipment available in only a few Universities and research establishments. One possible alternative is the greater use of liquid/liquid separation for recovery of biological polymers including proteins. There is also plenty of scope for the greater use of membranes for separation of both water-soluble components and slurries.

Similarly there is a need for a much better understanding of the interactions between individual recovery operations, including the effects that changes in the fermentation conditions have on downstream operations.

4. *Instrumentation and Process Control*

The success of many fermentation processes owes much to the increase in product titres resulting from close monitoring of the fermentation. The introduction of computers has already revolutionised the fermentation industry. Most fermentation companies are using computers for process monitoring and data logging of on-line measurable variables such as temperature, pH, dissolved oxygen tension, oxygen consumption and carbon dioxide production rates, and in some cases for control of individual parameters. Production managers can now use the processed data, consisting of both measured and derived parameters, to steer the fermentation towards maximum product formation, even when remote from the production plant, by the use of telephone-linked terminals. For a few processes the computer is also used to guide the fermentation but most companies have not reached this position mainly because of a lack of understanding of the complex interactions between the fermentation variables. This situation is likely to change rapidly and computer control may make a major contribution to higher product titres in the future. Computers are also used for the control of entire process cycles including automatic start-up, shut-down and cleaning or sterilisation of the equipment. This sequential control function is widely used in the dairy and food industry.

Robust sterilizable sensors are a critical requirement for the sophisticated monitoring and control of biological processes. It is still not possible to get a reliable on-line measurement of microbial concentration especially for fungal cultures. Some progress has been made with enzyme electrodes and optical fibre-linked measuring devices but more effort is still required to make these and other monitoring devices more suitable for industrial use.

Indirect estimates of microbial growth and, in some cases, of product formation rates can be calculated from measurable variables, such as oxygen consumption and carbon dioxide and heat production, using appropriate correlations derived from mathematical models. Dynamic process models are proving to be valuable in the design, development and operation of processes but, as indicated above, our understanding of the interactions between fermentation variables and the behaviour and response of microorganisms to these is still limited. The importance of this aspect and the need for more information is also referred to on p. 48.

Chapter II

SCIENTIFIC, TECHNOLOGICAL AND RESOURCE CONSTRAINTS ON BIOTECHNOLOGY

Despite our firm belief that exciting developments resulting from biotechnology lie ahead, it would be wrong not to draw attention to some major scientific, technological and resource constraints.

A. Energy and Chemical Feedstocks

Foremost among the principles which need to be examined when assessing biotechnological innovation is the availability, existing and potential, of raw materials. A frequently vaunted advantage of biotechnological processes and products is the ability to base them on renewable resources and many commentators predict a revolutionary change in the chemical and related industries analogous to that which, earlier this century, accompanied the transition from a predominantly coal-based to a predominantly oil-based technology. The Dutch report on biotechnology [8] elaborates this point: during the past 40 years the world production of synthetic organic chemicals increased more than 100-fold to 350 Mte and from being 59 per cent coal-based in 1940 it became 79 per cent oil-based in 1979. The situation in the USA is even more extreme — over 90 per cent of the organic chemical industry's feedstock needs are met by petroleum. In Western Europe the 1979 consumption of chemical feedstocks was 100 Mte oil equivalent and an additional 83 Mte was accounted as processing energy; the latter represents 10 per cent of total energy demand. By the year 2000 the Western European energy demand is expected to increase by more than 30 per cent while petroleum consumption is predicted to be 15 per cent less than the 1979 rate. The world current annual energy consumption is estimated to be 9 to 10 TW (*ca.* 7 500 Mte oil equivalent). Approximately 10 per cent of this is related to gasoline and Diesel fuel requirements for transportation. Different industry sectors are characterized by their energy intensities (ratio of value of energy input to output): information of this sort is useful in identifying high-coefficient industries, establishing energy intensity rankings and defining strategies for energy and feedstock use. Analysis of a selection of OECD countries (Germany, France, Italy, Netherlands, Sweden, UK, USA) reveals that the energy industries *per se* have the highest coefficient among high-coefficient industries. The intensity rankings of six industries in these countries follow, the order (comparative values): energy industries (32), basic metals, chemicals and mining (4), wood/paper, and construction (1).

At present the average world per capita consumption of energy is of the order of 2 kW but ranges from *ca.* 10 kW per capita in the USA and Canada, 3 to 7 kW in other industrialized (non-centrally planned economy) countries to less than 2 kW in developing countries. In low-income developing countries the energy consumption falls to *ca.* 100 W or less. Total energy consumption has been predicted to rise to approximately 20 TW and 55 TW by the years 2000 and 2050 respectively.

The above facts provide part of the backdrop against which any biotechnological alternatives to energy and feedstock supplies, prompted by high costs and/or decreasing reserves of crude oil and natural gas, have to be evaluated. It has been proposed that Western Europe's increased energy demand to the year 2000 will be met from coal and nuclear sources. Currently only *ca.* 140 GW (*ca.* 1.5 per cent total

world energy requirement) are derived from atomic power stations and, although the EEC output rose by 24 per cent during 1979–1980 (due very largely to major developments in France), many other countries are curtailing their nuclear power programmes.

1. *Future Sources*

This is not the place to discuss extensively possible substitutes for petroleum as chemical feedstocks but brief reference is necessary in order that biomass-based alternatives can be seen in perspective. The major alternatives to biomass are coal gasification and liquefaction, development of synthesis gas processes and the exploitation of oil shales and tar sands. The fossil fuel reserves needed to sustain such activities are large (it is claimed, for example, on the basis of explored regions alone, that oil shales are available to meet current world petroleum consumption for 7 000 years) and technology is sufficiently advanced in some sectors for us to anticipate economically viable processes in the near future (*in situ* retorting of oil shales in North America possible by mid-1980s). On the other hand shale oil and tar sand need extensive refining which inevitably will produce cost increases. Outside the centrally planned economies, successful oil shale and tar sand operations have been managed but only when certain factors have combined:

- lack or depletion of domestic crude oil;
- desire for self-sufficiency;
- local environmental concern subjugate to national priorities;
- government support carefully harmonized with returns from private investment.

Coal gasification, including *in situ* operation, is not economically viable at present and prospects for it and various liquefaction processes remain uncertain due to the time factor for necessary R&D. Commercialization is thought to require decades rather than years despite the fact that reasonable technologies already exist or are at the development stage. Moreover, some processes might become viable only if almost unlimited supplies of energy were realized and, possibly, if a hydrogen technology was achieved. Other problems associated with coal conversion technologies include: high capital investment in plant; long construction times for plant; very large numbers of commercially-untried processes; massive environmental problems; and expense of handling large volumes of slag and ash. Biotechnological sulphur removal from coals could alleviate part of the latter problem if an inexpensive leaching process is developed. It appears justified to conclude, therefore, that coal conversion techniques will have little impact on world energy and chemical feedstock supplies during this century.

With regard to chemical feedstocks, one further point should be emphasized. Whereas petroleum can be substituted by other feedstocks for conventional petrochemical technology, the products of such technology (e.g. plastics, fibres, detergents) may be replaced by alternative natural or man-made materials, such as natural fibres and rubber, paper, wood and glass. Of particular interest is the possibility of introducing materials like starch and sucrose as industrial feedstocks. Thus, sucrose ester mixtures can be used for detergent formulations while purified esters could have outlets in the cosmetics industry; formulations of starch (up to 15 per cent) and polyethylene can be used to produce plastic films for packaging or mulching. In all cases additional advantages are conferred to the product: biodegradability, non-toxicity, nonallergenicity.

a) *Renewable Raw Materials*

In order that biomass might contribute significantly to feedstock requirements, the following criteria must be satisfied:

- a sufficiently large resource base to meet market demands;
- competitively priced products;
- appropriate technology; and
- high process efficiency.

Biomass for non–food exploitation comprises the complete range of plants, terrestrial and aquatic, including algae; agricultural and forestry surpluses; all organic wastes and residues from agriculture, fisheries, forestry and human communities.

Much has been written on the subject of wastes as biotechnology feedstocks and the problems (collection, seasonality, amounts available) attending their economic use. One point we would reiterate here is the need to make detailed studies of waste generation and geographical distribution as an essential step in the evaluation of waste exploitation and thereby enable decisions on location of conversion plants, conversion technology, and optimum scale. The recent survey of agricultural wastes in the UK (*ca.* 8.5 Mte oil equivalent per year and equivalent to 3.8 per cent national primary fuel consumption in 1980) illustrates the usefulness of this approach to resource planning. Wastes already are used, or being assessed, as fermentation feedstocks for production of organic acids, enzymes, fine chemicals, sweeteners, polymers, antitumor agents and biofuels, and such utilization does not invariably depend primarily on relief of environmental pollution. In passing, it should be noted that biotechnological processes themselves can generate potential pollution problems (a large brewery may produce 10 000 m³ per day of waste which is equivalent to a sewage load from a community of 200 000 people), a point which is not always appreciated by advocates of biotechnology.

The major component of biomass is usually carbohydrate — sugars (cane, beet, sorghum); starches (grains, cassava, potato); lignocellulose (trees, residues) — with nitrogen and phosphorus contents being very variable. Organic wastes also are generated by certain manufacturing industries and may be rich in aromatics and carboxylic acids, for example. Finally, in this context peat should be listed, world reserves of which are estimated at 100 Gte oil equivalent. However, maximum peat accumulation is only *ca.* 450 kg per hectare per year (*cf.* average cane yields 100 te per hectare per year) and most of those countries which possess large reserves are, for conservation reasons, concerned to develop them slowly (*e.g.* Finland has 9 per cent world peat reserves but until a crisis point arises, does not plan to increase usage by more than *ca.* 5 per cent).

Biomass conversion systems comprise combustion, pyrolysis, gasification and fermentation and, while the latter is our chief concern, its competitiveness *vis-à-vis* other routes of conversion becomes crucial when establishing energy and feedstock policies. Possible fermentation products include a range of acids, alcohols, esters and gases among which ethanol and methane currently have most interest. Several countries, notably Brazil, USA, Pakistan, Australia and South Africa, have launched major alcohol projects and many governments are assessing the opportunities for national power alcohol programmes. A switch to biomass–based processes will be determined largely by the comparative costs of biomass and petroleum on a carbon and energy basis. As the cost differential diminishes, the adoption of fermentation will rest on the efficiency of the process technology. The raw material cost differential varies as a function of geography and politics and, whereas inflation rates for petroleum have been greater than those for agricultural products, a 10 per cent increase in the former has, in general, produced only a 3 per cent increase in crop prices. Clearly the raw material issue will assume greater or lesser significance depending on whether bulk or fine products are considered.

Ethanol production is a well understood process but significant improvements are likely to follow from:

- continuous fermentation enabling increases in productivity over traditional batch operation;
- high substrate fermentations enabling increased ethanol concentrations;
- increased ethanol–tolerant producer organisms;

- use of ethanol producers other than yeasts, particularly thermophiles that can utilize a wider spectrum of raw materials;
- improvements in distillation technology (major energy drain on the process) and/or introduction of less energy demanding ethanol recovery techniques.

A serious difficulty concerns the utilization of lignocellulosic materials as fermentation substrates and unless there are dramatic improvements in pretreatment and hydrolysis techniques (enzymatic or whole organism), the preferred routes of utilization are likely to be pyrolysis (methanol), gasification and direct combustion (p. 40). In contrast, biomethanation can be based on a wide range of biomass materials while maintaining good gas yields and calorific values. Recent improvements in biogas productivity have been in the order of 1 m³ to *ca.* 10 m³ gas per m³ reactor capacity per day. The versatility of anaerobic digestion is considerable and, for example, seaweed-seawater processing is feasible.

Some data on biomass productivity are useful at this juncture. The world renewable source of biomass is equivalent to *ca.* 10¹¹ te per annum, most of which is lignocellulose with smaller proportions of starch (*ca.* 10⁹ te ex. grains) and sugar (10⁸ te). Land under cultivation (*ca.* 10¹⁰ ha) dominated by cereals (75 per cent). The food deficit in developing countries is predicted to be *ca.* 10⁸ te grain equivalent by the mid-1980s. Increased biomass production can be achieved by:

- i)* Increasing crop yields and stability to environmental stress;
- ii)* increasing the efficiency of energy intensive inputs;
- iii)* decreasing pre- and post-harvest losses;
- iv)* increasing the area of land under cultivation.

Unfortunately option *i)* is reliant on optimal agricultural management (energy intensive) while improvements under *iv)* can only come from the development of marginal land or desert (p. 38). The upper limit of world food production has been calculated as *ca.* 40 Gte grain equivalent, i.e. 30-times present rate, with a feasible target being set at 10-times present production. In Australia, for example, the capacity for increased productivity of sugar and starch has been put at 2 to 3- and 2- fold respectively. The development of catch crop agriculture (intercropping) has been advocated as a fifth means of increasing biomass productivity (planting of second crops after harvesting the main crop); for a country like the UK it has been claimed that a catch cropping system could provide up to 4 per cent of primary energy requirements. On the other hand, it has been argued strongly that there are neither products nor by-products of agriculture and forestry available to sustain the world's chemical industry in raw materials. However, industry could be forced to purchase the products of agriculture and forestry at elevated prices, a situation which would lead to increased ecological perturbation, increased misuse of land and more hungry people. This general conclusion will need some modification particularly with regard to geographical location. Thus, countries at present producing ethanol as a fuel and feedstock lie within the latitudes 40°N, 40°S where the considerable advantages of sugar cane cultivation are possible. In contrast, it seems unlikely that biomass fuels/feedstocks will contribute a large proportion of requirements in temperate zones unless lignocellulose can be mobilized. Even in the latter case, significant wood-based resources exist in only a few countries (Canada, USSR, Scandinavia, South America) and, as we have suggested, the preferred technology will be gasification unless hydrolysis techniques are greatly improved.

b) Constraints on the Use of Conventional Biomass

A check-list of constraints to using biomass as a biotechnology raw material include:

- i)* high water content (see pp. 41-42);
- ii)* frequently its availability is seasonal;
- iii)* high collection and storage costs;
- iv)* susceptibility to high pre- and post-harvest deterioration;
- v)* recalcitrant nature of certain materials;

- vi) competition with traditional demands (food, fibre, feed, fuel);
- vii) possible genetic vulnerability of very large hectare monocultures.

We have touched on several of these issues but further reference to vi) is necessary.

It is generally agreed that biomass production in regions such as Europe will not remotely meet the raw material needs of the chemical industry or permit significant biofuel production. Some commentators see biomass production in low-income developing countries as one or a part solution to this limitation, i.e. large scale production of ethanol, for example, for use in Europe. However, in our opinion, the present distribution of the world's population, wealth and agricultural bases are not propitious for providing the needs of the world's hungry and a programme of the type indicated would severely exacerbate the problem. Large changes in land use may induce considerable perturbations in national and international socio-economic structures, unforeseen at the time of initiation. Thus, the major switch of Brazilian agriculture to cane (for ethanol) has led to rises in sugar and, successively, food prices and has generated also a new pollution problem following the discharge of fermentation and spillage residues to rivers. Ratledge [44] has sketched a plausible scenario of supply and price effects that could follow altered land usage as a result of the gasohol programme in the USA — increased corn production, leading to increased price of soyabeans and cotton leading to synthetic fibres becoming competitive with cotton and ultimately leading to increased consumption of petrochemicals to satisfy the increased fibre demand.

c) *Energy Balance and Photosynthetic Efficiency*

The net energy ratio (NER) — energy output of useful products divided by energy costs (agricultural processes, fertilizers, pesticides) — is a major consideration when assessing a biomass feedstock project. A wide range of NER values have been reported but for most starch crops and sugar beet the NER is less than or approaching 1, while fermentations of cellulosic substrates produce extremely poor balances (NER = 0.1 - 0.2). The best values (3 to 5) are obtained for sugar cane. In order to achieve positive NERs it is essential to utilize the biomass in toto as epitomized by the “whole cane concept”, i.e. sugar as major feedstock, bagasse to raise process steam, recovery of additional products from hemicelluloses and lignin in the excess bagasse, digestion or spray drying of stillage residues for biogas or feed.

The practical maximum efficiency of solar energy conversion (ESEC) into biomass by traditional agriculture is quoted as 5 to 6 per cent. However, the photosynthetic efficiency (PE) of biomass growth (energy stored in biomass/light energy absorbed) is *ca.* 30 per cent, assuming a quantum demand of 8 and an average wavelength of 575nm. (ESEC = PE x fraction of solar energy available for photosynthesis corrected for photorespiration and maintenance; 0.39). Recent work in Pirt's laboratory has shown that in light-limited cultures of microalgae PE values as high as 43 per cent can be realized, yielding practical ESECs of 18 per cent [42]. While these findings put in question the accepted model of photosynthesis, they have considerable significance for biomass production: appropriately engineered photobioreactors could have solar energy capture efficiencies comparable with solar thermal and photovoltaic cells. Pirt has claimed that photobioreactors offer the only option with potential for development on sufficient scale to provide an alternative to fossil carbon feedstocks. He has calculated that photoreactors located in temperate zones would have maximum productivities of 180 te per hectare per year and NER of 3. An added advantage is the ability to manipulate reactor conditions in order that protein, fat or starch-rich biomass is produced as desired. The principles of photobioreactor design and operation largely remain to be established but it is evident that further research and development in this field could meet the industrial requirements for biomass which are unobtainable from agriculture and forestry.

In comparison with *in vitro* photobioreactors, the previously developed open pond or ditch systems are less productive and have poor control. However, in selected situations their development is entirely sensible and the “mari-culture on land” system (using phytoplankton) being developed for coastal deserts under the EEC's Solar Energy Programme is especially interesting. The system does not compete for fertile land, does not require fresh water and productivities in demonstration plants in South Italy have

reached 60 to 90 te per hectare per year. It has been calculated that 5 Mte biofuel per year could be produced from 1 700 km² of coastal desert (cf. land requirements for sugar cane, 9 000 km²; cassava, 3 800 km²). Suitable available coastlines in Europe, Africa and the world amount to approximately 1 000 km, 17 000 km and 25 000 km respectively and, if developed, could yield 2, 50 and 80 Mte biofuel annually. The Dynatech R&D report [43] to the US Department of Energy also concluded that land-based aquatic farms had potential for development whereas projected costs were prohibitive to the development of open-ocean counterparts.

2. *Biotechnology and the Location of Industry*

Spitz and Weiss of Chemical Systems Inc. have provided recently a useful assessment of the possibilities for changing location of the chemical industry [45]. The change from coal to oil and natural gas as chemical feedstocks led to intense industrialization in the USA (Gulf of Mexico), where inexpensive feedstock was located, and in Europe and Japan which were able to import unlimited quantities of crude oil and naphtha from the Middle East. It is pertinent to enquire, therefore, if large scale biotechnological innovation is likely to occasion a comparable relocation of industry. Among the factors favouring relocation will be: the finality of crude oil and natural gas supplies in and to the developed world; availability of coal; desire of OPEC countries to develop major petrochemical industries with a view to export; environmental constraints; and, favourable tax and monetary incentives. It is Spitz and Weiss' opinion that only slight changes in the present structure will be seen by 1990 and, thereafter, change is likely to be gradual rather than sudden. The effects of biotechnology on industrial location are even more problematical because of the long lead times required to develop commercial biomass-based processes. Significant impact may be seen in countries such as Brazil where strong government encouragement to produce biofuels on a massive scale has occurred.

3. *Research, Development and Demonstration*

The second coming of coal (with oil shale and tar sand exploitation following close behind) and nuclear power are likely to provide most of the energy needs of industrialized countries when the search for petroleum substitutes becomes really critical. For example, the OECD Member countries are aiming to increase their consumption of coal by *ca.* 7.5-fold by the year 2000 and, as mentioned above, some countries have opted for a major nuclear power programme. It follows, therefore, that renewable energy (and, probably, feedstock) technologies will remain very small activities in such countries for several decades to come. The pressure will increase on developing countries to experiment with large scale renewable energy/feedstock processes and there are signs already that major failures will occur unless extremely careful evaluations are made (see below). Attempts to switch to biomass-based processes before they have been thoroughly proven are likely to worsen economic conditions and needlessly impede the introduction of biotechnologies. Consequently, there is an urgent need for such technologies to be researched, developed and, above all, demonstrated at a production scale in industrially developed countries. The UNERG Technical Report [46] also draws attention to this problem and makes six specific recommendations for demonstration:

- i)* biogas production plants for countries without previous experience;
- ii)* gasification of biomass plants;
- iii)* global demonstration to show how gas can be used to generate electricity;
- iv)* energy farm and wood-fired electricity power station;
- v)* conversion of biomass to methanol;
- vi)* ethanol from lignocellulosic materials.

4. *Problems Peculiar to Developing Countries*

We have alluded to opportunities and problems that biotechnological innovation may present for developing countries and we wish to develop the latter issue further. Relationships between industrialized and developing countries could very likely be modified as a result of biotechnology, not least because of the trade in agricultural commodities (see pp. 37-38). It is predicted that by 1990 the developed world will account for approximately 24 per cent of the world's population, 85 per cent of its economic activity and about 50 per cent of total grain production and consumption. In order to redress the balance, low-income developing countries will require extremely large subventions together with research and training support enabling them to establish infrastructures that can manage not only food supplies but the impact of biotechnology as a whole. Traditional trading patterns between the industrialized and developing countries may become progressively dislocated by the former becoming self-sufficient in certain commodities as a consequence of high technology, e.g. high fructose syrup replacement of cane sugar; and by the keenness of industrialized countries to export biotechnology to the Third World without either trading partner being fully appraised of the result. Over enthusiastic advocacy of novel renewable energy processes may be a case in point and we emphasize that adequate demonstration systems, coupled with sophisticated resource analyses, must be essential first steps before developing countries are urged to switch from traditional renewable sources. It is worth recalling that traditional renewables (wood, dung) provide energy for about 2 000 million people (*ca.* 270 Mte oil equivalent, less than 4 per cent of the world's total energy consumption), that 90 million people are suffering acute fuelwood shortage and that over 800 million people are consuming fuelwood more rapidly than it is being replenished. It can be argued that fuelwood replenishment, not new technology, is the first and paramount priority for much of the Third World. Biotechnology should be wary of devising technical solutions and then seeking appropriate problems. Not only should the reliability of raw material supplies for biotechnological processes be assessed but the identity of captive markets for the products of such processes made certain. Developing countries are acutely aware of the latter, having seen commodity prices fall sharply during the last five to six years (e.g. since 1975 prices of jute, maize, cocoa, sugar and cotton have fallen by 71 per cent, 69 per cent, 60 per cent, 59 per cent and 53 per cent respectively).

The case of ethanol production in Kenya is a cautionary one. The oil import burden has adversely affected Kenya's trading competitiveness such that twice the quantity of coffee and tea needs to be exported to maintain oil supplies. Quite reasonably, therefore, the Kenyan government, like several others, has established an ethanol policy and expects to produce sufficient amounts to replace 20 per cent of the gasoline demand by 1985. Moreover, the intention is to provide ethanol as a feedstock for a plastics industry. In short, the situation and response is analogous — at least superficially — to that in Brazil. However, problems have arisen and are related to biomass feedstock supplies, end use technologies, chemical support complex and the total energy policy of Kenya. High yields of sugar cane are obtained from good quality, irrigated land but a consequence of switching 400 000 hectare to a fuel crop is a continuing and increasing food importation. Alternative crops (cassava, sorghum) will take a long time to develop, because of either harvesting difficulties and susceptibility to disease. or little experience of cultivation. A wood-based ethanol programme in Kenya is totally impracticable. The chemical support complex is seen by some commentators as an *ex post hoc* justification to enable more funding for the ethanol programme: its output, in addition to ethanol, is baker's yeast, vinegar and citric acid. Unfortunately, present local markets for these additional products are very small and the high cost of production vitiates export. In comparison, the successful ethanol programmes in Brazil and Zimbabwe reflect no food shortages and, respectively, a long tradition of ethanol production and the force majeure of an oil embargo.

B. The Trouble with Water

One of the main disadvantages of enzymic and microbial catalysis is the need to work in an aqueous environment. In most cases there are constraints, on the concentrations of catalyst and reactant that can be used and the product concentrations that can be achieved, which greatly influence the operation and economics of biological processes.

1. Reactor Operation

Fermentation processes are not very reaction intensive compared to many chemical reactions, for several reasons:

- the concentration of microbial catalyst is usually low. This is very often due to the limited rate at which oxygen can be transferred economically from air to the culture liquid (see p. 31);
- the product is often inhibitory to its own production. Thus for most yeast fermentations the rate of accumulation of ethanol falls when the concentration reaches 6–8 per cent (weight/volume);
- high concentrations of reactants may prevent product formation.

This is illustrated by the effect of glucose on microbial production of antibiotics. Catabolite repression by the glucose severely restricts synthesis so that it is necessary to maintain a low concentration of glucose in the broth to ensure product formation. This is done by controlled feeding of glucose to the fermenter.

Enzymic reactions also are done at relatively low reaction intensities, mainly for economic reasons. As it is normally very difficult to recover the enzyme from the aqueous solution, the minimum amount of catalyst is used compatible with an acceptable reaction time. Many enzymic reactions are reversible so that the reaction rate declines as the product concentration increases and equilibrium is approached. Commercial high-fructose glucose syrups usually contain 42 per cent fructose and 53 per cent glucose on a dry solids basis even though it is possible to convert about half of the initial glucose to fructose using glucose isomerase, because it is uneconomic to take the conversion further.

One important technique which has found only limited industrial application so far is immobilization of the catalyst, that is, its conversion from a water-soluble form by entrapping the catalyst in a solid or binding it to a solid. Not only does this allow reuse of the enzyme or microorganism but much greater reaction intensities can be achieved, for instance, in packed bed reactors. In some cases high reactant feed concentrations can be used with immobilized biocatalysts. Glucose feeds up to 45 per cent dry solids are supplied to packed beds of immobilized glucose isomerase for production of high fructose glucose syrups.

Although biocatalysts require an aqueous environment and are normally used in dilute aqueous solution or suspension, some enzymes and microorganisms can function at liquid-liquid interfaces or in the presence of a high proportion of a water-immiscible reactant or an organic solvent in which the reactant is dissolved. There are several possible advantages for such liquid-liquid reaction systems: 1) high reactant/product reactor concentrations; 2) reduction of inhibition by the product through its transfer to the non-aqueous phase; 3) ease of product recovery.

Inevitably there are also potential disadvantages such as catalyst denaturation or inhibition by organic solvents. There is much work to be done but this approach, if successful, could lead to a major step forward in some areas of biocatalysis. At least one process, using a lipase for the interesterification of fats dissolved in an organic solvent, has reached pilot-scale.

2. *Reactor Outputs*

Nearly all fermentation products, including most commercial enzymes, are extracellular and are recovered from the liquor after removal of the microbial mass. Despite the great advances that have been made in increasing the yields of many fermentation products, their concentrations (per cent, weight/volume) in the harvested broth are still low:

Citric and glutamic acids	10–12
Ethanol	7–12
Baker's yeast	5
Benzympenicillin	3
Riboflavin	1

This is a serious problem as the cost of removal of water is high. The contribution that this cost makes to the final product price depends on the type of product and its scale of production. It is certainly important in high volume–low/intermediate value processes where the product may be biomass, e.g. single cell protein and baker's yeast. If there is a resurgence of production of organic chemicals by fermentation, then for these the cost of the initial separation/concentration step will affect markedly the overall cost. On the other hand, the costs of research and development, initial testing, quality control and marketing of high–value pharmaceutical products are much greater and tend to dominate the overall cost.

Many products of biological processes such as beer are still very dilute solutions. Thus breweries incur heavy transport costs, essentially for moving water and this is a major reason why breweries have developed high–gravity brewing. Some products, such as glucose syrups, are concentrated to a point where they have to be maintained above ambient temperature for them to remain liquid in order to save on transport costs. This is also one reason for the increasing popularity of packaged dried foods that can be reconstituted.

One real advantage of reuse of biocatalysts, usually in immobilized form, is the large reduction in the amount of fermentation broth that has to be processed. The outputs from such reactors are usually much easier to process.

3. *The Supply of Water*

In this Section B, so far we have outlined the problems caused by the high water content of biological materials and the need for biocatalysis to be done in an aqueous environment. Industrial processes, both chemical and biological, use large quantities of water for cooling and other purposes. For any large scale process it is essential to consider recycling water. Apparently this has been done for some single cell protein processes where the spent broth stream is suitable for reuse.

In some countries water is both scarce and very expensive. This may make otherwise economic processes either inoperable or uneconomic. Similarly, shortage of water may greatly restrict the ability to grow the agricultural feedstocks for biological processes. For instance, two beet sugar factories in Israel have been closed because the cost of water was too high. The CSIRO Report [9] draws attention to the considerable attraction of salt–tolerant organisms in Australia where limitation of the availability of fresh water is likely to be the ultimate constraint to productive activities. Thus the introduction of processes based on biotechnology may depend on other technologies concerned with the production of fresh water.

C. **Product Recovery**

Commercial production of biological products varies greatly in scale. This is reflected in the size of fermenters used. These range from 1 500 m³ for biomass production, between 100 and 200 m³ for penicillin and 1–5 m³ for vaccines. Thus the problems of product recovery differ with the scale of product manufacture. In contrast to most chemical processes, there is a considerable volume reduction following

some steps in product recovery, so that the size of a unit operation depends on its position in the recovery process sequence.

Many biological products are unstable unless handled within a limited range of pH and temperature, and very few are volatile. This constrains the choice of unit operations that can be used. Gas phase processing which is widely used in the chemical industry is seldom possible. Recovery from liquids depends extensively on solid/liquid separation which is made difficult by the low relative densities of the biological solids and their compressible nature. Solid/liquid separation is normally done by vacuum or pressure filters or centrifuges. There is little likelihood of major changes in the operating characteristics of such machines although important alterations to the design of some centrifuges have reduced the heat input to the processed liquor and sedimented solids, which in the past has caused serious problems.

The instability of many products also dictates that product streams must be handled at low temperatures and with reasonable speed. It may be necessary to process in one working day a fermentation batch that has taken many days to produce. Unless many fermenters are being operated, this may mean that recovery equipment is only being used for part of the time.

Product recovery varies enormously in its complexity. Biomass for animal feed normally only requires separation from the broth followed by washing and drying. The solids content of separated biomass is usually 20–30 per cent weight/volume. The remaining water must be removed under carefully controlled conditions to minimise damage. In contrast, some products such as enzymes or blood proteins for clinical use require a sequence of many operations to achieve the required degree of purity. As it is difficult to obtain a step yield for each operation much greater than 90 per cent and sometimes less, the overall yield of product is linked to the number of steps necessary for adequate processing. The product recovery engineers have the onerous task of achieving adequate purity with minimum product loss.

D. Genetic Manipulation

1. Host Vector Systems

Cloning of foreign genes in the Gram-negative bacterium *E. coli* is routinely performed now in many laboratories and use of this model system has revolutionized molecular genetics. However, there are a number of disadvantages of the system. *E. coli* is an inhabitant of the alimentary tract and, hence, presents a potential hazard, although this has often been exaggerated. The cell wall contains a lipopolysaccharide endotoxin (poison) which could be difficult to separate from products intended for pharmaceutical use. Any product from a genetically engineered strain has to be separated from all the main proteins in the producer cell which is more difficult if the product is not excreted and the cell has to be disrupted. *E. coli* exports relatively few proteins compared with other organisms, although this disadvantage has been cleverly overcome by fusing the foreign gene to another whose gene product is exported, for example, proinsulin fused to the enzyme β -lactamase (penicillinase) is exported into the periplasm close to the cell wall. Other methods of improving excretion in *E. coli* are under investigation involving the use of fragile “leaky” cells genetically impaired in wall or membrane structure.

For these reasons attention has been turned to other bacteria. The Gram-positive *Bacillus subtilis* was the first non-pathogenic organism to be transformed by DNA in 1958 and subsequently the genetics of the organism have been well developed. Moreover *B. subtilis* strains are known to secrete over 50 different proteins. Furthermore, these organisms are consumed in food in large quantities in the Far East and used for the industrial production of antibiotics, insecticides and enzymes such as α -amylase. The reason *E. coli* was preferred initially over *B. subtilis* was that, in comparison to the latter, relatively little was known about its plasmids and bacteriophages. This situation has changed now and *B. subtilis* can be considered for the production of a variety of proteins. Perhaps the ideal vectors for transferring heterologous DNA will themselves be the products of genetic engineering involving DNA from *E. coli* and *B. subtilis*, their plasmids and phages.

Both the above organisms are prokaryotic and could present problems in the expression of eukaryotic DNA. For example, many human proteins are glycosylated (having specific carbohydrate residues attached at a defined point on the polypeptide). This process is rare in bacteria but can occur in other eukaryotic microorganisms such as yeasts by a mechanism similar to that found in human tissue. Moreover, *Saccharomyces* spp. are genetically well explored, not pathogenic and have widespread use in the brewing and baking industries and, hence, fermentation characteristics for large-scale growth are well established. Successful transfer experiments have been made using the 2 μ m DNA plasmid vector but, again, the most successful vector turned out to be a hybrid between *E. coli* and *S. cerevisiae* plasmids exemplified by the success of its use to produce interferon in yeast. Current work is concerned also with producing yeast vectors which are able to integrate into one of the chromosomes so that inserted genes become an integral and stable part of the genome.

Considering the diversity of microbes used in industrial processes it is clear that a major constraint is the absence of suitable host-vector systems in these organisms and, although there are benefits from cloning DNA from these microorganisms in well-developed model systems such as *E. coli* for its analytical value (e.g. DNA sequencing), the major impact of the technology will come from the use of host-vector systems endogenous to these microorganisms. Significant progress has been made with *Streptomyces* and *Pseudomonas* and, although considerable effort is now being applied to filamentous fungi such as *Aspergillus* spp., *Penicillium chrysogenum*, *Acremonium chrysogenum* and *Trichoderma* spp. as well as to the anaerobic bacterium *Clostridium*, no suitable plasmid or virus vectors have been reported yet for these commercially important genera. Nevertheless, it can be only a matter of time before comparable vectors are available. However, in comparison with *E. coli*, there remains one consideration which will hamper initially a full ingenious exploitation of the technology — often very little fundamental work has been undertaken on their genetics, physiology or elucidation of biosynthetic routes of important compounds produced from them. A good example of this is *Penicillium chrysogenum* which has been used industrially for nearly 40 years and, yet, the penicillin biosynthetic pathway is still not fully established and there are no details of genetic fine mapping; not even the number of chromosomes present in the organism is known!

Turning to the situation in mammalian cells, in contrast to the numbers of vectors available in bacteria, principally only two — the Simian monkey virus *SV40* and the mouse virus polyoma — have been developed so far. In *SV40* it has been shown that β -globin genes from mouse can be integrated, transcribed and translated during lytic infection of kidney cells. However, the ideal vector should lead to expression of the desired gene in the absence of cell lysis, which is not the case at present.

Although not widely reported, there are a number of other vectors being developed with an eye to gene therapy for genetic deficiency diseases. For this, or for the production of valuable compounds from mammalian cells, it is possible also to use somatic cell hybridization; microcell mediated gene transfer, where donor microcells containing only one or several chromosomes are fused with genetically intact recipients; chromosome mediated gene transfer, involving the endocytosis of cell free chromosomes by the recipient cell with only subchromosomal fragments being retained; and, even DNA mediated gene transfer, where protein free naked DNA is taken up by recipient cells. All of these methods have been described but need to be developed further.

With regard to the transformation (i.e. the unidirectional transfer of genetic information in which DNA originating from one cell is taken up by another and stably maintained), in many cases investigated so far in a wide range of organisms, it has not proved possible to demonstrate this process. However, in these cases and also to improve the efficiency of transformation when it does occur, the potential of using liposomes should be explored. When phospholipids are dispersed in an aqueous solution, vesicles (known as liposomes) form, encapsulating a discrete volume of the aqueous phase inside a bilayered lipid membrane. DNA or chromosomes can be encapsulated in such vesicles and have been fused with some animal cells, plant and microbial protoplasts, giving rise to transformed cells. The similarity of

composition between cell membranes and liposomes might, with appropriate modifications, make the technique applicable universally.

Finally to plants, where the biggest single constraint, though probably only a technical one, is the inability to regenerate mature plants from cells, including protoplasts and tissue cultures of important plant species (e.g. carrot, tobacco and oil palms). Although this has been done in some species, cellular proliferation is often accompanied by chromosome aberrations and changes in ploidy. Also, with the exception of alfalfa, very little success with regeneration of agronomically important crop plants (e.g. legumes and cereals/grasses) and most woody tree species has been achieved.

Transformation requires an efficient vector to bring about integration of foreign DNA into the host genome. A number of possible vehicles for use in plants are under investigation, including the use of the crown gall tumour inducing Ti plasmid from *Agrobacterium tumefaciens*, the double-stranded DNA caulimoviruses (e.g. cauliflower mosaic virus), the single-stranded gemini viruses (e.g. bean golden mosaic virus), tandemly repeated plant genes such as those for ribosomal RNA, and the transposable elements of maize. One of the main bottlenecks with any of these methods has been that, if the techniques are not to be restricted to vegetatively propagated plants, the desired transformed phenotype must be seed transmissible. This has in fact recently been achieved with a modified Ti plasmid. With cauliflower mosaic virus there is a need to find a site on the viral genome where exogenous DNA can be inserted and expressed during infection without interfering with viral replication. Also, there is a further problem in that the small size of the virus may limit the amount of DNA that can be added.

The work with the Ti plasmid is the most advanced and, although the key group of plants, the monocotyledons, are not susceptible to crown gall, at the whole plant level, promising work with the use of the plasmid with isolated cells has been reported. Moreover, this work has demonstrated, also, the expression in *Agrobacterium tumefaciens* of plant growth hormones such as cytokinins. The way is now open to produce other interesting plant products in microorganisms in a manner analogous to human proteins produced in *E. coli*. Many problems remain but the rewards are potentially so great that further work will more than repay the efforts.

2. Gene Expression

Sakaguchi [53] has listed the likely barriers which must be considered for each and every attempt to establish and express foreign DNA as follows:

- the nuclease barrier which destroys exogenous DNA before incorporation;
- the replication unit barrier where the evolved complex replicon machinery may prevent proliferation of foreign DNA;
- transcription barrier indicated by the specificity of attachment and promoter sites which may vary with every combination of RNA polymerase and DNA sequence;
- translation barrier requiring ribosomal recognition;
- protease barrier responsible for degradation of enzymes in a foreign environment;
- intervening gene barrier resulting from the fact that eukaryotic DNA gene sequences have intervening genes (or introns) which are removed in the formation of messenger RNA whilst prokaryotes lack both introns and the “processing” enzymes.

Future work will involve overcoming in novel ways these and other barriers for particular products in different organisms. Where expression of foreign DNA has been successful, it is interesting to note that examples can be found in which each of the above barriers have been circumvented. To give one example, an enzyme (reverse transcriptase) can be used to make a DNA copy of mature (i.e. processed) mRNA from a eukaryotic organism which can be introduced and transcribed normally in a bacterium.

Experiments have demonstrated that the level of gene expression is influenced principally by the number of gene copies and the efficiency of their transcription and translation. Transcription of a cloned sequence requires the presence of a promoter recognised by the host RNA polymerase and efficient

translation requires that the mRNA bears an appropriate ribosome binding site and a correct translational reading frame. This has been done in *E. coli* by placing the insert under the control of appropriate *E. coli* promoters such as those for β -galactosidase or β -lactamase.

With the success of cloning in both Gram-negative bacteria (e.g. *E. coli*) and Gram-positive organisms (e.g. *B. subtilis*), it has been suggested that a broad range vector which can express in both groups of bacteria would be desirable. However, although *E. coli* is known for its ability to express genes from *B. subtilis*, in contrast *B. subtilis* strongly discriminates against the expression of *E. coli* genes but expresses genes from other Gram-positive bacteria such as *Staphylococcus aureus*. This apparent asymmetry in the expression of heterologous genes between *E. coli* and *B. subtilis* is under active investigation and recently attempts to overcome this, by supplanting the native regulatory elements of an *E. coli* transposon derived gene for chloramphenicol resistance with *B. subtilis* DNA fragments to give an *E. coli/B. subtilis* co-integrate, have proved hopeful.

One further area is likely to be important in unravelling the regulation of DNA expression in eukaryotes. This concerns the role of the enormous amount of non-coding repetitive DNA found in eukaryotic genomes where, so far, recombinant DNA technology has not shed much light on the mystery. Improved knowledge here could lead *inter alia* to a general method for amplifying genes from eukaryotic organisms.

3. Stability

Strain stability is a long-standing problem in industrial fermentation. This is not surprising since the industrially useful strains have undergone, frequently, many mutational steps over long periods of time in order to maximise production of specific metabolites. When the genes coding for particular enzymes or pathways are carried on plasmids, as is the case for some industrially useful organisms, the problems of strain stability may be accentuated. The ability of the host cells to maintain the plasmid unchanged through several growth cycles may be affected by the genetic characteristics of the host cells, the culture conditions, the copy number of the plasmid and the gene(s) carried on the plasmid. The plasmid may be lost totally or undergo segregation, or other rearrangements, with the loss of significant gene regions. With multicopy plasmids, the copy number may change thus reducing the predicted gene amplification. Important investigations are in progress on the molecular biology of plasmid replications and control of copy number. It has been assumed, sometimes, that plasmid stability is related directly to size with small plasmids being less liable to spontaneous loss. That this simplistic interpretation is by no means true is obvious from recent studies on large plasmids in a variety of organisms, including *Pseudomonas* and *Rhizobium* which are highly stable in contrast to the situation with some small *E. coli* plasmids involved in, for example, somatostatin or insulin production. Knowledge gained over many years on the mechanism of recombination and the genes concerned with the process in bacteria has proved useful also. For example, plasmids from *S. aureus* introduced into *B. subtilis* have been used to clone heterologous genes. If the mutation *recE* (rendering the cell recombination deficient) is present within the recipient cell, homologous genes can be cloned on the plasmid also. In the absence of this mutation, the homologous gene can only be integrated into the chromosome of the host. Since mutations known to affect DNA repair and recombination are known in all organisms from man to microbes, including some industrially important strains, further work will be expected to lead to methods not only to improve stability of plasmid borne genes, but also for integrating exogenous genes into plasmid or chromosomal locations in eukaryotes. As with known instability problems, the exacting growth conditions obtaining in chemostat and large-scale culture are likely to exacerbate this and as such this area requires much fundamental study.

4. *Cell Secretion, Export of Gene Products and Downstream Processing*

This has been discussed briefly on p. 43 as a reason leading to the current interest in the use of *B. subtilis* rather than *E. coli*, although in the latter ingenious ways of obtaining the desired product in the periplasm have been found. However, genetic engineering is likely to play a major part in improving the efficiency of the often considered unglamorous but industrially important area of “downstream” processing. Genetic tricks are available now for making cells secrete proteins that would not otherwise be secreted but very little genetic work has been undertaken, so far, on such topics as secretion mechanisms, cell aggregation, cell fragility, and induced lysis, for those organisms where, at present, products have to be extracted by breaking open the cell.

5. *Mutagenesis*

Considerable progress has been made in this field, particularly with respect to the central role of DNA repair mechanisms. DNA repair pathways have evolved in all groups of organisms and may be considered as a series of “sieves” intercalated between production of a premutational lesion and realisation of a mutant clone. By interfering with DNA repair, either by chemical inhibition of repair enzymes or mutation of DNA repair genes, mutant yield can be increased. This is important for improving commercially valuable organisms and, also, for obtaining mutant lines, a prerequisite for genetic studies.

Here again, *E. coli* has served as a model system and over 25 different loci have been identified to be involved in DNA repair of damage induced by far ultra violet light. Different mutagens act principally through two separate pathways, one causing repair errors and the other replication errors. Other bacteria, fungi, algae and, more recently, mammalian cells (e.g. human *Xeroderma pigmentosum* cells) have been investigated but virtually nothing is known of the situation in plant cells. This is important since one of the major hurdles in developing transformation systems, or parasexual genetics for plants similar to those for microorganisms, is the difficulty of obtaining stable genetic markers such as auxotrophy and drug or heavy metal resistance. Deficiency of nitrate reductase in tobacco and proline auxotrophy in maize are two of the few good examples.

There are cases, also, where organisms have proved refractory to usual mutagenic procedures. Since a number of genes known to be essential for mutagenesis, and others whose presence enhance mutant yield in *E. coli* and *S. cerevisiae*, have been successfully cloned, these are good candidates for transfer by recombinant DNA technology.

Yet another tool which could be exploited further is the use of transposons. Transposable genetic elements are segments of DNA that can insert at many loci within genomes and they have been found in both pro- and eukaryotic organisms. In bacteria they can be used successfully as “mutagens” to mutate genomes, as genetic markers for DNA where there is no readily scorable phenotype and, also, they can mediate a wide range of recombinant events. Further research on these systems in a range of organisms combined with recombinant DNA technology and site specific mutagenesis will enable their enormous potential to be realised.

6. *Availability of Strains, Cell Cultures, Vectors and DNA Sequences*

The problem of preserving genetic variability has long been recognized by plant and animal geneticists and gene banks have been set up for this. Although a number of excellent well-stocked microbial culture collections exist, concern has been expressed by some countries (e.g. Australia) on the adequacy of this provision (see pp. 22-24). The recent situation of microbial diseases, such as smallpox, virtually being eradicated underlines the need for preserving variability. Interesting experiments with viral diseases such as hepatitis B show that it would be practicable and safer to preserve such pathogens as cloned fragments.

In addition to seed storage, there is a need, also, to set up banks to maintain plant and animal cell and tissue culture to facilitate access to important research material. It would be desirable, even, to support the collection and storage (perhaps as cloned samples) for distribution of rare material such as tumours capable of synthesizing abnormally high quantities of specific hormones.

This latter raises the question of banking DNA sequences and vectors. It is interesting to note that Fiers from Belgium started his ambitious project to sequence the complete genome of the bacterial virus MS2 in the early 1960s, when it was not possible to sequence fragments more than eight nucleotides at one time, and it was not until 1976 that the complete 3 569 nucleotide genome of MS2 was published. The combination of rapid sequencing techniques and recombinant DNA cloning methods has revolutionised this type of work and, today, sequencing has become rapid and commonplace and the problem arises of how to store and collate all this information. Scientific journals, not surprisingly, are loathe to print masses of ATCG permutations. Individual laboratories and, particularly, multinational industrial companies involved in the field recognise the value of this information and are taking their own initiative as is clear from job advertisements in scientific journals. Of course, the ideal answer would be a central data bank in which all sequences could be deposited and made available to the whole scientific community. There are good precedents for such a service in the Brookhaven Protein Data Bank and the Cambridge Crystallographic Data Centre. The “Molgen” service at the University of Stanford is a start in this direction.

This is an aspect of what has been termed “bioinformatics” representing the interface between information technology and biology which will play a major role in biotechnology. This is being pursued actively by, among others, the Commission of the European Communities which has set up a Task Force on “Biotechnology and Informatics” and, as part of the FAST programme, has identified the following four areas of application:

- i) *Data capture technologies*, at all levels from radiation spectrometries (NMR, lasers, X-ray), through enzyme electrodes in fermenters to satellite teledetection of land potential;
- ii) *Data banks* with facilities for storage, scanning and retrieval of details of biotic material at all levels from genetic and macromolecular (e.g. enzymes) to whole plants and animals;
- iii) *Mathematical models* of structure, function and dynamics from molecular biophysics to models of fermenter contents and downstream processing operations;
- iv) *Artificial intelligence/interactive software* as in medical diagnostics and computer-aided design.

Certainly an analytically predictive capability based on structural and functional information on macromolecules and microbial metabolites has already been demonstrated and among other things brings nearer the ability to design specific drugs, the “magic bullets” of Paul Ehrlich. Moreover, on-line computer control of fermentation processes using mathematical modelling and specific probes linked to instrumentation and engineering is a reality (see p. 33). The potential applications of bioinformatics are enormous. Even the science fiction idea of DNA tapes as the ultimate in miniaturization for general information storage cannot be discarded.

E. Extended Use of Biocatalysts

Successful biotechnological innovation depends on selecting the most suitable biocatalyst, optimizing its structure and deploying it in an environment which optimizes its function. For most processes, efficiency and overall economics are improved by extending the operational life of the biocatalyst. This may be done by using continuous-flow or fed-batch fermentations, or various types of immobilization technology. Immobilized biocatalysts (enzymes, viable or non-viable microorganisms or other cells) have received growing attention over the past 15 years or so and are attractive in several ways:

reuse, or use over long periods of time; continuous processing; higher product yields; and simple recovery of products. In this section we discuss some of the advantages of immobilization and highlight the need for further research and development.

Immobilized biocatalysts are or will be used in a wide range of contexts — chemical transformations, chemical syntheses, analysis, medicine, food and beverage processing, and effluent detoxification. There are hundreds of reports on enzyme immobilization but only a few consider scale-up problems and ways in which production scale operation can be achieved. Current industrial use of immobilized enzymes centres on:

- partial conversion of glucose to fructose by glucose isomerase to give a low cost sweetener with many uses in the food industry;
- conversion of benzylpenicillin by penicillin acylase to 6-aminopenicillanic acid, a precursor of a range of penicillins;
- production of L-amino acids from inactive racemic mixtures of D- and L- forms using aminoacylase.

Rather surprisingly, microorganism and cell immobilization technology was developed after enzyme immobilization but already the field has seen rapid development and some industrial applications. Thus, aspartic acid and malic acid are produced on the 100s of tonnes per year scale; immobilized bacteria are used to degrade malic acid in wine; and immobilized homogenized *Bacillus coagulans* is the major means of glucose isomerization used today. Immobilized cell technology also is applied to waste treatment (removal of nitrate) and for the bioaccumulation of metals out of waste process streams. The economics of immobilized biocatalyst systems depend on the costs of the catalyst and the immobilization, productivity and stability of the catalyst. It should be kept in mind that the catalysts used in many chemical processes have half lives of months or years, often at very high temperatures and pressures. The catalytic half lives of several immobilized cell systems also are very respectable: 120 days (aspartic acid production), more than 50 days (malic acid production), more than 270 days (malic acid degradation), and 60 days (glucose isomerization). The nature of the immobilizing medium is one crucial factor in metabolic stability. Thus the half life of aspartic acid production is increased from 150 days to 628 days when the immobilization medium is changed from polyacrylamide to carrageenan. Even with polyacrylamide immobilization, production is 40 per cent cheaper than the traditional fermentation process.

The choice of biocatalyst will depend on several factors and important among them are: whether the conversion entails one or more reactions; whether an enzyme cofactor is involved; and whether biochemical energy needs to be supplied to the biocatalytic system. The regeneration of cofactors is one of the greatest challenges confronting the design of enzyme reactors but it is possible that chemical alternatives to such cofactors will be found. Enzyme reactors also are advantageous when high activities per unit volume are required. This may be critical when other unwanted reactions are also occurring, such as colour formation during isomerization of glucose syrups and lactam ring hydrolysis during benzylpenicillin deacylation.

The promise of immobilized cell technology is considerable and probably much wider in scope than for immobilized enzymes. For example, preliminary reports indicate that productivities of acetone/butanol by immobilized bacteria can be increased by 200 per cent over the traditional batch fermentation, while the enhanced production rates of ethanol by continuous fermentation with entrapped yeast has encouraged pilot plant studies in countries such as Japan. The immobilization of plant cells for the production of secondary metabolites such as alkaloids is a topic which merits further research although the ultimate means of producing such chemicals is likely to follow gene transfer to microorganisms (production yields and rates will limit the usefulness of plant cell cultures *per se*).

The catalytic stabilities of enzymes and cells, and the means for their improvement, are critical for the further introduction of immobilized biocatalysts into industry. Equally it is an area that has been rather lacking in systematic research, indeed progress in cell immobilization technology has been almost entirely dependent on empirical research. An appreciation of the situations in which biocatalysts are to be

employed is obviously important and future applications are likely to move increasingly towards catalysis under non-physiological conditions, e.g. low water activity, high salinity, non-aqueous, high temperature, extreme pH environments. There is an urgent need to understand the processes of enzyme inactivation, both *in vitro* and *in vivo*, and to devise means for its prevention. One approach is via chemical modification of enzymes to produce stability and/or changes in the catalytic properties. Although work on chemical derivatization has been extensive, it exists as an art rather than a science and advances have been made on an empirical basis. Chemical modification of enzyme proteins can be considerable, such that desirable properties of several enzymes are assembled in a single protein. Ultimately, the preferred approach may be construction of synthetic enzymes; gene synthesis is becoming a relatively routine matter (pp. 27-29), the limiting factor remains our understanding of the relationship between primary structure, stability and activity. Similarly, the development of whole cell immobilization has been of a trial and error type. Nothing is known of the relationship between physiological state of the organism during its preparation (growth) and its performance following immobilization; of particular importance are the effects on activity and stability and how immobilization frequently leads to enhanced stability. In some cases the extended operational stabilities of immobilized microorganisms are the result of cell growth and *de novo* protein synthesis. This raises the much broader question of the extended use of microorganisms for multi-step biosynthetic reactions. For instance, at the end of a penicillin fermentation, the broth contains about 30 kg penicillin m³ and about the same amount or more dry mycelia which then is discarded because its penicillin-synthesising ability has deteriorated. This situation is similar to many other fermentations and emphasizes the need for greater investment in microbial physiology (p. 24). a rational approach to the maintenance of cellular activities, we shall never know how close we can get to having the perpetual biocatalyst.

Chapter III

IMPORTANT ISSUES AFFECTING DEVELOPMENTS IN BIOTECHNOLOGY

The social and economic pressures coming from increasing demand for better health and nutritional standards, environmental concerns for waste management, and increasing costs of oil based feedstocks, will ensure that biotechnology will have a major impact in the world. That impact will depend, of course, upon scientific and technological progress but some other considerations are discussed below.

1. Government Research and Development Policies

There is now a substantial literature devoted to this subject. The proportion of government spending on basic research and R & D differs from country to country, with small economies in general emphasizing the fundamental end. Nevertheless, whatever differences exist, it has been noted that in OECD Member countries overall, there has been a move towards a more systematic consideration of science and technology policies and priority assessment with increasing emphasis on international competitiveness. Therefore, initiatives in biotechnology are likely to cut across existing government ministries and departments and proper coordination of these needs to be ensured. In addition, the role of international cooperation in this field will need a careful examination (see pp. 20-21).

Biotechnology, together with microelectronics, optoelectronics, robotics and informational technologies generally have been stated by most governments as priorities but the assessments of time-scales for development and implementation have differed. What is clear is that strategic support given to biotechnology relative to the other areas will have a significant effect on the pace of developments.

Even within biotechnology, each government will have to decide which areas are appropriate to support. The strength of the health care sector may mean that it is the one least in need of government support. For example, the health system in the USA is probably the largest and wealthiest and has the most demanding market for drugs, instruments and equipment in the world. Here, and elsewhere, health care has played a role for biotechnology analogous to the military demand on the development of the electronics industry and so the costs of producing low-volume high added-value products have not been a significant constraint. This is unlikely to be the situation for high-volume industries such as bulk chemicals, energy production, agriculture and waste management. However, significant advanced technology arising from investment in the medical area should produce "spin-off" for these other fields.

2. Education and Manpower

In the OECD area, millions of people are already employed in biotechnological sectors as defined in this report. Unfortunately, precise manpower statistics are not available for these sectors. Future developments in biotechnology will not affect the estimated 28 million unemployed in OECD Member countries in 1982 but in the long term will make a positive contribution to employment. Whether it will have an impact analogous to that of electronics which created many millions of new jobs

between 1960 and 1975 remains to be seen. However, it would be prudent to note that new technologies may produce negative effects (i.e. job losses) in traditional sectors of employment.

The education, training and acquisition of the appropriate skilled people must be a major consideration in any biotechnological planning and countries would be expected to respond to this in different ways. Estimates of the number of people required vary from country to country but widely differing estimates can be found even within one country. It must be borne in mind that manpower forecasting is notoriously difficult and, in the absence of objective data, one is usually left to make the best of the subjective nature of inputs from experts using their specialised knowledge and judgment (i.e. the Delphi technique). As an example, using this approach, a recent report from the Royal Society of London [58] predicted that over the whole field of biotechnology in the UK, there would be a need over the next ten years for some 1 000 additional graduates and 4 000 technical support staff. It should be noted, however, that the UK numbers take no account of any "brain drain". Present indications are that a significant brain drain is taking place from some countries as certain skills are presently in high demand world wide and, indeed, some national reports (e.g. Canada) have called for relaxation of immigration quotas to combat the immediate shortage of biotechnological skills. Belgium has noted the progressive increased use of its trained research manpower by foreign companies, a situation arising from its own small biological industry. The lack of trained manpower is evident in some other smaller countries (e.g. Finland and Sweden).

In common with several national reports, we consider that education for biotechnology, at first degree level, should be based on existing courses in biological subjects and engineering (the disciplines underpinning biotechnology), with postgraduate conversion possibilities being made available in appropriate disciplines. We recognise the value of a limited number of degree courses, in disciplines such as biochemical engineering, designed to produce students specifically for the biological industries. It is worth noting here the confusion that often exists between the terms "biotechnologist" (see p. 18) and "biochemical engineer". As we indicated on p. 18, we consider a "biotechnologist" to be an all-embracing term describing any scientist or engineer engaged in applying his/her skills and knowledge to the processing of biological materials. A biochemical engineer is a process engineer whose role is to translate the knowledge of the biological scientist into a practical operation. He has been trained, therefore, in the scientific and engineering principles underlying the design and operation of biological processes.

There is a close correlation between the number of academic staff involved in biotechnology and the number of students that can be trained for the biological industries. It follows that, even under difficult economic circumstances, if biotechnology is expected to make significant contributions to a country's economic and social well-being, adequate numbers of staff and resources must be provided. Countries such as the USA, France, Germany and Japan have taken this issue seriously and, in some, special programmes in biotechnology are being sponsored.

The following relationships for the number of students that can be trained in biotechnologically-oriented degrees per number of academic staff are indicative of the situation in the UK:

	Length of Academic Course (years)	Number of Students per Academic Member	
		Total	Per Year
Undergraduates	3	12-15	4-5
Postgraduates:			
M.Sc.	1	5	5
Ph.D.	3	3-4.5	1-1.5

A similar relationship appears to be true in the USA. In Japanese universities, where teaching is done by Research Groups consisting normally of four academic members, the ratio seems to be somewhat lower.

Research in the higher education sector has a vital role to expand our understanding and knowledge of the scientific and engineering principles underlying biotechnology and it should be

concentrated on fundamental problems. This knowledge must be transmitted if it is to form a basis for scientific and technical advance, economic growth and improved welfare. National prosperity, among other things, will depend on the efficiency of the two-way exchange of information and coupling between research groups and industry, which is well developed already in several OECD Member countries. The relationship between business and academe is considered further on pp. 53-54.

Constraints arising from the high cost of research, both in terms of manpower and equipment and the need for an interdisciplinary mix of professional skills, have been overcome in many countries by focusing efforts in research, postgraduate teaching and continuing education on a limited number of centres, often, but not necessarily, involving collaboration of several institutions.

Another important facet of manpower training is technology transfer brought about by symposia, on-the-job training and various scientific exchange systems in operation.

There have been, and will continue to be, major impacts on everyday life from biotechnology. If a climate suitable for the uptake of biotechnology is to be fostered, all pupils at school level should be made aware of the role and importance of applied biology and engineering. In particular, for biotechnology, a broad scientific and technological education is essential and should encompass appreciation of economic, commercial and social factors in the creation of new products, processes and services.

3. *Finance and the Relationship of Academe with Business*

As well as government support for biotechnology, there are numerous other funds, foundations, charities and corporations financing programmes for discovery and innovation. There are signs that many of these have deliberately sought applications in biotechnological areas. In addition, support is available from industry and new venture capital involving specialist investment trusts, banks, finance houses and even private investors.

On the whole, industry is in favour of the continuation of a publicly supported and largely open basic academic research activity. The relationship between industry and academic scientists in the past has often involved consultancy arrangements where the scientist remains independent and can advise industry with a degree of confidentiality but without financial dependence. The arrangements have been healthy and mutually beneficial. Academic biotechnologists and, particularly, biologists have not generally had the relationship with industry and commerce that is common in some other professions including engineering, where collaboration has led to business arrangements. It has been suggested that for biotechnology, it is simply a matter of time for similar attitudes to mature since fundamentally there are no long-term conflicting loyalties.

However, some people have been concerned by a number of recent events — excessive secrecy, withholding publication of findings, refusal to make available strains and vectors relating to published work and increased motivation towards financial gain have been noted amongst academics — and it is argued that fundamental values and important freedoms of the academic life are at risk. Two causes principally have led to the current situation. The first is that with reductions in government support for research which have taken place in some countries as a consequence of the economic recession, academic scientists have had to interest others in supporting their work and, hence, are dealing more and more with funding agencies other than governments. The issue of patents arising from these changing relationships is considered separately on p. 55. The second, which has been dramatic, has involved the venture capital market.

The growth of venture capital companies has been greatest in the USA where, perhaps ten years ago, ten of these firms existed but now there are several hundred investing billions of dollars a year. Although this venture capital has spread abroad and has been paralleled elsewhere, countries with smaller economies have remained virtually free of it. It is not surprising, therefore, that potential conflicts between academe and business have been highlighted by experience in the USA and, to a lesser extent, in the UK. Small companies in biotechnology, particularly those involved in genetic engineering, set up with

venture capital, often carry out the type of work which makes the separation between industrial and academic research appear extremely artificial. Also, academics are joining the boards of such companies while retaining their faculty positions. The situation is reminiscent of the rapid development of electronics-based entrepreneurial research corporations set up in the USA many years ago, most of which were swallowed up subsequently by large companies.

Whether or not the outcome is the same in biotechnology, how academe copes with these issues will have a major impact on future cooperative ventures between commercial and non-profit making institutions and the way in which emerging technologies are transferred into the private sector and on the future conduct of basic research. Thus there is a need for a fuller investigation of the mechanisms whereby university/industry collaboration in biotechnology takes place. Certainly at present the number of legal and institutional solutions to these problems are so diverse that it is impossible to reach agreement on which one is best.

Finally, the general climate for innovation, entrepreneurial vision (involving the ability to judge a project realistically combined with an imaginative evaluation of its potential profitability), the supply of adequate funds for development and marketing expertise, are crucial factors for the successful exploitation of biotechnology.

4. *Safety and Regulations*

In the early 1970s, when the technology of genetic manipulation was first acquired, predictions of scientists ranged from panacea to pandemic. A public storm followed and it was not at all surprising that national authorities in many countries, having been told that this technology was capable of creating new forms of life and that scientists themselves had requested a moratorium for this type of research, responded by setting up groups and committees to consider the social and political acceptability of the risk. A public feeling of instinctive mistrust towards scientists promoting genetic engineering was widespread. However, finally, after considerable public debate and advice from scientists, medical and epidemiological experts, the general conclusion has been reached that, provided suitable precautions are taken, the benefits of the technology far outweigh any conjectural risks. One of the problems was that different countries prescribed different regulations or guidelines and, in some, no restrictions at all were imposed. It was not unheard of for both academic and industrial researchers to undertake the work in other more lenient countries to overcome national restrictions.

Despite the fact that the two countries that adopted stringent guidelines, the USA and the UK [from the US National Institutes of Health (NIH) and the UK Genetic Manipulation Advisory Group (GMAG) respectively], differed in their categorization of experiments, the forum created for discussions — involving scientists, the public and government — prepared the way for the necessary public acceptance of developments in the national interest. As models for societal decision-making on technological risks, they are likely to be followed in the future.

There has been a general reduction in restrictions in most countries, as knowledge and experience of the techniques have been obtained, and most of the recent debate has been concerned with pilot-plant and production-scale fermentation of the engineered strains, but we are still far from the ideal solution of a world agreement on guidelines and codes of practice. Great care, of course, would have to be exercised in implementation, because even if a code of practice were established for, say, scaled-up work involving the products of genetic engineering, there could be enormous variation from one proposed application to another in terms of competence and experience in operating large-scale fermenters.

When considering the safety of biotechnology in general, it must be remembered that pathogenic microbes are a normal feature of our environment, held in check by public health measures and an informed food technology, and that any dangers from large-scale biotechnology with processes using 1 000 to 30 000 or more litre fermenters must be seen against this background. It has been suggested that the special hazards rest only in the potential of some microbes to infest higher species and cause disease. With the exception of a few that affect plants (e.g. the xanthan gum producer *Xanthomonas campestris*,

which is pathogenic for *Brassica* spp.) and insects (e.g. *Bacillus thuringiensis*, effective against mosquito and black fly larvae), it is unlikely that dangerous pathogens will be used on a large industrial scale.

However, it should be remembered that pathogenic microorganisms, in contrast to the conjectural hazards of recombinant DNA technology, do present real risks and it is extraordinary to note that at the time when pressures on governments to legislate to prescribe safety measures for genetic engineering were at their greatest, and even the Commission of the European Communities was considering a directive, many countries had no regulations concerning the handling of dangerous pathogens! For diagnosis, research, and vaccine production, growth of some highly dangerous human and animal pathogens will have to continue but (as mentioned on p. 48) recombinant DNA technology, in fact, is making these procedures safer than ever before.

Public safety must be a prime concern, of course, in designing and operating industrial processes. All countries should have regulations concerned, on the one hand, with health and safety at work and, on the other hand, with protection of the public and their environment. Unification of standards for good laboratory practice and manufacturing procedures should be encouraged. But, increasingly demanding legislation and excessively restrictive regulations must be avoided as these will impose major constraints on developments in biotechnology. Our concern is not restricted to regulations concerning safety, as an excellent biotechnological example from the European Economic Community demonstrates. Policy decisions taken within the framework of the Common Agricultural Policy to protect the beet farmers have seriously curbed the development of isoglucose, a product with a wide potential market as a sugar substitute. It should also be noted that Governments have and can favour developments in biotechnology by introducing appropriate legislation and tax incentives as occurred, for example, with the Gasohol programme in the USA. Recent changes in FDA regulations abolishing the need to test batches of antibiotics have removed a costly constraint involved in stock-piling antibiotics prior to putting them on the market.

5. *Patents*

The patent system provides an inventor with a temporary monopoly on the use of the invention in return for disclosing the knowledge to others in a specification that is intended to be both comprehensible to, and experimentally reproducible by, a person skilled in the art concerned. Others in society may then use the knowledge to develop further inventions and innovations.

For patents in the field of biotechnology, there is a wide spectrum of opinion ranging from those who consider that inadequate legal protection is the most important constraint on developments to others who feel it to be almost irrelevant, believing that secrecy, backed by aggressive marketing of the final product to build a quick lead over potential rivals and achieve market dominance, is preferable. They further point to the dramatic increase in the cost, time and difficulty of patenting an invention, both through national offices of individual countries as well as through multinational schemes, and to litigation costs arising from the need to challenge or defend a patent. The former would argue that trade secrecy and long technological lead times, traditionally effective ways of protection, are much less likely with biotechnological investigations and that only a strong patent system provides an adequate safeguarding of intellectual property. However, it is certainly true that biological inventions have not fitted easily into patent laws framed mainly on inventions in physics and chemistry.

In the past, animal varieties and essentially biological processes for the production of plants and animals have been unpatentable while special arrangements for protecting plant varieties (sometimes but not necessarily by patent laws) are common. Microbiological processes or the products thereof have been patentable (although in some countries product claims are still not acceptable) and special provisions for microbiological inventions have been introduced in many countries following initiatives of the World Intellectual Property Organization (WIPO) and agreements reached in the 1977 Budapest Treaty. An invention is only regarded as being sufficiently disclosed if a culture of the microorganisms involved has been deposited and the Treaty allows for deposit in a single officially recognised culture collection,

which for the purposes of the Treaty will acquire the status of an International Depositary Authority, to suffice for multiple patent applications on a broad international scale. The greatest controversy has arisen so far on the interpretation of Rule 11 of the WIPO regulations: “The certified party (requesting release of a culture) has a right to a sample of the microorganism under the law governing patent procedure before that office”. Whilst it is clear that the availability of the organism is essential for full disclosure and verification and, indeed, to put the microbiologist in the same position as his counterpart investors in other disciplines, a way has to be found of preventing a competitor obtaining a culture which could immediately be used in a commercial process even before a patent has been granted. Solutions to the dilemma include making a separation of the act of verification (e.g. by the use of an independent expert microbiologist) from the act of release of the strain (European Patent Convention) and availability of the strain at the stage when there are legally enforceable rights (USA and Japan).

In addition to claims for microbial products and processes, the recent US Supreme Court ruling to allow the General Electric Co. “Chakrabarty” patent for a genetically manipulated (but not using recombinant DNA technology) strain of *Pseudomonas* which has potential for degrading oil spills has created a new dimension to scientific novelty in that microorganisms *per se* can now be protected in the U.S.A. Test cases are expected to see whether or not this applies to other cases of what lawyers are pleased to describe as “animate nature”. While lawyers and philosophers debate the subtleties raised by private ownership of living organisms, the ramifications of Stanford University’s offer of non-exclusive licences to exploit the Boyer-Cohen patent which covers a now universally employed method for the preparation of recombinant plasmids, their use for the transformation of bacteria and the replications and transcription of the recombinant plasmids in transformed bacteria, ensures that the debate will continue.

Stanford’s action has only been possible as a result of changes in policy in the USA to permit universities to retain patent rights on all inventions arising from federally supported research. Whilst providing universities with an independent source of income, some people have expressed the concern that the introduction of this type of reward for what they have always done as part of an accepted and supported scholarship (i.e. contributed to society and commerce) is one further step towards losing traditional academic freedoms (see also pp. 53-54). Although this may not be true, difficulties are likely to arise, however, since the objectives and incentives of industry and academe differ, for example, the need for patenting as opposed to rapid publication of results.

We are pleased to learn that OECD is undertaking an examination of this whole area of patent protection to provide a more comprehensive international review of existing patent laws and their application, as well as of planned changes, and to define the main difficulties and constraints likely to influence the successful development of biotechnology.

IN CONCLUSION

The industries based on biotechnology, such as those concerned with food, pharmaceuticals and waste treatment, are closely linked with the world's major problems of malnutrition, disease and environmental pollution. The solution to these problems will depend, among other things, upon the continued successful development of these industries, not only within OECD Member countries but also elsewhere. Biotechnology is fundamental to the future optimal use of the world's renewable resources. It also will make an increasing contribution to meeting energy requirements, both indirectly through the use of improved processes, and directly by providing substitutes for existing fuels, but this contribution, although valuable, should not be overestimated (see pp. 34-39).

We firmly believe that biotechnological studies must continue to be fostered by OECD, not only for the benefit of its Member countries but also for the rest of the world. Biotechnology will almost certainly result in changes to trading relationships with Third World countries, as many of the commodities traded are agricultural or organic (see p. 40). We support strongly in this context the view expressed in the Report of the Independent Commission on International Development Issues [67].

Many Governments are in the process of determining what initiatives they should take concerning biotechnology and what impact biotechnology will have on their countries in the future. Analysis even of the limited data in Appendix II shows that each of the OECD Member countries has a different mix of biotechnologically-related industries. This is not surprising and indicates that there is no unique approach to biotechnology in the future.

In Chapter I we have attempted to indicate the kinds of advances that may be made in the major areas underpinning biotechnology. Not all of these will be achieved, but the chances of success will depend greatly on the willingness of Governments to support fundamental research related to biotechnology and to encourage its exploitation by industrial companies.

In Chapter II we have drawn attention to some scientific, technological and resource constraints on the development of biotechnology. We believe that answers will be found to many of these but realise some may be insuperable. There are also many other factors that may constrain developments in biotechnology and these are described in Chapter III. The check-list of issues provided in Appendix VII is offered as a focus for starting strategic planning so that each country can exploit to its best advantage the great potential of biotechnology.

APPENDICES

Appendix I

SOME RECENT DEFINITIONS OF BIOTECHNOLOGY

1. Biotechnologie, Eine Studie über Forschung und Entwicklung, Dechema (1976) (translation).

Biotechnology is concerned with the use of biological activities in the context of technical processes and industrial production. It involves the application of microbiology and biochemistry in conjunction with technical chemistry and process engineering.

2. Biotechnology — Report of a Joint Working Party, UK (1980).

The application of biological organisms, systems, or processes to manufacturing and service industries.

3. Biotechnology in Canada: Promises and Concerns (1980).

The application of biological organisms, systems, or processes to manufacturing or service industries.

4. Biotechnology: A Development Plan for Canada (1981).

The utilization of a biological process, be it microbial, plant or animal cells, or their constituents, to provide goods and services.

5. Biotechnology: a Dutch Perspective (1981).

The science of applied biological processes.

The science of the production processes based on the action of microorganisms and their active components, and of production processes involving the use of cells and tissues from higher organisms (narrower definition). Medical technology, agriculture and traditional crop breeding are not generally regarded as biotechnology.

6. Biotechnology for Australia (1981).

The devising, optimising and scaling-up of biochemical and cellular processes for the industrial production of useful compounds and related applications. This definition envisages biotechnology as embracing all aspects of processes of which the central and most characteristic feature is the involvement of biological catalysts. Plant agronomy falls outside this definition but plants provide the raw material for most biotechnological processes, so research in plant breeding and productivity is of direct importance.

7. OTA Report – Impacts of Applied Genetics (1981).

The collection of industrial processes that involve the use of biological systems (in glossary).
The use of living organisms or their components in industrial processes.

8. FAST (Forecasting and Assessment for Science and Technology)
Sub-programme Bio-society – research activities.

The industrial processing of materials by microorganisms and other biological agents to provide desirable goods and services.

9. European Federation of Biotechnology (1981).

The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells, and parts thereof.

10. IUPAC (International Unions of Pure and Applied Chemistry), (1981).

The application of biochemistry, biology, microbiology and chemical engineering to industrial processes and products (including here the products in health care, energy and agriculture) and on the environment.

11. This Report (1982).

The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.

Appendix II

STATISTICS FOR BIOTECHNOLOGICALLY-RELATED INDUSTRIES

Table 1

Some Comparative Production Statistics (1978)

Country	Food ^a	Chemical Products ^b	Drugs & Medicine ^c
	Production Value as % of GDP		
France	10.5	12.8	n.a.
Germany	7.4	15.1	1.1
Japan	8.9	14.9	1.4
New Zealand ^d	15.5	6.0	0.3
Norway	13.2	8.8	0.2
Sweden	7.9	7.1	0.5
United Kingdom	9.8	14.8	1.0
U.S.A. ^d	9.1	13.6	0.8

a) b) c) According to ISIC classifications 311/2, 3500 and 3522 respectively.

d) Data for 1977.

Source: OECD.

Table 2

**Contribution of Food and Beverages to the External
Trade of OECD Countries (1977)**

Country	Food and Beverages	
	Exports ^a	Imports ^b
Australia	31.3	6.6
Austria	3.7	7.9
Belgium	9.7	13.2
Canada	11.7	8.4
Denmark	34.1	12.8
Finland	4.1	8.6
France	14.6	13.9
Germany	5.0	16.1
Greece	32.6	8.5
Iceland	76.8	10.4
Ireland	40.1	13.5
Italy	7.7	17.6
Japan	1.2	16.8
Luxembourg	n.a.	n.a.
Netherlands	21.7	16.1
New Zealand	46.1	7.0
Norway	11.3	6.8
Portugal	15.6	17.9
Spain	21.2	15.5
Sweden	2.5	8.8
Switzerland	4.1	11.5
Turkey	51.3	1.3
United Kingdom	6.9	17.9
USA	18.9	9.9
Yugoslavia	11.8	9.0

a) As % of total merchandisable exports.

b) As % of total merchandisable imports.

n.a. = Not available.

Note: Data excludes non-food agriculture.

Source: OECD, World Table, 2nd Edition, 1980.

Table 3

**Contribution of Medicinal and Pharmaceutical Products
to External Trade of OECD Countries (1978)**

Country	Exports ^a	Import ^b
Denmark	2.1	1.4
Germany	1.6	1.1
Japan	0.3	1.4
Sweden	1.4	1.5
United Kingdom	2.5	0.7
United States	1.4	0.5

a) As % of total exports of merchandise.

b) As % of total imports of merchandise.

Source: calculated using data from OECD and World Tables, 2nd edition, 1980.

Appendix III A

BIOTECHNOLOGY: ACCORDING TO INDUSTRIAL SECTORS

<i>Sector</i>		<i>Activities</i>
Chemicals:	organic (bulk)	ethanol, acetone, butanol organic acids (citric, itaconic)
	organic (fine)	enzymes perfumeries polymers (mainly polysaccharides)
	inorganic	metal beneficiation, bioaccumulation and leaching (Cu, U)
Pharmaceuticals		antibiotics diagnostic agents (enzymes, antibodies) enzyme inhibitors steroids vaccines
Energy		ethanol (gasohol) methane (biogas) biomass
Food		dairy, fish and meat products beverages (alcoholic, tea and coffee) baker's yeast food additives (antioxidants, colours, flavours, stabilizers) novel foods mushroom production amino acids, vitamins starch products glucose and high fructose syrups functional modifications of proteins, pectins toxin removal
Agriculture		animal feedstuffs veterinary vaccines ensilage and composting processes microbial pesticides Rhizobium and other N-fixing bacterial inoculants mycorrhizal inoculants plant cell and tissue culture (vegetative propagation, embryo production, genetic improvement)
Service Industries		water purification effluent treatment waste management oil recovery analytical tools

Appendix III B

BIOTECHNOLOGY: BASED ON VOLUME AND VALUE

<i>Category</i>	<i>Activities</i>
High volume, low value	methane, ethanol biomass animal feed water purification, effluent and waste treatment
High volume, intermediate value	amino and organic acids food products baker's yeast acetone, butanol polymers metals
Low volume, high value	antibiotics and other health care products enzymes vitamins

Appendix III C

BIOTECHNOLOGY: BASED ON TECHNOLOGICAL LEVEL

<i>Category</i>		<i>Activities</i>
High level:	High capital investment; sophisticated plant and processes often requiring strict containment; high maintenance costs; high operator skills.	High value-added products and products destined for health care and human food and food additives. Large-scale continuous processes.
Intermediate level:	Moderate capital investment and less complex operations.	Fermented foods and beverages. Animal feedstuffs. Biofertilizers and pesticides. Crude enzymes. Waste management processes which entail sophisticated operation and control.
Low level:	Small capital investment and scale of operation; simple and usually indigenous equipment; labour intensive operations; often septic systems. Village level technology.	Low value products frequently related to alleviation of pollution, sanitation, fuel and food provision. Extensive use of naturally adopted mixed fermentations. Biogas; microbial protein from agricultural and food wastes; traditional fermented foods and beverages; mushroom production.

Appendix IV

**MARKET PREDICTIONS FOR IMPLEMENTATION IN PRODUCTION
OF GENETIC ENGINEERING PROCEDURES**

Product category	Number of compounds	Current market value in million \$	Selected compound or use	Time needed to implement genetic production (Years)
Amino Acids	9	1,703	Glutamate Tryptophan	5 5
Vitamins	6	667.7	Vitamin C Vitamin E	10 15
Enzymes	11	217.7	Pepsin	5
Steroid Hormones	6	367.8	Cortisone	10
Peptide Hormones	9	268.7	Human growth Hormone Insulin	5 5
Viral Antigens	9	n.a.	Foot-and-mouth disease virus Influenza viruses	5 10
Short Peptides	2	4.4	Aspartame	5
Miscellaneous Proteins	2	300	Interferon	5
Antibiotics	4*	4,240	Penicillins Erythromycins	10 10
Pesticides	2*	100	Microbial Aromatics	5 10
Methane	1	12,572	Methane	10
Aliphatics (other than methane)	24	2,737.5	Ethanol Ethylene glycol Propylene glycol Isobutylene	5 5 10 10
Aromatics	10	1,250.9	Aspirin Phenol	5 10
Inorganics	2	2,681	Hydrogen Ammonia	15 15
Mineral Leaching	5	n.a.	Uranium Cobalt Iron	
Biodegradation	n.a.	n.a.	Removal of organic phosphates	

n.a. = Not available.

* Number indicates classes of compounds rather than number of compounds.

Sources: U.S. Congress Office of Technology Assessment; Genex Corp.
Industry Week, September 7, 1981, p. 68.

Appendix V

SOME INTERNATIONAL AGENCIES PARTICIPATING IN BIOTECHNOLOGY PROJECTS

<i>Agency</i>		<i>Activities</i>
UNESCO	UN Educational, Scientific & Cultural Organization	Scientific and technological research and training in applied microbiology and biotechnology. Major regional project for Africa and Arab States*
UNEP	UN Environmental Programme	Conservation of microbial gene pools; environmental management*
UNIDO	UN Industrial Development Organization	Provision of information and assistance
UNITAR	UN Institute for Training & Research	Provision of specialist training
UNCSTD	UN Conference on Science & Technology for Development	Vienna, 1979: awareness function and formulation of a Programme of Action which has influenced programme proposals of other UN agencies such as UNESCO
UNERG	UN Conference on New & Renewable Sources of Energy	Nairobi, 1981: awareness function and formulation of development strategies including biomass-based energy; intergovernmental committee to report to UN General Assembly in 1982
ICRO	International Cell Research Organization	Research and training in applied microbiology*
IOBB	International Organization for Biotechnology & Bioengineering	Global network of collaborating laboratories*
WFCC/WDC	World Federation for Culture Collections/ World Data Centre	Communication between culture collections and their user; aid establishment of culture collections and regional collaboration in developing countries; training in maintenance and management of culture collections*
CSC	Commonwealth Science Council	Rural development and alternative energy projects
IDRC	International Development Research Centre	Financial support of research projects
IDB	Inter-American Development Bank	Biofuels programme in Central America
EFB	European Federation of Biotechnology	Workshops, conferences and strategic planning
CEC	Commission of the European Communities	Research and training support in Biomolecular Engineering, Energy and Environment programmes; FAST (Forecasting & Assessment for Science & Technology) Sub-programme "Bio-Society" dealing with strategic perspectives in which EFB is contracted to prepare "A Community Strategy for European Biotechnology"(1982)
IUPAC/ IUB/IUM	International Unions of Pure and Applied Chemistry; Biology and Microbiology	Conferences

*The activities of these agencies are closely coordinated and frequently made in cooperation with others such as the United Nations University, International Federation of Institutes of Advanced Studies, and the UN Economic & Social Commission for Asia and the Pacific. The Panel on Microbiology is the instrument by which the UNESCO/UNEP/ICRO programme is guided and implemented.

Appendix VI

SOME EXAMPLES OF APPLICATIONS OF GENETIC MANIPULATION IN BIOTECHNOLOGY

A. HUMAN HEALTH

1. Monoclonal antibodies (for purification techniques, assays, tissue typing, in vivo tumour location, clinical diagnosis, therapy including targeting of chemotherapeutic agents).
2. Interferon (possible use for cancer treatment, antiviral therapy, inflammatory diseases).
3. Vaccines (against e.g. hepatitis B, influenza, malaria, encephalitis, cholera, herpes, adenovirus).
4. Hormones (e.g. growth hormones, insulin, prolactin, relaxin, gastrin, erythropoietin, thrombopoietin, chorionic gonadotropin, menopausal gonadotropin, steroids).
5. Enzymes (e.g. urokinase, heparinase, alcohol dehydrogenase).
6. Other proteins (e.g. specific antigens, blood factor, albumin, antithrombin, fibronectin).
7. Improved and new antibiotics, drugs, vitamins.
8. Gene therapy for genetic diseases.

B. FOOD, AGRICULTURE AND HORTICULTURE

1. Enzymes (e.g. amylases, rennin, β -galactosidase, invertase, glucose isomerase, pectinases).
2. Food additives (e.g. sweeteners, aromas, flavours, colouring matter, thickeners and stabilizers, vitamins, amino acids, antioxidants, preservatives, surfactants).
3. Additions for animal feed (e.g. new antibiotics).
4. Improved and new plant varieties (including enhanced yields, crops specifically designed for particular land use, genes for proteins such as casein introduced into carbohydrate predominant crops).
5. Pesticides and herbicides with increased specificity (e.g. use of *Bacillus thuringiensis* products, *Verticillium*, Baculoviruses, parasitic nematodes, nematocides, protozoan pathogens, piperidine derivatives).
6. Vaccines (against e.g. diarrhoeal colibacillosis, foot and mouth).
7. Plant growth hormones (e.g. cytokinins).
8. Fertilizers, microbial nitrogen fixation and manipulation of symbionts.
9. Diagnostic reagents for plant and animal diseases.

C. ENERGY, RAW MATERIALS, CHEMICALS AND ENVIRONMENTAL MANAGEMENT

1. Biomass from chemicals, wastes, residues and fuel crops (including production of ethanol, methanol, methane and SCP).
2. Enhanced oil recovery (e.g. xanthan gum, surfactants).
3. Improved algal cultures for use in photobioreactors (production of e.g. carbohydrate, protein, lipids, hydrocarbons).

4. Hydrogen and carbon dioxide production.
5. Chemicals and solvents (acetic acid, adipic acid, butanol, isopropanol, acetone, furfural, glycerol, waxes, polymers, alkene oxides and glycols, lubricants).
6. Metal extraction (e.g. copper, uranium, nickel, zinc, lead) from low grade minerals and recovery of valuable metals (e.g. mercury, cobalt).
7. Decomposition and detoxification of chemicals (e.g. oil spills, Dalapon, pentachlorophenol).
8. Improved microbial systems for environmental control of air, water and soil.

Appendix VII

**A CHECK LIST FOR STRATEGIC PLANNING
IN BIOTECHNOLOGY**

1. RESOURCES

Raw materials including feedstocks, water and minerals; energy; land availability; competing technologies; manpower.

2. SCIENTIFIC AND TECHNOLOGICAL INFRASTRUCTURE

Education; training; research base and R&D priorities; information transfer.

3. CLIMATE FOR INNOVATION

Invention — innovation time lag; industrial base; competition; finance; regulations; patent laws; social acceptability.

4. TRADING POSITION

Commodity prices; import–export balances, particularly for food; markets.

5. ENVIRONMENTAL CONSIDERATIONS

Land use; pollution, effluent and waste, its location and management.

Appendix VIII

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Appendix IX

GLOSSARY

Aerobic:	Growing only in the presence of oxygen.
Amino acids:	The building units of proteins; amino acids are linked together in a particular order which determines the character of different proteins.
Anaerobic:	Growing only in the absence of oxygen.
Antibody:	A protein component of the immune system in mammals found in the blood; monoclonal antibodies are those which are derived from a single source clone of cells and which recognise only one kind of antigen.
Antigen:	A macromolecule (usually a protein or carbohydrate) which when introduced into the body stimulates the production of an antibody that reacts specifically with it.
Autotroph:	An organism that obtains its energy from a source other than the oxidation of organic compounds (that is from inorganic compounds or light) and obtains carbon for biosynthesis from carbon dioxide.
Auxotroph:	A nutritional mutant.
Bacteriophage:	Commonly referred to as phage; a virus for which the host cell is a bacterium.
Batch culture:	Growth in a closed system on a finite amount of nutrient medium; the specific growth rate in such a culture tends to zero with time.
BOD:	Biological oxygen demand.
Catabolite:	A chemical product of catabolism, i.e. of energy-generating or degradative metabolism.
Chemostat culture:	Growth in an open system into which fresh nutrient medium is fed continuously at a constant rate and the culture volume is kept constant by the continuous removal of culture; the specific growth rate in this system is independent of time and is controlled externally by limiting the rate of supply of one of the nutrients.
Chromosomes:	Linear or circular molecules of DNA with or without associated proteins.

Clone:	A collection of genetically identical cells or organisms which have been derived asexually from a common ancestor; all members of the clone have identical genetic composition.
Cofactor:	A requirement of some enzymes for non-protein structures in order to be catalytically active; the cofactor may be a metal ion or an organic molecule, the latter being called a coenzyme.
Down-stream processes:	All unit stages in a process (e.g. harvesting of the biomass, product recovery, separation and purification, product formulation, packaging) which occur after the fermentation or bioreactor stage.
Endocytosis:	The ingestion of solid particles or droplets of materials in solution by cells which are devoid of walls.
Entomogenous microorganisms:	Those which attack insects by growth within their bodies.
Enzyme:	A protein that catalyses a chemical reaction.
Eukaryotic:	The highly differentiated cell which is the unit of structure in animals, plants, protozoa, fungi and algae.
Genome:	The chromosomal complement of an organism.
Gram reaction:	Differential staining procedure used in the identification of bacteria; bacteria either react as Gram positive (resist decolourisation with alcohol) or as Gram negative (decolourised with alcohol).
Halophile:	Microorganism that requires sodium chloride for growth.
Heterokaryon:	A cell in which genetically different haploid nuclei may co-exist and multiply.
High gravity brewing:	Fermentation of wort of very high initial gravity with dilution occurring after distribution.
Inoculants:	The purposeful addition of microorganisms as in the sowing of legume seeds together with the appropriate strain of nitrogen fixing bacterium.
Kilobase:	A chain of nucleotides 1 000 monomers in size (see nucleotides).
Ligase:	An enzyme which catalyzes the joining together of two molecules, as in the joining of DNA molecules.
Lipase:	An enzyme which catalyzes the hydrolysis of lipids such as fats.
Liposome:	An enclosed vesicle formed artificially from phospholipids.
Lysis:	The bursting of a cell with the release of its contents.

Mari-culture on land:	A process which makes use of non-arable land (especially in arid coastal areas) for biomass production, using phytoplanktonic algae and sea water.
Multicopy plasmid:	A plasmid which is present in many copies within a bacterium or other host cell.
Mutagen:	A chemical or physical agent which brings about mutation.
Mutant:	Organisms one or more of whose properties differ from the parent organism from which it was derived by mutation.
Mutation:	An event that alters the genetic materials; used here to denote a change in the sequence or chemistry of the purine or pyrimidine bases contained in DNA molecules.
Mycorrhiza:	“Root fungus”; an association of fungi with the roots of forest trees and other plants which increases the plant’s capacity to absorb nutrients from the soil.
Nuclease:	An enzyme which catalyzes the hydrolysis of nucleic acids.
Nucleotides:	The monomeric units of nucleic acids; they consist of adenine, guanine, cytosine, thymine and uracil to which are attached sugar-phosphate groups.
Oncogenic:	Cancer-causing.
Periplasm:	The region between the plasma membrane and the cell wall.
Phenotype:	The characteristics of an organism that results from the interaction of its genetic constitution with the environment.
Phytopathogen:	An organism pathogenic to plants.
Ploidy:	The number of sets of chromosomes present in an organism or cell.
Polynucleate:	Having more than one nucleus.
Prokaryotic:	The less differentiated cell which is the unit of structure in bacteria.
Promotor:	Region of a genome to which the enzyme RNA polymerase attaches in order to transcribe the structural gene(s).
Protoplast:	A cell which lacks a wall.
Racemic mixture:	An equimolar mixture of D- and L-stereoisomers of a chemical.
Replicon:	A DNA molecule capable of replication.
Reverse transcriptase:	An enzyme that catalyses the synthesis of a single strand of DNA from a messenger RNA molecule, that is the reverse of the normal direction of processing genetic information.
Rheology:	The study of the flow and deformation of matter, such as fermentation liquids.

Ribosome:	A subcellular particle composed of RNA and protein which is concerned with the synthesis of proteins.
Somatic cells:	Cells of an organism other than the germ or sex cells.
Thermophile:	A microorganism that requires high temperature for growth (usually in excess of 50°C).
Tissue culture:	A method for growing cells from animal and plant tissues in vitro.
Titre:	The quantity of a given compound or activity.
Transposon:	A fragment of DNA which can occur in a number of different chromosomal or extrachromosomal positions.
Vector:	An agent of transmission; for example a DNA vector is a self replicating molecule of DNA that transmits genetic information from one cell or organism to another.
Xenobiotic chemical:	Man-made chemical, foreign to the environment.