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MICROBIAL PHYSIOLOGY AND BIOTECHNOLOGICAL INNOVATION IN THE EEC COUNTRIES, PORTUGAL AND SPAIN

(State of the art in microbial physiology as related to biotechnology in the EEC countries, Portugal and Spain)

Deutsche Gesellschaft für chemische Apparatewesen e.V.
Frankfurt/Main

Contributors : G. Gottschalk, W.H. Hamilton, W. Harder, A. de Leeuw, O.M. Neijssel, C. Ratledge

Final report : study contract no. ECI-1000-B-7210-83-D

Division Genetics and Biotechnology

Directorate Biology, Radiation Protection and Medical Research

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COMMISSION OF THE EUROPEAN COMMUNITIES

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SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

1. An analysis was made of the basic scientific and technological disciplines required for biotechnological innovation. This analysis identified Microbial Physiology, which is concerned with the performance of the metabolic machinery of microbial cells, as one of the key disciplines of biotechnology (Chapter I)
2. A survey of the current status of Microbial Physiology in the EEC countries, Portugal and Spain revealed that it is generally healthy (in a number of areas world-leading) in the community as a whole, although it is not evenly distributed among the Member Countries. There is a comprehensive coverage of major physiological groups of organisms with an emphasis on basic studies (Chapter II).Thus a solid platform of knowledge and expertise in Microbial Physiology is available in the EEC countries that can be brought to bear on the development of innovative applications in biotechnology
3. An analysis of current scientific and technological constraints on biotechnological innovation identified several areas of Microbial Physiology where our current level of understanding is insufficient to effectively support the development of novel applications (Chapter III)
4. Hence we recommend that the following areas of Microbial Physiology be further developed (Chapter IV):
    1. Physiology of organisms from diverse (unusual) environments, including those that are able to grow at high temperatures, at high or low pH values and low water activity (extremophiles)

2. Selection of organisms with desired properties including stability and expression of (foreign) genetic elements
3. Post-translational modification of proteins, including selective import into cellular compartments and excretion
4. Micro-environmental regulation of the cell cycle and metabolic fluxes with a view on improving product formation
5. Interactions in, stability and performance of consortia, including biofilms and microbe-plant interactions
6. Physiology of microorganisms in immobilized systems
7. Performance and function of production organisms under simulated process conditions
8. Performance and function of organisms in relation to environmental problems

5. The efforts of each individual country in Europe are not adequate to ascertain a sufficient level of competence. In view of the developments in the USA and Japan, we recommend that a coordinated effort in Europe is undertaken to stimulate both basic and applied research in selected areas of Microbial Physiology so as to successfully meet current and future world-wide competition

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PREFACE

In recent years dramatic new developments in the ability to manipulate biological systems and parts thereof have sparked unprecedented interest in the industrial uses of living organisms. This has led to an impressive number of documents on biotechnology and, when invited to prepare a state of the art report on Microbial Physiology as related to Biotechnology for the EEC. we were initially rather reluctant to add yet another document. However, most reports that have appeared so far, while recognizing the importance of Microbial Physiology for furthering Biotechnological Innovation, have not attempted to substantiate this in a comprehensive manner. Thus we felt that a balanced and critical analysis of Microbial Physiology in the context of Biotechnological Innovation was both timely and useful and we have accordingly endeavoured to analyze the current state of the art of Microbial Physiology in the EEC countries, Portugal and Spain in a world context.

We have also attempted to disclose current problems in the industrial application of biological systems that might be solved through a better understanding of the science of Microbial Physiology. Unfortunately the available information was far from complete, so that this analysis must not be considered fully comprehensive. Because of this, it has been our intention to uncover general areas of Microbial Physiology that are in need of further development in order to adequately stimulate large scale industrial development, production and use of microbial cells and their products. Rather than becoming unduly specific, we have tried to emphasize what we consider to be important trends and issues and this has led us to formulate a number of recommendations.

This report focusses on the significance of Microbial Physiology for Biotechnological Processes and Biotechnological Innovation (Chapter I). We have attempted to evaluate the current status of Microbial Physiology in Europe (EEC countries, Portugal and Spain) in comparison to other major centres in the world (Chapter II), draw attention to possible scientific and technological constraints that may hamper the development of innovative Biotechnological Processes and indicate how these may be overcome (Chapter III). This has formed the basis for the recommendations in which we indicate those areas of Microbial Physiology that are in need of immediate stimulation (Chapter VI).

Although this report has been discussed and is supported by the members of the Preparatory Committee of the EFB Working Party Microbial Physiology (annex 1), it expresses the opinion of its authors and is their sole responsibility. We hope that it will stimulate both our colleague Microbial Physiologists, other scientists and non-scientists and will lead to an appreciation of the important role that Microbial Physiology has to play in Biotechnological Innovation.

We wish to acknowledge the useful discussions that we had with many colleagues throughout the EEC Member Countries, the help from DECHEMA and the assistance of Mrs Marry Pras.

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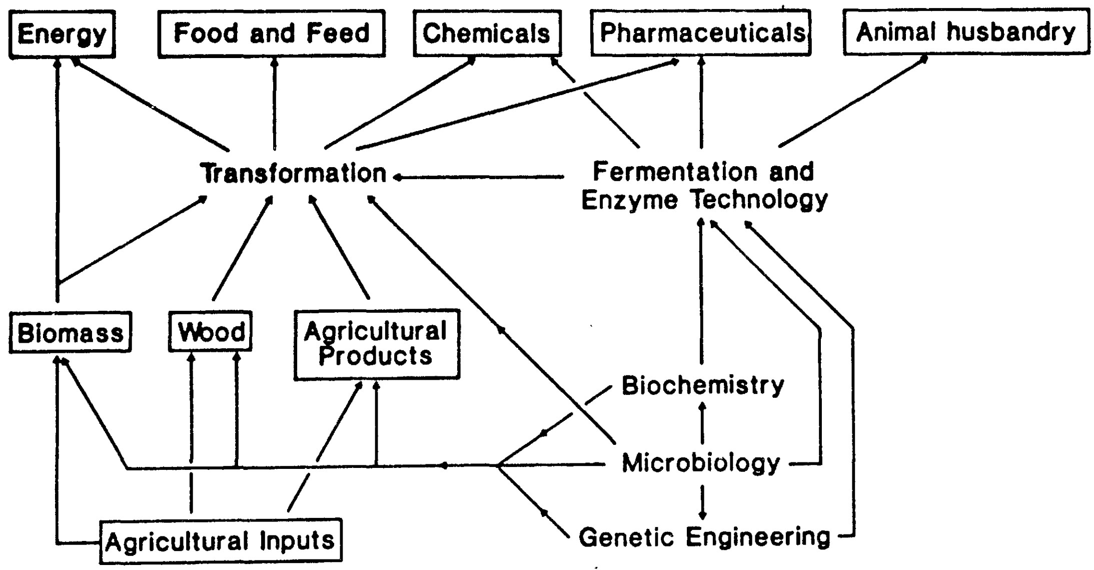
CHAPTER I INTRODUCTION: SIGNIFICANCE OF MICROBIAL PHYSIOLOGY FOR BIOTECHNOLOGICAL PROCESSES AND BIOTECHNOLOGICAL INNOVATION

1. Scope of Biotechnology

In the past decade dramatic new developments in the ability to select and manipulate organisms and their genetic material have led to an unprecedented interest in the industrial uses of these organisms or parts thereof. This new activity, collectively called biotechnology, draws upon the advances in a number of biological sciences including biochemistry, genetics, microbiology, physiology, ecology, biophysics etc. The practical application of these sciences, which, in addition to being critically dependent upon the interdisciplinary interaction between the various biological sciences, also requires input from various technological sciences, is generally considered to become increasingly fundamental to the ability of man to control his environment and to promote health care, bio-industries and agriculture (Fig. 1). It has been suggested (1) that '... the biotechnology which is relevant to the removal of current scientific and technical barriers to the promotion of health and the development of industry and agriculture be designated as new biotechnology' and this interpretation is used in this report.

fig.1 BIOTECHNOLOGY: A MULTIDISCIPLINAIRY APPROACH TO THE DOMESTICATION OF LIVING ORGANISMS (adapted from 1)



The importance of this new biotechnology has been stressed in numerous reports of various organizations and working parties (see for instance 1,2,3,4,5) and in these the view is generally expressed that many significant achievements may be expected to occur before the end of this century. Of the advances in sight which are of immediate relevance in the context of the present report, the following (taken from ref. 1) are considered to be the most important:

- the isolation or construction (through the use of recombinant DNA techniques) of strains of organisms that display new properties or are able to accomplish specific functions essential for the production of food, feeds, pharmaceuticals, chemicals and energy
- the development of new types of bioreactors in which the above organisms can be grown and manipulated so that their desired properties are optimally exploited

From this it appears that the application of biocatalysts as agents of chemical transformation depends critically on the ability to select an appropriate organism (or biocatalyst derived from it) and to optimize its useful properties in relation to the environment in which it is required to function. In the past the development of industrial processes in which biocatalysts were to be used has mainly been based on empirical knowledge. But in recent years it is becoming increasingly clear that the ultimate success of a biotechnological process is critically dependent upon advances made in the fundamental sciences which underpin it; there is ample evidence to show that short cuts, empiricism and superficial attention to basic scientific principles will lead, at best, to poor process performance, at worst, to expensive failures. At present, the majority of biocatalysts as agents of chemical transformation are microorganisms and microbial enzymes. Consequently, knowledge of the properties of microbes and the way in which they change in relation to changes in the (growth) environment in which they occur (which is precisely the business of microbial physiology) is fundamental to the current and future performance of biotechnology.

2. Microbiology and Biotechnology

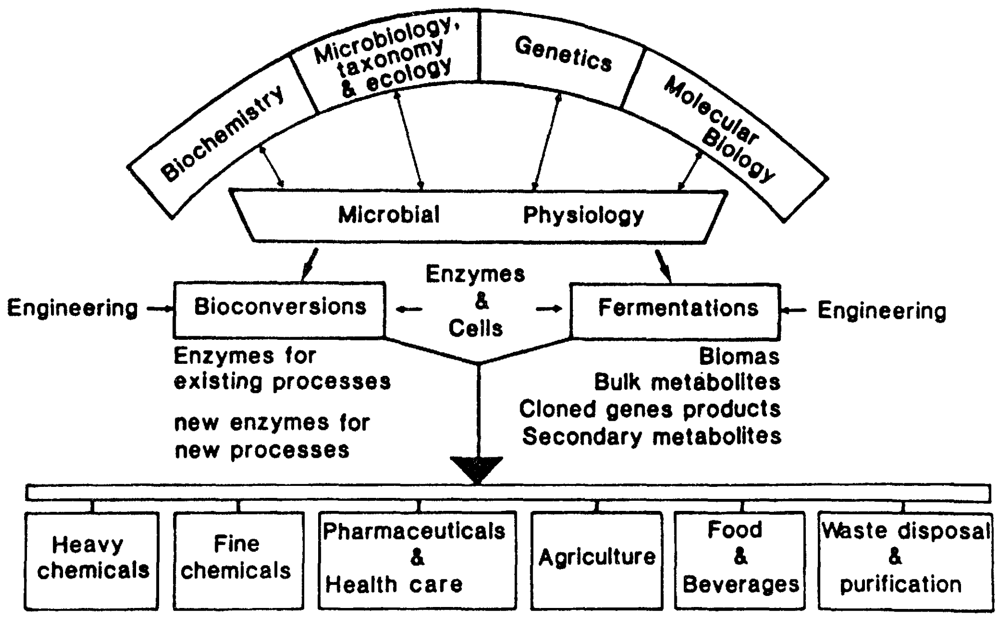
Microbiology is, by definition, the study of microorganisms. It is important to recognize, that as a group, microorganisms are very heterogeneous and are similar only in the fact that they are invisible to the unaided human eye. Compared with the vast number of known species (and the possibly even much greater number that have not yet been explored), the number of organisms that have been used commercially is extremely small. Although the situation is slowly changing due to the demands for organisms with particular biocatalytic properties, there is a general lack of awareness among applied scientists and technologists of the tremendous metabolic potential of microbes. Yet, exploration of the richness of microbial types and activities in the past decades has adequately demonstrated the importance of continued efforts in this area for biotechnological innovation. This necessitates the availability of competent general microbiologists who are able to critically examine and observe phenomena that occur in natural ecosystems and have expertise in isolating the organisms in pure culture. In addition, active participation of microbial physiologists, biochemists and geneticists is required to further exploit the potential of both known and newly discovered organisms. In this way the activities of special microbial genotypes that exist in nature may be brought to bear upon a particular biotechnological problem. If necessary the possibility now exists to purposely change the selected genotype by genetic engineering. Again a multidisciplinary approach is required to fully exploit this commercially.

Exploitation of the way in which microbes of a given genetic constitution change their metabolic potential in response to changes in their environment is probably equally important as the exploration of natural environments for novel organisms. This notion has come from physiological studies of known organisms that have been conducted during the last decades. These studies have shown that a microbial cell is not simply a bag of enzymes encoded by DNA and surrounded by a membrane, but that microbes possess the potential to adapt, functionally and structurally, to changes in their environment. This latter capacity is uniquely associated with the life-style of microbes; they have a limited ability to control their environment and generally respond to environmental change by changing themselves. Thus most microbes potentially possess an enormous phenotypic variability that confers great versatility. This variability may involve merely one or more quantitative changes in some cellular components or radical qualitative changes in cell structure or function. Consequently, an organism of a given genotype is very much a product of its environment, so much so that '... it is virtually meaningless to speak of the chemical composition of a microorganism without at the same time specifying the environmental conditions that produced it' (6). The same applies to the functional properties of microorganisms (7). The potential of this phenotypical change and its importance for biotechnological processes is considered in the next section.

3. Microbial Physiology and Biotechnology

Microbial physiology is concerned with the study of the interaction between an organism and its environment and more particularly with attempts to unravel the relationship between metabolic capability and changes in the environment in which the organism exists, either in a growing or non-growing state. Its central position in biotechnology is depicted in Fig. 2. As indicated in the previous section, the ability of microorganisms to modulate their cell structure, chemistry and function is remarkable and this phenotypic variability has already been exploited commercially. It is generally expected that further exploration of this phenotypic variability will lead to a much wider application in biotechnological processes. In recent years enormous investments have been made in seeking to understand and manipulate the microbial genotype in order to develop new products and processes (8).

fig. 2 CENTRAL POSITION OF MICROBIAL PHYSOLOGY TO BASIC SCIENCES AND BIOTECHNOLOGY



In contrast, studies of phenotypic variability have been comparatively neglected. This may cause delay in the full exploitation of genetically engineered organisms in industrial processes because their use is probably not as straightforward as is commonly thought.

There are three major areas where Microbial Physiology may be expected to contribute significantly to the further development of biotechnology. These are a) exploitation of phenotypic variability; b) exploration of the performance of organisms during large scale production processes and c) application of genetically engineered organisms in industrial processes. These aspects will be briefly considered below.

a) The vast majority of current processes in biotechnology employ batch or fed-batch culture conditions. In most cases these processes have been developed without detailed knowledge of the physiology of the organisms involved. One reason for this is that it is difficult to analyse the continuously changing physiological states of the organisms in such culture systems and therefore optimization of such processes has often been based on empirical knowledge.

Most of our current fundamental knowledge on the effect of culture conditions on the properties and performance of microbial cells has come from continuous culture studies. This cultivation technique enables an analysis of physiological states of organisms under steady state conditions, but can also be used to investigate microbial behaviour in transient states. In the past continuous cultures have been used in the development of batch and fed-batch production processes, both for medium optimization and for improving process performance through an understanding of the effect of growth conditions on organism behaviour. The success of this approach has been extremely encouraging and an increased use of continuous flow systems to understand and explore phenotypic variability of microbes is strongly recommended. This type of work is also necessary for the development and use of on-line analytical methodology which is required for adequate process control.

Continuous flow systems thus are indispensable research tools for biotechnological innovation. But they also possess enormous potential for specific production processes. Because of the fact that the environmental conditions that prevail in a continuous flow system are quite different from those in batch culture, microorganisms growing in continuous culture generally express properties that they do not elaborate in batch culture, so that new and unexpected features of microbial behaviour may be exploited. Examples are metabolite overproduction, overproduction of enzymes and vaccines and manipulation of metabolic fluxes by intelligent use of mixed substrate conditions. Further exploration of this phenotypic variability may lead to the development of new continuous processes, particularly on a small or medium size scale.

It is expected that continuous flow processes will also find large scale application in specific cases, in particular for the production of biomass, endproducts of anaerobic energy metabolism such as ethanol, and certain enzymes. Here the higher productivity of continuous processes is of advantage. However, due to the highly competitive environment that continuous flow system impose upon organisms, the technique cannot always be used and continued work on batch and fed-batch systems is necessary for the development of new biotechnological processes. The current renewed interest in the behaviour of microbes exposed to environmental constraints extant in fed-batch cultures may lead to rewarding new insights.

b. Large scale cultivation of microorganisms invariably suffers from problems of culture inhomogeneity. Basically three different types of culture inhomogeneity can be distinguished, namely: organism heterogeneity, genetic heterogeneity and environmental inhomogeneity. The first is due to the fact that cells in growing cultures are in different phases of cell duplication and since it is know that their physiological properties are different in the different phases of the cell cycle, it is important to develop methods to maintain most of the cells in the proper phase. Genetic heterogeneity is due to spontaneous mutation. This may not be too much of a problem in certain processes but can become quite serious in processes that employ genetically engineered organims. Studies of strain stability under simulated process conditions is therefore of great importance and for this continuous flow methods are most appropriate. These techniques can also be used to investigate the effect of environmental inhomogeneity which is due to the fact that the physico-chemical properties of the culture medium in a large scale culture differ from place to place. Thus microbes placed under such conditions are continuously exposed to changes in their environment and this we know profoundly affects their performance. Continuous culture studies specifically aimed at analyzing microbial response to environmental constraints typical of large scale production processes, although small in number, have shown the dramatic effect of environmental inhomogeneity. Generally these effects reduce process performance. Clearly a better understanding of the nature of these effects will lead the way to improved productivity through either physiological or technological means.

c) There can be no doubt that genetics has made a substantial contribution to existing biotechnology and the emergence of *in vivo* and *in vitro* recombinant DNA technology has added new dimensions to this genetic capability. However, one of the major problems in the use of genetically engineered organisms in industrial processes is that they not always express their newly acquired genetic information as expected. An example comes from work carried out by ICI Ltd where a gene for an energy conserving nitrogen assimilation pathway taken from *E. coli* was transferred into their SCP organism *Methylophilus methylotrophus* strain AS1 where it substituted for a functionally similar but energy consuming pathway originally present. Although in laboratory experiments this led to a significant improvement in yield, the constructed organism was not stably maintained under large scale process conditions. The basis for this instability is probably the genetic inhomogeneity of the population and the selection of a specific genotype better adapted to large scale cultivation conditions. This example illustrates the requirement for a rigorous analysis of the performance of recombinant organisms under simulated process conditions. Advances made in our knowledge of the environmentally controlled expression of constructed genotypes may well be of crucial importance for the industrial application of recombinant organisms.

The advances in molecular biology, genetics and biochemistry made in recent years must be integrated to further our understanding of the behaviour of intact microbial cells when they are placed under different conditions in their growth environments (Fig. 2). This is now recognized of being of crucial importance because this aspect of the physiology of microbes largely determines the performance of biotechnological processes. However, the comparative neglect of Microbial Physiology in recent years has led to a global shortage of well-trained microbial physiologists. There is little doubt in our minds that this will create a bottleneck in biotechnological innovation, particularly in North America (this is considered in the recent OTA report - ref. 8 - which perhaps paints a too optimistic picture of the role of recombinant DNA techniques in the development of new products), but also to a lesser extent in Japan and Europe. This is a serious problem that can only be solved by adequate training programmes. Increased support for basic research in microbial physiology also appears to be essential to overcome the current trend of overemphasising the role of recombinant DNA technology for biotechnological innovation.

CHAPTER II CURRENT STATUS OF MICROBIAL PHYSIOLOGY IN THE EEC COUNTRIES, PORTUGAL AND SPAIN IN A WORLD CONTEXT

1. Introduction

Scientists from the European countries have played a leading role in the early development of Microbiology. The initial discovery of the "kleyne diertgens" was made in the Netherlands by Antonie van Leeuwenhoek, while two centuries later Pasteur in France and Koch in Germany established the causal relationship between microbial activities and chemical changes in their environment. Subsequent work by Beyerinck and Winogradski led to the discovery of the many metabolic types among microbes, while in the first half of the present century Kluyver and several of his European colleagues initiated studies on the physiology and biochemistry of the newly isolated organisms. These latter studies were instrumental in paving the way for the development of modern biochemistry and molecular biology, but also laid a firm basis for the development of Microbial Physiology as a special branch of microbiology. The recent world-wide interest in molecular biology and recombinant DNA technology has had the disadvantage that it depleted resources for research in Microbial Physiology. This is particularly evident in the USA and Canada, but also in Europe the effects are being felt. Because of the differences in funding, the present efforts in Europe are not as heavily affected as in North America and the current level and quality of research in Microbial Physiology in Europe does provide a reasonably firm basis upon which biotechnological innovation can be built. This relative advantage should be exploited by selective stimulation through appropriate funding. Japan also has an established tradition and strong ongoing interest in Microbial Physiology and this country must be considered as Europe's main competitor in the field.

Most of the research programmes in Microbial Physiology in Institutes and University Departments throughout the EEC countries are deliberately designed to be basic (fundamental) in nature. There is almost unanimous agreement that this continued emphasis on fundamental aspects is most effective in stimulating biotechnological innovation, particularly since the 'ivory tower syndrome' that was characteristic of much of the European Academic research of the recent past has now been replaced by an increasing awareness among Academic research workers that the results of their fundamental studies may be important for practical applications. At the same time they show an increasing interest in fundamental aspects of problems of an applied nature. On the other hand Industry realizes more and more that it is impossible to do all the fundamental research necessary for the development of new processes or the improvement of processes under her own steam. The recent support given by the National Governments of a number of EEC countries for innovative research in biotechnology serves as a powerful catalyst in improving the relationship between Academic and Industrial research in Microbial Physiology.

2. Current Status

In an attempt to update the existing information on the nature and extent of research in the area of Microbial Physiology in the EEC countries, Portugal and Spain, a questionnaire (annex 2) was sent to University and Polytechnic Departments and Government-financed Institutes in the various countries. It was decided that there was little point in contacting the Biotechnological Industries within the EEC, because it was felt that this would not yield pertinent information on research topics or number of scientists involved.

For some countries the return was very satisfactory, while for other countries, notably Belgium, Denmark, Greece, Luxemburg and Portugal the response was rather disappointing. Although this may indicate that there is hardly any microbial physiology in some of these countries, we know from other sources that there is certainly significant activity in both Belgium and Denmark. The reason for the inadequate response from these two countries is unclear to us.

The information received was analyzed as follows. Since it was our intention to obtain quantitative information on the number of research groups within Academic Departments and Government-financed Institutes and the research areas in which they are currently involved, the work with important physiological groups of organisms was grouped (Table I). In addition research on a variety of topics of importance for biotechnological innovation was taken together and tabulated (Table II). The tables present the information on a per country basis. Activities in Microbial Physiology in Departments associated with Medical Schools are not considered in this study, although the effort is comparatively large and the contribution to the topics considered in this report is significant.

The tables show that there is a strong commitment to research in Microbial Physiology in Europe as a whole. However, the effort is not evenly distributed over the various nations. The research activities in the Federal Republic of Germany, France, Italy, the Netherlands and the United Kingdom cover most groups of organisms and topics. Of these

Table I. Major Institutes (I) and Research Units (RU) involved in the study of the physiology of a particular group of organisms in the EEC countries, Portugal and Spain. The average size of a research unit is 2-3 academic staff + 1-2 technicians

	Belgium		Denmark		Rep. of Ireland		FRG		France		Greece		Italy		Lux.		Neth.		Port.		Spain		UK		Totals	
Physiological group	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU	I⁺	RU
1. Fermentative anaerobes	-	-	2	4	4	8	4	11	2	4	1	2	1	3	-	-	6	7	-	-	-	-	23	28*	43	67
2. Sulphate reducers and methanogens	2	2	2	3	2	5	8	17	4	6	-	-	1	1	-	-	4	6	-	-	-	-	13	15	36	55
3. Phototrophic bacteria (+ cyanobacteria)	-	-	1	1	-	-	5	8	2	3	-	-	1	3	-	-	3	4	-	-	1	2	9	10	22	31
4. Chemolithotrophs and methylotrophs	-	-	-	-	-	-	6	11	2	2	-	-	1	4	-	-	3	4	-	-	-	-	9	12	21	33
5. Extremophiles	-	-	-	-	-	-	3	7	-	-	-	-	2	3	-	-	1	1	-	-	2	4	9	12*	17	27
6. Yeasts	2	2	4	[illegible]	4	6	7	10	5	7	1	1	4	10	-	-	5	6	1	3	4	15	26	31	63	97
7. Filamentous fungi	-	-	3	6	1	1	4	5	3	3	-	-	1	1	-	-	5	5	-	-	2	12	23	36	42	69
8. Actinomycetes	-	-	1	1	-	-	4	8	2	2	-	-	-	-	-	-	-	-	-	-	1	3	14	20	22	34
9. Protozoa + eukaryotic algae	-	-	1	1	-	-	2	4	-	-	-	-	1	1	-	-	1	1	-	-	2	4	12	20	19	31
10. Aerobic degradative bacteria	1	1	3	3	3	6	16	30	6	10	1	1	2	11	-	-	1	2	-	-	-	-	7	10*	40	74

⁺ Research Institutes and University Departments

* Excludes Central Public Health Laboratory and National Institute for Medical Research which did not specify allocations to individual projects but have, between them, 193 permanent and temporary staff involved in projects associated with microbial physiology

- The figures are approximations because not all Institutes indicated the group of organisms studied
- Although the data are not exhaustive for all countries, for Europe as a whole more than 80% of the field is covered

Table II. Major Institutes (I) and Research Units (RU) involved in the study of microbial physiology related to specific topics of importance to biotechnology in the EEC countries, Portugal and Spain. The average size of a research unit is 2-3 academic staff + 1-2 technicians

Topic	Belgium		Denmark		Rep. of Ireland		FRG		France		Greece		Italy		Lux.		Neth.		Port.		Spain		UK		Totals	
	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU	I+	RU
1. Walls and membranes	1	1	1	1	2	3	8	19	7	8	1	1	-	-	-	-	8	17	-	-	4	21	35	47*	67	118
2. Export of metabolites and biopolymers	-	-	1	1	4	9	6	10	8	10	-	-	2	2	-	-	3	3	-	-	2	5	21	25	47	65
3. Secondary metabolism and antibiotics	1	1	2	6	-	-	8	15	2	2	-	-	3	17	-	-	1	1	-	-	2	7	11	15	30	64
4. Biodegradation, waste treatment and xenobiotics	3	3	4	6	1	1	5	8	10	19	-	-	1	2	-	-	14	18	-	-	1	1	34	48	73	106
5. Biodeterioration	-	-	1	1	-	-	4	5	1	4	-	-	1	3	-	-	1	1	-	-	-	-	8	9	16	23
6. Metal leaching and accumulation	-	-	-	-	-	-	2	4	-	-	-	-	-	-	-	-	1	1	-	-	-	-	12	12	15	17
7. N_2 fixation and denitrification	1	1	2	2	1	3	8	12	2	4	-	-	2	5	-	-	5	10	-	-	5	10	17	30	43	77
8. Immobilized biocatalysts	2	3	2	4	-	-	3	4	2	4	-	-	2	4	-	-	4	5	-	-	-	-	6	8	21	32
9. Regulation of microbial metabolism and product formation	2	8	4	5	4	6	18	27	6	6	1	1	5	19	-	-	11	18	1	2	7	18	23	32	82	142
10. Photosynthesis	-	-	1	1	-	-	5	7	2	2	-	-	2	2	-	-	2	3	-	-	4	6	6	8	22	29
11. Process development	-	-	3	4	1	2	8	14	2	2	1	1	2	3	-	-	8	12	-	-	1	2	9	33	35	73
12. Toxins, antigens, vaccins etc.	1	2	1	1	-	-	2	4	2	5	1	1	-	-	-	-	3	6	-	-	-	-	17	19*	27	38
13. Stability and expression of foreign genetic elements	-	-	5	6	4	11	21	37	4	6	-	-	5	24	-	-	2	5	-	-	1	2	15	30*	57	121
14. Cell cycle, differentiation, morphogenesis and sporulation	-	-	2	4	2	3	6	11	1	1	1	1	1	2	-	-	5	8	-	-	-	-	10	22*	28	52
15. Interactions in mixed cultures and biofilms	1	1	3	6	1	3	4	6	1	2	-	-	1	2	-	-	3	3	-	-	-	-	20	26	34	49
16. Others	-	-	-	-	2	8	-	-	4	7	-	-	-	-	-	-	6	8	1	1	4	7	28	31*	45	62

+, * : see Table I

countries, the UK and the Federal Republic of Germany are particularly prominent. In the UK approximately half of the activity in University Departments and Polytechnics is carried out in major groups which have a significant impact in the field; these groups are predominantly in the University sector. The remaining half of the research effort is widely spread throughout the system. Within the Polytechnics there is generally a greater commitment to working on applied problems, often in direct collaboration with industry. There is also a major concentration of microbial physiologists in UK Government Research Institutes (approx. 450 scientific staff). Although all areas of Microbial Physiology are being actively pursued in the UK, particular areas of strength are walls and membranes, biodegradation, waste treatment and xenobiotics, N_2-fixation and denitrification, regulation of microbial metabolism and product formation, process development, stability and expression of foreign genetic elements, cell cycli, differentiation, morphogenesis and sporulation and mixed population studies (Table II). Also the various groups of organisms of potential importance for biotechnology are adequately covered (Table I). There is particular strength in physiological studies of anaerobes, yeasts and filamentous fungi and actinomycetes.

In the Federal Republic of Germany during the last decades several new Departments of Microbiology were founded at various Universities. This brought about a strengthening of research activities, particularly in Microbial Physiology. In addition a number of Government-financed Research Institutes, some of which have a specific involvement in biotechnology, have a strong commitment to research in Microbial Physiology. The current research efforts cover almost all major physiological groups of organisms (Table I) and many physiological processes (Table II). Research on anaerobes and anaerobic processes, chemolithotrophs, yeasts and aerobic degradative bacteria is particularly abundant. Particular topical areas of strength are walls and membranes, secondary metabolism, N_2 fixation and denitrification, regulation of microbial metabolism and product formation, process development and stability and expression of foreign genetic elements.

At the time of writing this report the information available for France was incomplete (only 40% response to our questionnaire). The impression obtained from the respondents and from the 1984 Actions Thématiques Programmées is that most research on Microbial Physiology in France is concentrated in a relatively small number of major centres (located both at Universities and Government-financed Institutes). There is a comprehensive coverage of the physiological groups of organisms (Table I). Areas of particular strength appear to be walls and membranes, export of metabolites and biopolymers, biodegradation, waste treatment and xenobiotics, regulation of microbial metabolism and product formation, toxins and antimicrobial agents and stability and expression of foreign genetic elements (Table II).

Research in Microbial Physiology in Italy is located both in Academic Research groups and in Research Institutes and encompasses most physiological groups of organisms (Table I). There is partiular strength in studies of yeasts and aerobic degradative bacteria. The research covers a variety of topics (Table II), of which secondary metabolism, regulation of microbial metabolism and product formation and stability and expression of foreign genetic elements are of major importance.

According to a recent evaluation by a Government Committee, research in Microbial Physiology in the Netherlands is in a healthy state. Inspection of Table I shows that there is an almost complete coverage of the physiological groups of organisms (a notable exception is work on Actinomycetes), with particular strength in anaerobes and anaerobic processes, chemolithotrophs and methylotrophs, and yeasts and filamentous fungi (Table I). The research covers a variety of topics (Table II) of which walls and membranes, biodegradation, waste treatment and xenobiotics (in particular anaerobic processes), regulation and energetics of microbial metabolism and product formation, and process development are of major significance. In addition to academic research, which is mainly concentrated in 6 University Departments, research in applied microbial physiology of a more process-oriented nature is carried out in various Institutes that are either wholly supported by Industry, by the Government or by both.

Although it is apparent that both Academic Research groups and those in Research Institutes in Belgium, Denmark, The Republic of Ireland and Spain do make significant and valuable contributions to Microbial Physiology, both the extent of coverage of physiological groups of organisms and the efforts in the various topics considered to be of importance for biotechnological innovation is not as comprehensive as in the other countr es ciscussed above. The information that was made available to us indicates that in Belgium research in Microbial Physiology encompasses only a few groups of microorganisms. The emphasis is on biodegradation, immobilized biocatalysts and regulation of microbial metabolism and product formation. In Denmark most of the work is with anaerobic bacteria, yeasts and filamentous fungi and this covers a number of topics of which secondary metabolites, biodegradation, regulation of metabolism and product formation, stability and expression of foreign genetic elements and interactions in mixed cultures and biofilms are of particular importance. In Ireland there is emphasis on the physiology of anaerobes (including Lactobacilli), yeasts and aerobic degradative bacteria, while the following topics receive most attention: export of metabolites and biopolymers, regulation of microbial metabolism and product formation, and stability and expression of foreign genetic elements. Not unexpectedly a considerable amount of activity within both Denmark and Ireland is devoted to agriculture. The research in Spain, as can be deduced from our survey, has a strong emphasis on the physiology of yeasts and filamentous fungi, in particular on walls and membranes. Other areas that are studied include N_2 fixation, denitrification and regulation of microbial metabolism and product formation (including secondary metabolites).

Compared to the countries discussed above, research in Microbial Physiology in Greece and Portugal is relatively under-represented. In Greece the research activities are almost exclusively carried out in Departments that belong to Medical Schools and thus show a strong emphasis on medical aspects. On the basis of the information received for Portugal, it appears that research in Microbial Physiology in the country is concentrated mainly in one Research Institute and has a strong emphasis on yeasts in which in particular metabolic regulation and product formation is studied.

In addition to research in Microbial Physiology in University Departments and (Government) Institutes, a considerable body of research is found in Europe's major oil, chemical and pharmaceutical industries and many of the major breweries and food manufacturers including the dairy industry. Many of these firms have been active in the development of biotechnology; their success in the past decades (particularly in the pharmaceutical and food industries) and their continued health no doubt is due (at least in part) to considerable research and development programmes including those in microbial physiology. There is also significant activity in processing and upgrading of waste materials, and biogas and energy production.

Although there is an apparent strong tradition in Microbial Physiology in most of the above European companies with an active interest in biotechnology, there are probably not more than a hundred or two persons who would consider themselves as microbial physiologists. Moreover, the majority are not engaged in long-term fundamental research. Not unexpectedly in recent years industry in many European countries has turned increasingly outside for expertise for biotechnological innovation. With the advent of genetic engineering and the mushrooming of venture capital genetic engineering firms in the USA, most European companies responded by seeking alliance with or a share in centres of recombinant-DNA know-how in the USA. In the area of Microbial Physiology, however, many of the already existing relationships between Industry and Universities and Institutes in Western Europe were further developed and are expanding. This is due to the fact that Microbial Physiology in the EEC countries commands a relatively strong position in the world. In fact this is so much so that many US-based firms, including those involved in genetic engineering, are now actively trying to persuade European microbial physiologists to come to the US, or attempt to acquire skills and expertise in the field by collaborating with European groups.

3. European Microbial Physiology in a world context

In the preceeding section the view has been expressed that the expertise in Microbial Physiology in the EEC countries, Portugal and

Spain is generally healthy and in a number of areas may be considered as world-leading. An attempt has been made to substantiate this view by comparing the output of scientific papers that contain information of physiological relevance by research groups in the EEC countries and other countries in the world. For this the Excerpta Medica database* was used (Table III). The information contained in this table includes a relatively high percentage of genetically- and biochemically-oriented papers that contain data that are of physiological relevance. The data show that there is a considerable effort in Microbial Physiology in Europe. Table III also confirms the analysis given in the preceeding section of the relative contribution of the various countries within the Community towards research in Microbial Physiology.

In view of this we believe that the existing strength in Microbial Physiology in the EEC countries, when properly organized, for instance through Community action as detailed in (9), forms a solid base from which microbial physiology can contribute to biotechnological innovation. For the proper exploitation of the available expertise, it is desirable that future research efforts in Microbial Physiolgy are tuned to industrial strategies and market opportunities. In this respect the current situation in Japan may serve as an example.

* This database is a retrieval system covering scientific publications in the field of all medical and biological sciences world-wide

Table III. Number of research papers published in scientific journals that contain information that is of relevance for Microbial Physiology published by research groups in various countries

Country	Scientific publications (1980 - 1983)	
	Number	% of total
USA	6506	43.3
EEC + Portugal and Spain	5322	35.2
Japan	1789	11.9
United Kingdom	1677	11.1
Federal Republic of Germany	1395	9.3
France	903	6.0
Canada	758	5.0
Netherlands	510	3.4
Sweden	276	1.8
Switzerland	244	1.6
Italy	230	1.5
Spain	227	1.5
Belgium	176	1.2
Norway	105	0.7
Denmark	91	0.6
Austria	70	0.5
Republic of Ireland	49	0.3
Portugal	21	0.1
Greece	14	0.1
Luxembourg	0	0

CHAPTER III CURRENT SCIENTIFIC AND TECHNOLOGICAL CONSTRAINTS ON BIOTECHNOLOGY THAT MAY BE OVERCOME THROUGH A BETTER UNDERSTANDING OF MICROBIAL PHYSIOLOGY

1. Introduction

Almost all reports on Biotechnology by National Committees and Working Parties (see 1-5) contain recommendations for research and development in one of the disciplines that underpin biotechnology. In most of these a case is made for promoting basic biotechnology which is considered essential for biotechnological innovation in industry. In a recent report (5) the following statement is made with respect to microbiology: "... (Microbial) metabolism is particularly important in regard to its regulation and kinetics as applied to process engineering and control, scale up, and overall productivity. The major contribution to be expected from microbiology lies in the exploration of the full (phenotypic and genotypic) diversity of organisms for the biosynthesis of a wide range of metabolites ..... The application of continuous culture will be necessary for the continuous production and recovery of bulk chemicals". This essentially is a plea for an increased effort in Microbial Physiology and similar compilations of the strategic importance of Microbial Physiology for biotechnological innovation can be found in several other recent reports.

In an attempt to be more specific with respect to the areas of Microbial Physiology which can be expected to be of particular relevance for the future development of biotechnology (Chapter IV), we have tried to identify potential problems in applied areas of biotechnology where our current level of knowledge of Microbial Physiology is considered to constrain the development of innovation processes unduly. This information is based on discussions with representatives of some major Biotechnological Industries in Europe and although it is far from complete, it does indicate in which direction Microbial Physiology must be developed to adequately face current and future challenges.

2. Survey of applied areas and potential problems

Chemical Industry. A distinction is made between the so-called heavy (or bulk) chemical industry and those industries involved in the production of fine chemicals. The reason for this is that the contribution of biotechnology to these two sectors is expected to be different.

With respect to the Heavy Chemical Industry, the impact of bioprocesses and their significance to technological innovation is not considered likely to become important in the next decades. Most processes in this sector are conducted in the gas-phase and run at high temperature and pressure. It is anticipated that biotechnology can only contribute in a very limited number of specific cases, but it may become more important if bioprocesses can be developed that can be run under conditions of high concentrations and high productivity and for this biocatalyst that function at high temperatures and high pressures will be advantageous. The situation is expected to change when this industry becomes more dependent upon renewable feedstocks. For feedstocks likely to be available in the foreseeable future (i.e. starch, straw, wood, etc.), research should be initiated on organisms that can either use them directly or utilize products derived from them, for instance by chemical modification.

In the Fine Chemical Industry one of the main problems is the selection, development and optimization of economically competitive biocatalysts that catalyze a specific chemical reaction or reactions. Furthermore, product recovery and the development of a new generation of bioreactors for heterogeneous catalysis are considered of major importance. Since knowledge of the potential properties of microorganisms and how they are expressed under a variety of process conditions is essential to the further development of bioprocesses for the production of fine chemicals, several areas of Microbial Physiology are of importance. Biotechnological Innovation in this area is dependent upon advances made in basic Microbial Physiology in the areas 1, 2, 4, 6 and 7 of Table V (Chapter IV).

Traditionally, process development in the Fermentation Industry has been critically dependent upon the selection, development and optimization of production organisms. Compared to the number of known species of organisms, the number .hat is currently in industrial use, is surprisingly small. There are several reasons for this, the most important being the lack of knowledge of the physiology, biochemistry and genetics of more unusual organisms and lack of insight in the potential for exploitation of their phenotypic variability. This latter aspect is best studied in continuous flow systems. These techniques are also expected to yield the information that is required to adequately monitor and control product formation. Introduction of continuous flow systems in industrial processes requires that research is undertaken to establish the stability of pure and mixed cultures under such conditions. In addition to these aspects it is anticipated that studies of the growth and behaviour of organisms in multiphase systems may lead to improved processes because of the potential for improved product recovery. In view of this areas 1 through 8 of Table V (Chapter IV) are all considered of importance for innovative applications of biotechnology in the Fermentation Industry.

Much of what has been said above with respect to the Fermentation Industry also applies to Health Care and Pharmaceuticals. In this sector important developments are expected from the use of recombinant DNA organisms, particularly in the production of therapeuticals. It is anticipated that physiological studies of these organisms are required before they can be used on an industrial scale. These studies must address the stability and expression of the foreign genetic elements and also should investigate post-translational modification of proteins, including selective import in cellular compartments (in yeasts) and excretion. In addition to this the development of novel vaccins and toxins (including bio-insecticides), and of diagnostics is expected to rely on continued advances in Microbial Physiology. Thus it is considered that all areas of Table V (1 through 8; Chapter IV) are of importance for future innovation in this area.

In Agriculture current advances made in plant breeding, for which plant tissue culture techniques and recombinant DNA technology are increasingly used, are considered of major importance for future development. The techniques used are similar to those employed in Microbial Physiology and it is expected that specially adapted procedures must be developed in collaboration with microbial physiologists. Further developments in the use of phototrophic bacteria and algae as a source of biomass in certain countries in the EEC is also of importance. Improvement of microbe-plant interactions (in particular Rhizobia) may lead to increased productivity and for this a better understanding of the basic mechanisms involved is imperative. Increased concern about environmental hazards of the widespread application of agrochemicals has lead to the exploration of biological agents for the control of various pests. Use has already been made of the potential of microorganisms for this purpose, but increased efforts are necessary in this area. Finally the projected use of agricultural products (and wastes) as renewable feedstocks for the chemical industry requires that research is initiated for organisms that either effectively use these feedstocks directly or after chemical modification. All these activities require specific research efforts in various areas of Microbial Physiology, in particular areas 1, 2, 4, 5 and 8 of Table V (Chapter IV).

The Foods and Beverages sector has a long-standing tradition in biotechnology. Probably because of this, a major concern of industries in this sector is to maintain the characteristics and quality of existing products. Consequently much effort is devoted to solving problems in current processes (particularly in the dairy industry) through a better understanding of the behaviour and role of microorganisms, and to improve existing bioprocesses. It is expected that this continues to be a main emphasis in the remaining part of this century. Surprisingly little is known of the physiological basis of the performance of microorganisms in many of the bioprocesses currently in use (in fact most of them depend very much on empirical knowledge), so that a rewarding contribution towards improvement of existing processes is expected from studies of the physiology of pertinent organisms (i.e. lactic acid bacteria, brewer's yeast etc.). Industries in this sector are significant users of enzymes of microbial origin and many of the existing processes could be improved if better enzymes would be available. In view of the fact that microorganisms have been and still are a major source of enzymes, efforts should be undertaken to either find better enzymes by exploring the properties of novel organisms or by making them through procedures that have been developed in the study of experimental enzyme evolution or protein engineering. Another area of current and future importance is that of the production and use of (natural) colorants, flavours and fragrances. Advances in our knowledge of specific areas of Microbial Physiology again no doubt will contribute to innovation in this particular field. It is considered that areas 2, 4, 5 and 8 (Table V, Chapter IV) are of particular importance for new developments in this sector.

Current problems in Waste Disposal and Waste Purification include attempts to develop biotechnological methods for the removal of existing pollution of soil and water. In nature, microbes have always been the main agents of the mineralization of dead organic matter, even before the emergence of man. Introduction of synthetic chemicals (xenobiotic compounds) into the environment and their subsequent degradation by microorganisms has made us appreciate the versatility of microbial metabolism and indeed how microbial enzymes change (that is evolve) to handle these novel materials. Most man-made chemicals (but not the plastics) can now be degraded by microorganisms. For specific situations introduction of specially constructed organisms may be necessary, but adverse environmental conditions are probably mainly responsible for most of the existing problems. These may be overcome if the nature of these conditions is more fully appreciated. A special problem is that of pollution of fresh water and drinking water because the concentration of the compounds to be removed is generally exceedingly low. This must be carried out by so-called oligotrophic microorganisms; these have been little studied and their physiology is poorly understood. Improvement of anaerobic waste purification processes is also urgently required. The newly developed systems employ mixed cultures of organisms that have colonized carrier (for instance sand) particles so that the organisms are maintained in the digestor. Little is known about the physiology and performance of the mixed biofilms present on the particles or of the possibilities to incorporate specially constructed organisms into the films for special applications. The areas 1, 2, 5 and 8 (Table 5, Chapter IV) of Microbial Physiology are considered of importance for innovation in this particular field of application.

The above information is summarized in Table IV. As stated earlier, we realize that it is far from complete. However, it was felt that it would be impossible to obtain a much more detailed picture in the time that was available to us. Moreover, we were confident that such a detailed analysis was not likely to give much more information about the areas of Microbial Physiology that are in need of further developments in order to adequately support Biotechnological Innovation in the EEC countries, Portugal and Spain. And this, we considered, was our main task.

Table IV. Current and future focal points in various applied areas of Biotechnology which relate to Microbial Physiology

Applied Field	Examples	Areas of Microbial Physiology considered of importance (see Table V)
Heavy Chemical Industry	Development of bioprocesses that can be conducted under conditions of high concentrations and high productivity (high T and P, preferably in the gasphase) and those that use renewable feedstocks	1, 2, 6, 7
Fine Chemicals	Selection, development and optimization of specific biocatalysts for use in heterogenous bioreactor systems	1, 2, 4, 6, 7
Fermentation Industry	Selection, development and optimization of production organisms (incl. rec-DNA organisms); stability of pure and mixed cultures in continuous systems; performance of organisms in multiphase systems; product recovery	1, 2, 3, 4, 5, 7, 8
Pharmaceuticals and Health Care	See Fermentation Industry; development of therapeuticals, diagnostics and vaccines	1, 2, 3, 4, 5, 6, 7, 8
Agriculture	Development of biological agents for the control of pests; new processes for upgrading agricultural products and agricultural wastes	
Foods and Beverages	Improvement of existing bioprocesses, availability of improved enzymes; production of (natural) flavours	2, 4, 5, 6, 7
Waste-Disposal and Purification	Development of processes for the removal of existing pollution (soil, water); improvement of anaerobic waste purification	1, 2, 5, 8

CHAPTER IV IMPORTANT AREAS OF MICROBIAL PHYSIOLOGY THAT MUST BE DEVELOPED TO STIMULATE BIOTECHNOLOGICAL INNOVATION

In the previous chapter an attempt has been made to discern current (and future) scientific and technological constraints in applied areas of biotechnology. Its purpose was to identify a possible role (if any) for Microbial Physiology to help overcome existing (and future) problems and this has led to the construction of Table V. A total of eight areas of Microbial Physiology emerged from this exercise. Interestingly, the answers received for questions 7 and 8 of our questionnaire (annex 2) identified precisely the same areas as being of particular importance both for current and for future innovative development of biotechnology in Europe.

Earlier in this report (Chapter II-3) we have advocated increased use of continuous culture techniques as a research tool not only because it enables full exploration of the phenotypic variability of microorganisms but also with a view of increasing productivity of certain industrial biotechnological processes. Increased attention should be given to an analysis of non-steady state (transient state) conditions. In fact, the majority of the areas of Table V can only be properly researched by using continuous-flow methods and **we therefore recommend a stimulation of the use of continuous culture techniques in studies of Microbial Physiology.**

When considering the eight areas of Microbial Physiology that are thought to be of importance (Table V) in the context of the information contained in Tables I and II in which the current effort in the EEC countries is given, areas that are in need of stimulation emerge. These are briefly considered below:

Table V. Areas of Microbial Physiology considered of importance for Biotechnological Innovation; no order of priority given

1. Physiology of organisms from diverse (unusual) environments, including those that are able to grow at high temperatures, at high or low pH values and low water activity (extremophiles)
2. Selection of organisms with desired properties including stability and expression of (foreign) genetic elements
3. Post-translational modification of proteins, including selective import into cellular compartments and excretion
4. Micro-environmental regulation of the cell cycle and metabolic fluxes with a view on improving product formation
5. Interactions in, stability and performance of consortia, including biofilms and microbe-plant interactions
6. Physiology of microorganisms in immobilized systems
7. Performance and function of production organisms under simulated process conditions
8. Performance and function of organisms in relation to environmental problems

1. There appears to be a considerable interest in the EEC countries in studies of the physiology of unusual organisms from diverse environments, in particular of anaerobes. Many of these have properties of considerable potential importance to biotechnology and hence further exploration of the richness of microbial types and activities appears to be a great value. This may lead to novel applications such as desulfurization of coal, utilization of producer gas, production of chiral compounds and the development of fermentations at high temperatures under non-sterile conditions. We recommend a continued effort in this area of exploration of microbial types and activities. With respect to the so-called extremophiles, comparatively little work is currently being done in the EEC countries and in view of their potential importance (particularly with respect to the thermophiles) it is recommended that this particular area of research is stimulated.

2. Selection of organisms having desirable properties for biotechnological processes is hardly studied outside industrial laboratories. In view of the importance of this, and of that of in vivo enzyme modification by appropriate selective pressure, it is recommended to stimulate research in this area. Although there already is a significant research effort on the stability and expression of foreign genetic elements, the importance of this for the application of recombinant DNA organisms in biotechnological processes is such that this type of research should also be stimulated.

3. Post-translational modification of proteins and their selective import into cellular compartments of eukaryotes or selective excretion in prokaryotes is of crucial importance for the future production of polypeptides and proteins of pharmaceutical, medical or veterinary importance. Research in this area in the EEC countries is worthy of

significant stimulation. particularly since the existing strength in this field may serve as a platform for the discovery of new principles which may lead to the development of innovative processes.

4. An understanding of microbial metabolism, in all its aspects, is fundamental to the development of biotechnological processes. Major improvements in product formation may be achieved by increased effort in this area and it is recommended that further research efforts be made into the micro-environmental control of metabolic fluxes by the growth environment. This will include study of the uptake of nutrients into the cell, the various transformations of those nutrients, the formation of macromolecules and, when appropriate, the transport of materials out of the cell. In many cases, these studies will be best carried out with cells growing in steady state conditions and the increased use of continuous cultures is therefore advocated. However, some biotechnology products arise by virtue of changes in metabolic fluxes and in these cases the study of metabolic fluxes in non-steady (transient) states will need to be undertaken. Only then can the full potential of microbial phenotypic variability be fully exploited. This work should also include investigations on relatively little studied groups of organisms, such as the chemolithotrophs, methylotrophs and fermentative anaerobes and should focus on the use of novel feedstocks and renewable carbon sources.

5. In various areas of applied biotechnology there is an increasing interest in the use of microbial consortia, particularly with respect to anaerobes. These generally have both increased metabolic potential and increased stability so that in specific applications non-sterile conditions may be used. Consortia are also of great importance in bio-films for which there is an enormous potential for instance in waste

treatment. Research in this area should be strongly stimulated because the current efforts are insufficient. Similar arguments can be advanced for increased research on microbe-plant interactions.

6. Continued performance of immobilized cells critically depends on changes in their physiological properties with time. Very little is known about this particular aspect and in view of the projected importance of the use of immobilized cells in future biotechnological processes, it is recommended that research in this area is stimulated.

7. In order to adequately support the new biotechnological activities, fermentations should be controlled and reproducible processes. The application of newly developed analytical techniques for the study of the heterogeneity of cell populations, their segregated macromolecular composition and biochemical activities should be fostered. This may also yield more effective monitoring systems for fermentation process control. Besides, microorganisms growing in large fermenters are continuously exposed to gradients of essential nutrients (i.e. oxygen, carbon source etc.) and it is known that these changing conditions profoundly affect culture performance and stability. Very little is known about the basic physiology of microbes growing under such conditions and in view of its importance it is recommended that research is undertaken with respect to the performance, function and stability of production organisms under simulated process conditions.

8. Waste management, including sewage treatment, is by far the largest application of biotechnology in tonnage terms. Much attention is currently placed on anaerobic treatment processes in which Europe plays a leading role in the world. For the further development of these processes. the potential of microbes for anaerobic degradation of both natural and unnatural compounds must be further explored. In particular emphasis should be given to the purification of specific indu-

strial waste-water streams for which new organisms must be either isolated or constructed and their physiology studied. Biotechnological processes must be developed for the cleaning up of heavily polluted dump sites and this also requires further knowledge of the metabolic potential of microbes, in particular of microbial consortia, and their effectiveness.

Stimulation of the above areas of microbial physiology with the view of advancing the basic research upon which competitive and innovative applications of biotechnology within the Community can be based should go hand in hand with attempts to promote closer relations between Academic and (Government) Research Institutes on the one hand and Industry on the other. Within the Community both aspects can be effectively administrated by CUBE (Concertation Unit for Biotechnology in Europe - ref. 9), while the scientific platform for stimulation and concertation of all aspects of Microbial Physiology as it relates to Biotechnology can be provided by the EFB Working Party Microbial Physiology.

CONCLUSIONS

In the recent proposal for a research action programme in Biotechnology in the Community (9), the need for a new initiative to '... create conditions for competitive and innovative applications of biotechnology within the Community, and to advance the basic and pre-competitive research on which such innovations must be founded' has been put forward. In the context of the present report, the priority action on Research and Training in Biotechnology is of immediate importance. It consists of two subprogrammes which deal with 1) the establishment of a supportive infrastructure for biotechnological research in Europe and 2) the elimination, through research and training, of bottlenecks which prevent the exploitation by industry and agriculture of the materials and methods originating from modern biology.

Microbial Physiology is generally considered to be one of the cornerstones of new biotechnology (Chapter I). The existing strength in the field within the Community (Chapter II), when adequately supported in a number of selected areas (Chapter IV), is expected to be able to contribute by supplying the expertise and know-how upon which tomorrow's biotechnology can be founded. The areas of Microbial Physiology that have been identified in the present report as being in need of stimulation (Table V, Chapter IV), can be considered as subprogrammes of sector 2.2.2.3 entitled "Physiology and genetics of species important to industry and to agriculture" of the research action programme in Biotechnology in the Community (9). This sector identifies 2 broad priority areas for research namely a) Study of poorly understood basic functions in microorganisms (partially chemo-autotrophs, methanotrophs, mycorrhiza) which appear to be promising for future industrial use and b) Analysis of factors covering the yield (productivity) and stability of microorganisms during industrial exploitation or in relation to agriculture.

No European country by itself can be confident that it shall be able to compete against the USA and Japan in developing and applying Microbial Physiology to biotechnology. Therefore, co-ordinated implementation of a programme for the development of Microbial Physiology through research contracts in the areas detailed in Table V. for instance as envisaged in (9), would no doubt significantly stimulate biotechnological innovation, particularly if, as part of the excercise, collaboration between Industry and Academic research groups could be improved for certain parts of the programme that come half way between Academic and Applied research. Transfer of basic knowledge to industry and agriculture should be improved by training, both 'in house' but also by courses, summer schools and workshops. We strongly recommend that the Community also supports this latter type of activity. With regard to activities in Microbial Physiology, in addition to National Societies, the EFB Working Party Microbial Physiology is ready to undertake initiatives to support the Research Action Programme in Biotechnology in the Community.

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Annex 1

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annex 2

Draft letter to Head of Department or Head of a Research Group in an Institute

Dear

We are engaged in writing a report for the European Economic Community on "The current state of the art in microbial physiology in the EEC countries and Portugal and Spain". The objective of this study is to provide:

a) An evaluation, in the world context, of current European strengths in the field
b) An inventory of gaps in our knowledge
c) A discussion of technical improvements which might be sought and problems which might be solved through a better understanding of microbial physiology
d) An inventory of the research in microbial physiology necessary to stimulate large scale industrial development, production and use of microbial cells and their products.

In order to update the existing information on microbial physiology in your country, we invite you to help us by filling in the enclosed questionnaire. In case you wish to convey to us specific views on any of the areas a) through d) please do so so that we can incorporate this in our report. Thank you very much for helping us.

Yours sincerely,

Prof. G. Gottschalk, FRG
Prof. W.A. Hamilton, UK
Prof. W. Harder, Neth.
Dr. A. de Leeuw, Neth.
Dr. O.M. Neijssel, Neth.
Dr. C. Ratledge, UK

annex 2

To be returned to:

Questionnaire EEC Study on Microbial Physiology
(please print or type)

1. Department or Institute:

2. Name and position of respondent:

3. Is your Department (Institute) involved in research in Microbial Physiology? If yes, indicate the areas of research.

4. How many permanent members of staff are involved in the research detailed in 3.?

5. How many post-docs, research students and technicians are involved in the research detailed in 3.?
   post-docs :
   research students :
   technicians :

6. Which of the research topics given in 3. do you consider to be a) of an applied nature; b) basic, but of possible importance for biotechnology?.

7. What areas of microbial physiology do you think are currently of particular importance for the further development of biotechnology?

8. Which areas of microbial physiology do you think must be further developed in order to successfully stimulate biotechnological innovation?

Please return this questionnaire before January 18, 1984. Many thanks for your cooperation.