13th INTERNATIONAL TNO CONFERENCE

Biotechnology
a Hidden Past, a Shining Future

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Ladies and Gentlemen,

It is a pleasure to welcome you to this 13th Conference on behalf of the Board of the Netherlands' Organization for Applied Scientific Research TNO. A special word of welcome to the foreign guests, who are attending this symposium in greater numbers than ever before, and to the speakers who are kind enough to let us have the benefit of their experience. We are particularly grateful to Dr. Ginjaar, Minister of Health and Environmental Protection, for his willingness to address the Conference, the more so because of his former close ties with TNO.
During the past few years the TNO Conference Preparatory Committee has based the selection of subjects on the changing attitude of the Organisation towards the interaction between applied science and technology on the one hand and society on the other. This has led to conference subjects of rather general interest, socially as well as scientifically, subjects that can be approached from many sides and starting from many different points of view. I consider the theme of the present Conference: Biotechnology - a Hidden Past, a Shining Future - as an excellent example of this approach.
Biotechnology has been defined in various ways. The definition that comes closest to my own perception of the subject is the one that will be given by Dr. De Flines in his forthcoming lecture at this Conference, and I quote: "Biotechnology is the science which studies the integrated application of biochemistry, microbiology and process technology on biological systems for their use in industrial processes and environmental protection." - unquote. This definition also includes modern forms of ancient methods applied in producing e.g. wines, beer, cheese, vinegar and many other natural products. For centuries, the methods evolved by experience were handed over from generation to generation; the men operating these processes - or rather: techniques - were craftsmen who practiced their craft without much knowledge of the scientific background but often with surprising skill and high-quality results. It is both the scale on which present-day operations must be carried out and the variety and sophistication of the product spectrum that require a scientific approach, that is: make it necessary to replace the craftsmen by scientists and engineers.
The definition tells us that biotechnology is first and foremost an interdisciplinary science, with component sciences that are rather different in character. Applied biochemistry and microbiology on the one hand and process technology on the other have been practiced for many years, and the question arises why the combination of these sciences has recently attracted so much attention, the more so because they have been jointly practiced for many years. The only valid explanation is, I think, that new developments have occurred in the various component fields of biotechnology which makes the combination more interesting than before. For microbiology and biochemistry this is clearly true: our capacity for analyzing and understanding complex molecular and cellular phenomena has increased tremendously during the past few decades. Analytical instruments can now be combined with ever more sophisticated techniques of data acquisition and data processing. Also, new methods of introducing certain new and hereditary properties into micro-organisms, in other words genetic engineering, add a new dimension of applications to the many existing possibilities. Even these existing possibilities have not yet been fully explored and exploited. The programme of this Conference includes several contributions on this topic. It will also deal with possible side effects of techniques such as genetic engineering which appear to have aroused some, perhaps even wide-spread anxiety. Being some sort of an outsider, I am not going to take sides on this issue, but I feel I should say that I did observe in some con-

tributions traces of yet another public phobia. I sincerely hope that this Conference will contribute to a more balanced approach in which the pros and cons of genetic engineering can be weighed realistically.

Process technology, the other component of biotechnology, starts from classical Newtonian continuum physics. More specifically, it is based on the conservation equations for mass, energy and momentum. Its basis clearly differs from the corpuscular, or rather molecular and cellular approach used by most biochemists and microbiologists. Much progress has been made in the field of process technology in the past 25 years: new devices for chemical and biotechnological conversions have been developed and new materials of construction introduced. Perhaps the most striking change is that static as well as dynamic mathematical models were set up for describing complex equipment, process steps and even entire process systems. This systems engineering approach is not limited to the study of the effects of process conditions on process performance, but it is also very useful in process optimization and for on-line control purposes. The area where most advances are being made is in chemical reaction engineering and the allied field of biochemical reaction engineering. These extensions of our capabilities became possible only after adequate computers and the requisite methods for solving the often complex partial differential equations had become available. Areas in which, in my opinion, more progress is desirable include methods of separation as well as techniques for continuous and on-line measurement of reactant and product concentrations especially adapted to biotechnological systems.

Before discussing the applicational side of biotechnology, I feel I should say a few words about education. Most scientists active in the field under discussion have been educated in one of the component monodisciplines and often have insufficient knowledge of the other fields. I think that retraining programmes for scientists and engineers working in industry are urgently needed; a similar conclusion applies to academic staff. Much attention should also be given to reprogramming universities because otherwise insufficient manpower may become available for the rapid developments expected in biotechnology. It is, in my opinion, most desirable to teach the principles of process technology to biochemists and microbiologists and to establish new programmes for biotechnologists. In this connection, the current shortage of process engineers in the Netherlands and the expected shortage of chemists is certainly going to be a major handicap.

Yet another reason for the increased attention given to biotechnology stems from the awareness that our reserves of fossil fuels are not unlimited. Since the mid-1800s the Western world has come to depend on the easy access to and the unlimited availability of these resources. The high rates at which this accumulated form of solar energy is being depleted and changes in the world's economic relations make it necessary to look for other sources of energy and chemical feedstocks, if we are to maintain our present Western way of life. Eventually, we may have to fall back upon the annual influx of solar energy, not only by converting it directly into other forms of usable energy but also indirectly, by utilizing biomass formed by photosynthesis. The quantity of solar energy converted annually by photosynthesis exceeds the human energy requirements of the year 2000, even though the efficiency of photosynthesis is quite low. It is not unlikely that at some time in the future biomass will become the dominant source not only of food but also of energy and raw materials for organics. Biotechnology is expected by many to play a major role in converting biomass obtained by what is often called raw materials farming and energy farming. The large-scale production of alcohol in Brazil and the USA are early examples of this approach.

However, this solution is not without its problems. First of all, large-scale production of biomass and derived products may well have immense environmental effects. In the case of alcohol production these effects are already beginning to appear, in the form of severe water pollution. Moreover, it is far from certain that bioprocessing to bulk products such as alcohol and ammonia - provided that a suitable new method can be

developed for this product - can compete with present-day chemical processes operating at high temperatures and pressures which show a far superior energy efficiency. Gasification of biomass followed by conventional synthesis of chemicals, e.g. methanol, may well be the better alternative.

A more promising field of application is, I think, the production of sophisticated products by highly selective reactions using immobilized enzymes as catalysts. Since these catalysts show a very high intrinsic activity and operate at or near ambient conditions of temperature and pressure, they are ideally suited for the conversion of raw materials derived from biomass. Similar possibilities are believed to exist in the production of new drugs, vaccines and the like. Processes for this type of product will be small in size but high in sophistication and are the most likely field of rapid progress in the near future. It is in this area that new engineering solutions must be found to optimize conditions as regards mixing and mass and heat transfer.

Application of small-scale bioprocesses for the production of less advanced materials may be feasible in developing countries, even if the products are made elsewhere by large-scale processes from fossil raw materials. Necessary conditions are abundant supplies of cheap biomass and derived feedstocks, and a market that is relatively undeveloped but growing. If national policies are such that local industry is protected from cheaper imports or if transportation costs are relatively high, these small industries in developing countries may have a chance of survival. It is in this area that biotechnology could prove to be beneficial, the more so because bioprocesses may be accepted more readily than the large-scale chemical technology which so clearly bears the mark of the foreign Western culture. If this supposition is correct, biotechnology may help pave the way for a more effective transfer of technology.

Ladies and Gentlemen,

For TNO, the Netherlands' Organization for Applied Scientific Research, biotechnology offers an inspiring challenge as well as actual everyday tasks. The broad span of its activities includes Health, Food and Food Processing and Industrial Technology. This makes our Organisation uniquely placed for new developments in the field of biotechnology, an area in which co-operative and multidisciplinary work is so essential. I do hope that its co-operative atmosphere will be present with you throughout this Conference.

Thank you for your attention.

The Role of Government in the Development of Biotechnology

Dr. L. Ginjaar
Minister of Health and Environmental Protection
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More than 50 years ago the famous Dutch microbiologist Professor Albert Kluyver made the following statement:
"There is an altogether too prevalent notion that microbes, as the irreconcilable enemies of man, plants and animals, deserve attention only in order to make it possible to combat them better. However understandable such a concept may be in view of the beneficial effects resulting from the brilliant discoveries of the part played by microbes in numerous diseases, this nevertheless represents an extremely distorted view of reality."

The quotation confirms the fact that the subject of this conference - biotechnology - is not new, even from a scientific point of view. It is, however, true to say that it is a subject on which special interest is focused at the moment - not only at this conference but in scientific periodicals, in research and development work and so on. Notably in Japan, Germany and the EC steps have been or are being taken by governments to set up biotechnological programmes, and private industry has also been busy.
In Japan the emphasis is on the application of enzymes, both immobilized and otherwise. The Japanese Government's biotechnology programme embraces both fundamental scientific research and the development of applications. Germany has a very extensive programme which stresses the development of biotechnological processes for practical purposes, for instance in the production and application of proteins and other biological macromolecules, and the production of bulk chemicals. The EC programme focuses chiefly on the research needed to develop a second generation of enzyme reactors and the practical applications of genetic manipulation.

Important areas of application

The world currently faces three main problems: energy, food shortage and health.

It is an established fact that per capita consumption of energy and food (particularly animal protein) in the rich countries is so high that there is virtually no possibility of achieving similar levels for the world as a whole with the technology used at present. However, it is becoming evident that biotechnology can play a part in the solution of these problems: our dependence on fossil fuels, for example, can be reduced by producing energy from plant and animal waste - both in the wealthy West and in the poor, non-oil-producing developing countries. Furthermore this solution will at least partly relieve the problem of disposing of agricultural waste in countries such as the Netherlands.

The world food shortage could also be reduced by means of fuller utilization of plant and animal substances, for instance in the production of animal feed. This would not only mean using a greater number of production factors to feed the world's population, but it would also help to solve the distribution problem - food would be produced where it should be consumed. The important point here is that biotechnological processes are often particularly suitable for application on a small scale.

Finally, I should mention new possibilities of producing effective and sometimes cheaper medicines and the immense significance this may have for mankind requires no further elucidation.

Taking everything into consideration, it is certainly no exaggeration to say that scien-

tific breakthroughs such as the immobilzation of enzymes and the work on recombinant DNA have created tremendous prospects for the application of biotechnology, although a "shining future" is by no means assured at this stage. The needs which have been identified have still to be translated into actual biotechnological processes and products, and this is one of the areas where government has an important role to play, although its responsibilities are not limited to this role.

In my view there are three main ways in which governments should act to promote promising biotechnological developments - and indeed developments in other fields of technology as well:
1. they should stimulate fundamental research so as to increase the potential of the branch of technology concerned (technology-push);
2. they should specify the needs which exist in society but which are not automatically expressed in terms of purchasing power on the market (demand-pull);
3. they should take steps to limit the risks and other possible adverse consequences of new technology at the earliest possible stage.

Risk control

Risks are inherent in biotechnology - as they are in so many other activities in our society. I should like to distinguish between risks of three different types:
1. risks associated with the use of or contamination by pathogenic micro-organisms;
2. risks associated with the harmful side-effects of biotechnological products;
3. risks associated with unforeseen changes in the quality of biotechnological products.

Before going on to discuss these risks I should like to say a few words about the relationship between science and society, which is an essential element of the background to this conference.

Scientific activities are increasing both in range and intensity and at an accelerating rate because they themselves produce the means whereby they can go on developing. This is a continuous process, which contrasts with the lack of continuity which characterises the discussion of these issues by the general public. This is because the regular warnings voiced by individual scientists very seldom provoke any response from the public, but when they do the response is usually a vehement one because science is so difficult for the layman to grasp.
This conference cannot ignore the fact that there is a certain degree of general disquiet concerning the development of science. Research scientists have a tendency to point out the enormous benefits scientific advance has brought for our material welfare and possibly even for our well-being. They are sincerely convinced that the results of their work will serve the common good. However, in my view we have reason to regard these people with a measure of suspicion. I believe that as we penetrate further and further into the nature of matter, into the essential nature of reproduction and the secrets of the mind, there is an ever-increasing chance of our being able to interfere with life in a way that leaves us at a loss as to what to do next. This is what makes science such an unknown quantity, so elusive, if you like so horrific. I can therefore sympathize with people who call for curbs on scientific research. But who, then, is responsible for the development of science? The government and parliament, who can not always fully understand and digest scientific developments? Or the scientists themselves, who cannot be called to account by society and whose involvement in their research may lead to their seeing only one side of it? I will come back to the question of government responsibility a little later on; at this point, however, I should like to draw attention to the responsibility which scientists have to society. It is of the utmost importance for the development of biotechnology that the existence of this responsibility should be fully recognized.

It is my belief that society's acceptance of certain new technologies - work involving recombinant DNA being a case in point - depends to a large extent on whether or not

the public is convinced that the risks are being kept within tolerable limits. It is therefore necessary for the government to have a convincing policy on controlling the risks. Some of you may think the need for such a policy is exaggerated or that it might slow down progress; it is nevertheless one of the most important contributions government can make to the development and stimulation of biotechnology - because when anything goes wrong it has to intervene anyway and such intervention is likely to be stricter, with a danger of overreaction and of a less balanced consideration of all the factors involved.

So what form should a risk policy take to be both responsible and sufficiently convincing? To start with, the more dangerous the toxic properties of the micro-organisms and substances produced, or which can be released in the production process, the stricter the system of statutory controll will have to be.

A second point concerns the distribution of these micro-organisms or substances. The greater their distribution, the greater the chance of uncontrolled exposure of human beings and other living organisms to them. Serious ethical objections arise in particular when people or the environment are found to have been exposed to a danger of which they had not been previously informed, against which they could not defend themselves, and the consequences of which do not become evident until after the damage is done. Such a situation often evokes a sharp response - and rightly so. The strictness of the control system will therefore also have to increase as the extent of the potential exposure increases.

In general risk control systems - which apply also to biotechnology - have to include:
1. rules relating to the organizations, installations and production processes;
2. rules on the nature and composition of products;
3. rules for the use of biotechnological products;
4. rules on whether biotechnological products may be put on the market.

Let me give you a few examples:

The risk associated with DNA work, that is of the uncontrollable spread of genetically modified micro-organisms, used to be - and to some extent still is - considered so great that strict rules apply at organizations engaged in work at CI and CII level, while the number of laboratories allowed to perform experiments at CIII level is restricted.

In the first instance it was evident that undesirable side effects would be serious mainly because it would be impossible to control the spread of genetically modified micro-organisms. The government, with the cooperation of the scientists concerned, recognized without having to employ any statutory measures that it was necessary to investigate the seriousness of this type of risk. Some people think that such a policy constitutes an unnecessary restraint causing Dutch DNA research to lag behind that carried out in other countries. On the other hand it must be said that accidents will often serve as a much more powerful brake on the development of this type of research. Examples can be cited to show that there is ample justification for caution with respect to activities with unknown but potentially very great risks. In the Netherlands for instance we have always been fairly prudent about adding antibiotics to animal feed; in retrospect, however, it transpires that one cannot be careful enough.

As far as the recombination of DNA in the laboratory is concerned there is a growing impression that in the past the risks have been overestimated. In the Netherlands we have a special committee responsible for ensuring that safety guidelines for the recombination of DNA at CI and CII level are established, observed and kept up to date with the current state of knowledge. In addition my colleague the Minister for Science Policy is to set up a broadly-based committee to study the social aspects in greater detail; obviously it can be assumed that changing ideas concerning the risks and implications of this will be reviewed on a continuous basis with respect to CIII research

as well, and I believe that, all in all, this guarantees a well-balanced policy in this field.

Another example concerns the use of certain dangerous pathogenic micro-organisms, antibiotics and other biotechnical products or intermediate products. Generally speaking, these call for a policy of admission: certain conditions must be met before permission can be given for them to be produced or brought on to the market.

I will take the use of antibiotics as an example. It is known that a disturbing secondary effect of this important group of biotechnological products, which are used in large quantities both to combat microbial infection and in the production of meat and fish, is the development of resistance to them; for example in an increasing number of cases, gonorrhoea cannot be treated with penicillin, some forms of meningitis can no longer be treated with chloramphenicol, streptococci have been found which are resistant to so many antibiotics, that the therapy often fails, and so on. There is no need for me to point out what we would seem to be risking squandering important medical achievements here. This cannot be allowed except for the sake of some very substantial gain to society. Approval of the products must be restricted and subject to strict instructions for their use in order to limit the risks involved.

I would refer in this context to the hospital infections which are often caused by micro-organisms resistant to more than one of the usual antibiotics, making it necessary to give some patients a number of different antibiotics at once, which puts even more selection pressure on multiresistant strains of bacteria.

Another matter of serious concern is the applications of antibiotics in the production of meat and fish to achieve uninterrupted animal growth. Animal feed accounts for a significant part of the total consumption of antibiotics in the Netherlands. To give you an impression of the scale of this practice: it is estimated that approximately half of the millions of tonnes of animal feed consumed annually contain so much antibiotics that bacteria might be selected, that are resistant to drugs of human therapeutic relevance.

The development of resistances is of course disturbing; on the other hand there are indications that, at least in certain cases, resistance can be broken down again: after the addition of tetracyclines to animal feed was prohibited the number of strains of human salmonella bacteria resistant these substances fell, from 80% in 1973 to around 30% in 1978. In my view it is necessary to pursue a firm policy on antibiotics based on existing legislation and guidelines and with the help of such measures as the Veterinary Medicines Bill which is currently in preparation in the Netherlands.

The third category of risks I mentioned concerned the risks associated with unforeseen changes in the quality of substances during biotechnological processes, which I think may lead to very severe restrictions on biotechnology. First, products in which unforeseen changes would appear cannot be approved for human consumption, from which it follows that there must be great hesitancy about using them as animal feed. I think there is an important job for research here to safeguard biotechnological processes against such changes and when they do occur, to discover them at an early stage, even if it is not known which substances or micro-organisms have to be traced.

Stimulation of biotechnology

I now come to the two questions on which I want to concentrate the rest of what I have to say:
1. What can the government do for the development of biotechnology?
2. Which biotechnological processes should the government do most to promote?

One of the most important things the government should do is to identify the needs exist-

ing in society. It can stimulate demand-pull innovations by distributing information about such needs at an early stage. I shall list a few of the sort of needs I mean in a minute. At the same time it must act to remove any factors which stand in the way of innovation.

An example of a policy which has frustrated the development of biotechnology in Europe is the EC policy on the manufacture of glucose-fructose syrups. When the production of new sweeteners on a competitive basis emerged as a feasible proposition, the EC imposed a prohibitive levy in order to protect established interests - a policy which by no means benefited public health since glucose-fructose syrups have a lower calory content than ordinary sucrose. I would therefore endorse the policy recommendation made in the British report on biotechnology that new biotechnological processes should no longer be discriminated against in this way. However, biotechnology is of such immense significance for our society that the development of biotechnology is vitally important to many fields of government policy.

Particularly important are those applications which can help to produce substitute for products or processes constituting environmental hazards or alleviate waste problems or achieve more efficient utilization of raw materials. Especially interesting biotechnological applications exist for the processing of plant and animal waste, for instance methane fermentation and the production of animal feed. The raw materials for these applications are in plentiful supply and they are also renewable - so there is a double advantage. The quantities of waste involved here by far exceed the quantity of household waste and their processing could therefore mean a considerable reduction in the overall waste problem. Nor are the quantities negligible in terms of energy, they account for several per cent of total energy consumption.

A second example relates to waste water purification - not just the removal of biodegradable material but also of inorganic nitrogen and phosphorus compounds, heavy metals and oil residues, and all the other problems connected with waste water about which there is increasing concern. I am convined that there is a big market here for new biotechnological processes.

As a practical example let me tell you about a purification project currently being carried out by one of the largest Dutch biotechnological firms in cooperation with the government to investigate how water with a fairly high concentration of waste can be purified anaerobically. The technical problem is that the sulphates and solvents present in the water can influence the process. The aim of the research is to apply powerful anaerobic degradation processes by subsequently treating the effluent by aerobic purification in accordance with modern knowledge of the kinetics of fermentation processes. By comparison with traditional waste water treatment this method will require less space and will produce less sludge. If the project is successful - as we expect it to be - it will revolutionize waste water purification techniques.

But it is not only on account of waste disposal problems that biotechnological processes are important: another aspect, which has not received the attention it deserves, is that of external safety, particularly as regards certain production processes where biotechnological alternatives exist, and are safer than the corresponding chemical processes because changes take place at lower temperatures and pressures.

I should, finally, like to draw attention to the important achievements of biotechnology in the medical field, in producing drugs such as steroids and antibiotics. More recently, valuable progress has been made in producing proteins like the growth hormone and interferon by means of the recombination of DNA molecules in the laboratory. Tremendous possibilities are emerging in this field and it is precisely the promising character of the work of DNA which makes it so difficult to weigh up the advantages against the risks.

Another important area in which governments should be active in my view is the creation of a framework within which biotechnological projects can be placed to allow proper attention to be paid to technology-push projects. The Dutch Minister for Science Policy announced recently in a policy document on innovation that special attention will be devoted to biotechnology, bringing the Netherlands into line with a trend which has existed for some time already in other countries, notably - as I said earlier - in Japan, Germany and in the EC.

Conclusion

Mr. Chairman, it is easier to say you are going to stimulate biotechnology and limit the risks it involves than actually do something about it. A very special circumstance in the Netherlands is the fact that biotechnological experience is concentrated in a very few organizations, notably one or two companies and only to a very limited extent in government departments and higher education institutions. There is no broad base from which biotechnology can receive new impulses, which as a multidisciplinary field combining the different approaches of biologists and technologists it must have.

Biotechnology could be stimulated for example by improving educational and training facilities, improving the framework for scientific research and providing financial support for individual projects.

The innovation programmes planned by the Minister for Science Policy include a biotechnological programme directed primarily towards scientific research and the promotion of cooperation between industrial, university and government laboratories.

So far I have been talking about the role of government in the development of biotechnology; in fact the role of government will be very limited. It is primarily the responsibility of science and industry to ensure that their own experiments, products and processes are safe. Where safety is concerned the man in the street will not give either of them the benefit of the doubt, which in turn places a further responsibility at their door: public relations. It is vitally important that proper information is provided to make biotechnology acceptable to the general public. After all, the most important impulses for innovation do not stem from the government but from within society itself - something clearly demonstrated by the fact that the TNO has organized this conference. I should like to conclude by expressing the hope that the development of biotechnology will contribute to a shining future for the new TNO, and quote again the words of Professor Kluyver, which I feel are most appropriate here today: "The microbiological industry of the future," he said, "will be a key industry."

Thank you.

Biotechnology - Its past, present and future

Dr. J. de Flines
Member of the Board of Management of Gist-Brocades NV
Delft
The Netherlands

Biotechnology, what is it? I believe it is preferable not to begin with an exact elaborate definition but rather indicate, in a broad sense, that it is the discipline combining biology and technology.

Within the biological sciences, microbiology is understandably the most important, at least at the present time. Microorganisms multiply faster than cells of higher plants or animals. They can live in a relatively simple nutritional environment and they are able to produce an astonishing variety of products. Some - not all - are unicellular and easy to handle. Their adaptive capabilities are usually very impressive. Their multiplication rate and high capacity for the generation of new substance is probably due to the much more rapid transport of nutrients and metabolites into and out of the microbial cell compared with the cells of higher organisms. Partially this may be explained by their smaller dimensions and hence the higher surface to volume ratio.

As the causative agents of the plague and other pestilences, microorganisms - especially bacteria - have a very bad reputation. Since time immemorial, however, man has used microorganisms - though unknowingly until fairly recently - for the production of useful goods often with very pleasant properties and effects (except when used excessively). Wine, beer, cheese and other foods are well-known microbial products, as is - in a sense - our daily bread.
These, and many other examples constitute what one may call the hidden past of biotechnology.

Since the middle of the 19th century, biology and also microbiology have developed into the sciences as we know them to-day. They were followed later by biochemistry, biophysics and biotechnology. These latter three disciplines connect biology to other sciences: chemistry, physics and technology. To name alle biologists who have contributed to this evolution of microbiology and thereby to the creation of to-day's biotechnology would be impossible.
But I want to make three exceptions. Firstly for Antoni van Leeuwenhoek, a Dutchman from Delft, who was the first to observe microbes and describe them and thus the originator of microbiology as a science. Secondly, of course, for Louis Pasteur, the founder of modern microbiology who not only detected the causes of many infectious diseases but also performed some very early experiments in the field of applied microbiology. And finally for Albert Jan Kluyver - and at the risk of sounding too chauvinistic, I mention that he too worked in Delft - who demonstrated the significance of applying biochemistry to microbiology, thereby greatly facilitating the large scale use of microorganisms.

This brings us to the recent past - hardly hidden, and perhaps already shining - and to the present. Where are we now; where are we going? Currently, microorganisms are applied in four different ways:

1. They are produced for the use as whole cells. Probably the most important example is baker's yeast. For the making of our daily bread the world-wide requirement for compressed yeast is about 2 million tons per annum. Mushrooms are increasingly becoming part of our diet. Also to this category belongs single cell protein, produced for feed and possibly food purposes. This topic will be discussed separately and more fully during this conference.

2. For the production of metabolites. To this class belong all antibiotics that are produced by fermentation, and also such organic compounds as citric acid and glutamic acid. These latter two are produced at annual rates of 450,000 and 300,000 tons respectively; impressive quantities selling for a few dollars per kilo which is possible only because the scale of production is relatively large. From this series, ethanol should not be omitted. Annually, billions of liters are produced by fermentation not only for the enjoyment of drinking it but also for the pleasure of driving cars on so-called gasohol. I shall come back to this later and others will deal with the subject during this conference.

3. As biocatalysts in specific reactions. This may be done in two different ways: the organism as such may be the catalytic system or a specific enzyme may be isolated from it and applied. The use of certain fungi for the introduction of oxygen at a specific site of a steroid molecule is an example of the first kind and the application of glucose-isomerase in the production of fructose from glucose for sweeteners exemplifies the second.

4. For the purification of waste water. Organic substance must be degraded by the microorganism to carbondioxide and water or to methane, CO_2 and water in an aerobic or anaerobic process. In recent years, attention is more and more focussed on the latter because little sludge is formed and energy is supplied rather than consumed.

To this point I have only discussed the microbiological component of biotechnology. The role of technology itself, particularly that of chemical technology and in an increasing way that of physical technology should not be overlooked. Not only have all recovery processes and the construction of fermenters needed the expertise of chemists and process engineers but also the fermentation per se requires their input. In recent years the use of mathematical models of the production process have proved to be of help in optimizing fermenter output.

It does not seem interesting to attempt a complete survey of all biotechnological processes and products. Their number is quite considerable. A few of the most important topics will be discussed at some length by other speakers. Instead, it is perhaps worthwhile to look at some of the peculiarities of using microorganisms on a large scale.
The largest conventional fermenters of the moment are about 450 m^3. Therein, the organism is cultured in a liquid medium, in a so-called submerged fermentation. In the past surface cultures on solid media have been used but since they are usually more expensive, most have been abandoned for production on industrial scale. In aerobic fermentation, air supply and usually stirring are necessary to provide the microorganism with the required oxygen. Stirring is particularly important when fungi or actinomycetes are the production organisms because these are filamentous. To maintain high production rates usually large volumes of air are needed. A desired temperature has to be maintained. Contamination by other microorganisms must be avoided, hence supplied air and the culture medium must be sterile and aseptic conditions must be maintained throughout the fermentation. That this may be a difficult task may be seen from the following example. Suppose the duplication time of a contaminating organism is half that of a production organism, say 20 and 40 minutes respectively. Suppose also that at the start the infectious cells are present in a ratio of 1 to 1 billion of the production strain. It is then easily calculated that the faster growing organism will be equal in number to the other within 20 hours. Sometimes it is possible to favour growth of the production strain by choosing extreme conditions, such as methanol as the carbon source in SCP-production, or low pH in growing yeast.

It may occur that the production of an antibiotic, e.g. penicillin, protects the fermentation. But if an infection is a producer of penicillinase, which converts penicillin into the inactive penicilloic acid, the fermentation may be completely lost within a few hours. Therefore, sterility of operations is usually a prerequisite. As a rule this is

realized by heating, using steam at 120° C and steam-sealing all valves connected to the fermenter during the processing. Air will be usually sterilized by filtration. Continuous feeding of nutrients during the fermentation - a normal occurence in batch operations - requires the feed to be sterile and also additions of acid or base for pH-control and the antifoam.

All this might appear rather easy. But those experienced in the field will agree that it is no light task to run a large fermentation plant or process, utilizing a slow grower on an opulent medium at a neutral pH for about a week's time and repeat this the year round without at least a few percent loss through infections. One must also bear in mind that the inoculum of a big tank must be built up in stages, beginning with a mother culture followed by one or more shaken flasks and one or more smaller fermenters, to obtain the quantity needed for efficient operation of the expensive production unit. This lengthy sequence, of course, amplifies the risk of contamination.
It will be evident that fermenter design and that of the auxiliary equipment is of the utmost importance. Specialty applications may require special designs. Sometimes, continuous fermentation is possible. But this is more the exception that the rule, contamination and strain deterioration through spontaneous mutation being the stumbling blocks.

Fermenters may be universally applicable in biotechnology; the isolation of the fermentation product is another matter. Here diversity really starts. Many unit operations of chemical processing are used. Preferably these are continuous, making it a necessity that several batch fermentations become available consecutively in order to obtain a constant stream of input to the recovery process and of end product.
Let us have a closer look at a practical example: the production of benzylpenicillin. In our plant at Delft we use fermenters of 100 m^3 nett volume. Fermentation time is about 200 hours, and it takes about 15 hours to recover the penicillin from the fermenter. So as to have a constant input in broth to the continuous isolation process, about 200/15 (two hundred over fifteen) = 14 fermenters are needed. The fermentation is a, so called, fed batch process in which sugar solution is continuously fed into the fermenter. Phenylacetic acid, as a solution of its potassium salt, is also added as a precursor for the sidechain of benzylpenicillin. At the completion of the fermentation, the thick broth is filtered through a continuous, rotating filter, the mycelium is washed and filtrate plus washings are extracted with butylacetate in a countercurrent extractor. The extract is supplied with a source of potassium ions in order to obtain the crystalline potassium salt of benzylpenicillin. This is filtered off on a rotating filter, slurried in butanol, filtered and dried, yielding a constant stream of the potassium salt of penicillin at 99.5% purity.

Let us, for a moment, consider the economics of this process.

Manufacturing cost of benzylpenicillin in percent of total

	Fermentation	Recovery	Total
VARIABLE COSTS			
Raw Materials			
carbohydrate, phenylacetate etc.	44	-	
solvents etc.	-	5	
	44	5	49
Utilities			
steam, electricity etc.	8	4	
	8	4	12

FIXED COSTS			
labour, maintenance, overheads, depreciation etc.	26	13	39
total	78	22	100

It may be seen that raw materials and energy constitute about 60% of the total cost. The so-called fixed costs account for the other 40%. This is the usual picture for fermentations of this type. The cost does not include expenditures for waste water treatment. When this is required, aerobic or anaerobic treatment is available for this waste which is readily biodegradable. The treatment adds considerably to the cost because the spent fermentation liquor has a high BOD content. A plant of reasonable capacity may be equivalent in BOD to a city of, say, 300,000 inhabitants.
The penicillin thus obtained sells for about $ 35 per kilo.
Obviously, a very important factor in the process profitability is the amount of penicillin produced per unit volume per unit time.
Doubling the productivity of the mould would reduce production costs to about 60% of the original value. Such improvement of productivity has been very impressive since the beginning of the penicillin era. From the forties until today an increase in the order of 1000 times has been achieved. How was this brought about? To a large extent by strain improvement through selection of spontaneous or induced mutants. So far, this has been a hit and miss affair based on the screening of thousands upon thousands of mutants.

From the example presented it will be evident that relatively short fermentations in large volumes with high product yields will result in low cost prices. This is the situation with citric and glutamic acid. In contrast, for the production of a rare chemical, such as hydrocortisone which sells at about $ 800 per kilo, fermentation although expensive will be competitive when chemists are not clever enough to introduce readily an oxygen atom at the so-called 11β-position. This also applies to degradation of the side chain of sitosterol, a process carried out on a large scale by three companies in the world, yielding the steroid intermediate androstenedione.

I believe it is not exaggerated to state in summary that biotechnology up to the present has made tremendous contributions to industry.
A number of very sophisticated fermentation industries has become established, particularly in the United States, Japan and Western Europe.
A diversity of processes is used in the manufacture of many different products. The following slide lists those that I consider most important.

wine
beer
baker's yeast
single cell protein

ethanol
L-amino acids
citric acid
xanthan gums
antibiotics (penicillin, streptomycin, gentamycin etc.)

steroid hormones (hydrocortisone, prednisolone etc.)
enzymes (proteases, amylases, pectinases, glucose oxidase etc.)

vaccines

aerobic and anaerobic waste water treatment

microbial mining

The technology used is greatly diversified and applies a variety of equipment and apparatus including computers, nowadays often dedicated, to direct the fermentation process. All this makes a fermenter with its auxiliary installations a rather expensive piece of equipment.
At present, a 200 m^3 fermenter for antibiotic production costs about 2 million $. The advantages of biotechnological processes over purely chemical operations are: the specificity of the desired reaction, the moderate reaction temperature, the capability to synthesize complex molecules. Disadvantages are: the still limited number of applications, the low concentration in which products are often obtained, and the considerable risk of fluctuating yields.

A review of the present status of biotechnology would be highly inadequate without mention of the fantastic recent achievements in molecular biology. By manipulating the material of the cells which directs their metabolism, the DNA, it is possible to add extra instructions for the synthesis of proteins. By this "genetic engineering" cells may be constructed that make enzymes and other proteins which they do not normally produce, and hence possibly other metabolites as well.
The next speaker, Dr. Ronald Cape, will tell us more about this fascinating new field. Also the social and political implications will be discussed. More and more evidence accumulates to show that initially the possible dangers have been grossly overestimated. It remains clear, however, that appropriate safety and control systems have to be maintained. In my opinion, for really large scale production processes we should only use nonpathogenic organisms of which the acceptability can be demonstrated.

Let us now try to look from the already shining present of biotechnology into the future 10 or 20 years in which its brilliance may still increase.

1. The price of oil has risen dramatically in the seventies. There are no indications for a lasting price stability in the coming years. Rather late, the industrialized countries have realized that alternative energy sources must be developed. Coal, tar sands, nuclear energy - hotly debated - and solar energy will become more important.
   Solar energy, turning into plant material by photosynthesis, has over millions of years been converted into fossil fuels. However, the present plants may be used as a direct energy source - which is renewable - by burning them or using them indirectly via bioconversion into easily transportable methane or ethanol. Of all photosynthesized carbohydrates, three are of major importance: cellulose, starch and saccharose. The latter two are easily converted but with cellulose the situation is different. Conversion of wood with its lignin and cellulose constituents by enzymes or whole microorganisms is still an unsolved problem, at least if processing is to be economically justified. Very likely, methods will be discovered that solve the problem. But it is obvious that in the near future only certain regions of the world - Brasil, the Mid-West of the USA and Canada - will be suitable for the economic, large scale production of an energy carrier, such as ethanol, from agricultural sources. Marine organisms are being studied as well, as a source of energy and chemicals. For western Europe the opportunities are probably less. Through the use of waste and some biomass a small but not insignificant part, in the order of a few percent, of our energy requirements might be met. Dr. Bruin will elaborate on this subject to-morrow.

2. Biomass, produced through photosynthesis, again with as its major constituents the three carbohydrates mentioned, could be used as the starting materials in biotechnological production processes for bulk chemicals.
   The 1978 Chemrawn Conference in Toronto estimated, however, that this use of biomass will not reach significance before the year 2000. Possibly the recent increases of oil prices will accelerate the switch over.

3. Production of more complex molecules by fermentation. Without any doubt, genetic engineering will greatly extend the possibilities in the next decade.

Human insulin is about to be produced by fermentation; interferon will probably follow soon. Other proteins, vaccines and later secondary metabolites will probably be produced in this way. A vast new field seems to be opening up with opportunities for fine chemicals and pharmaceuticals.

4. Enzymes will be used increasingly. At present, the most important products in quantity and total sales perform hydrolytic reactions: proteases in washing powders and amylases in the degradation of starch. It is to be expected that more complex systems will be introduced for the acceleration of different reactions, such as oxidations, reductions, isomerisations. Immobilized enzymes with their improved stability, often at higher temperatures, and hence their repeated or prolonged use should gain in importance. Existing systems are, for example, penicillin acylase and glucose isomerase. Very interesting is the more complex conversion of ethylene and propylene into their oxides and glycols by an immobilized enzyme system developed by Cetus Corporation.
   Very sophisticated multiple enzyme systems with built in regeneration mechanisms for supporting reactions will probably be developed in the coming years.

5. Waste water treatment is today often carried out by aerobic fermentation. This is unsatisfactory because the process consumes much energy, creates a large quantity of sludge, is expensive and requires heavy investments. Anaerobic treatment and more sophisticated aerobic processes will certainly be developed.

6. A few words about using cells of higher organisms. Vaccine production by using special cell lines of e.g. mice is well known. Plant cells can be cultured and are able to produce desired substances. Their slow growth and rather low production rates argue against use on a industrial scale. But progress will be made in this area, possibly also through genetic engineering. And finally, I will not omit to mention possible developments in the area of symbiosis between microorganisms and higher plants which might result in e.g. better utilization of nutrients or nitrogen fixation.

From this review you may conclude that my expectations concerning the role of biotechnology in the coming decades are very optimistic indeed.
But allow me, at the end of my talk, to sound one warning. Recent reports in the press and the other media but also those from learned institutions have become almost jubilant about the possibilities of applied biotechnology. This might make the false impression that realizing these opportunities will be an easy task. In my opinion, the contrary is true. A great deal of fundamental and applied research work will be needed, followed by development. Hence considerable scientific and financial resources will have to be made available. Only a concentrated effort will enable us in the foreseeable future to reap all the benefits of biotechnology.

That we shall succeed in reaching that objective and that this conference may form a modest contribution to it, is my wish for all of us.

The Future of Biotechnology and the Role of Genetic Engineering

Dr. R.E. Cape
Chairman of Cetus Corporation
Berkeley
U.S.A.

Tremendous excitement in biology has been generated of late in the industrial sphere, especially with respect to the development of new products and processes utilizing microorganisms or their enzymes. An immense accumulation of basic biological knowledge, has not been reduced to practice until recently. Now, industries with prior dependence on biotechnology and, more significantly, perhaps, others which historically have had no biotechnological base, are deeply involved in exploring completely new biological opportunities. To some it is the perceived utility of new biological "tools" which provides the incentive. To others it is the compelling need to develop low energy, clean alternative routes to industrial chemicals; the need for renewable resources as alternates to expensive, disappearing oil; and the need for processes with improved economics to enable survival in an inflationary economy.

Before dealing with specifics, perhaps it's appropriate to deal first with basic definitions. Biotechnology in one form or another has been with us for a long time. The word "biotechnology", itself, is a good one in that it is almost self-descriptive. It's a collective term - we think of it that way - because it embraces the disciplines of microbiology, biochemistry and chemical engineering. Indeed, the biotechnologist applies and integrates each of these branches of science in developing industrial processes based on the use of microorganisms or their enzymes. And, the role of genetic engineering - again, a good term, especially if one stresses "engineering" - is to construct or modify microorganisms to serve as efficient, environmentally acceptable, industrial "factories". Thus, the role of genetic engineering is basic to the future of biotechnology - one is inseparable from the other - the reduction of biological dreams to practice depends on both.

Now, we know we've said nothing new to this audience - at least nothing obvious profound. But, those of us involved in creating new biologically - based businesses continue to be surprised at those who continue to fail to comprehend that, almost invariably, new organisms or new applications of "old" organisms dictate great opportunities for new chemical engineering. Conversely, few chemical engineers understand that today's biology is in dire need of innovation from their discipline. Fortunately, today's experienced industrial geneticist understands clearly the demanding interdependence between a newly engineered microorganism and optimized process development. It's obvious to him, for example, that a continuous process with cell recycle for the production of ethanol places different demands on a yeast than does the conventional batch fermentation. So, for now it falls on him in large measure to demand, lead and coerce others to provide the means for translating esoteric research into practical products. It follows that those that listen and act, i.e. his associates or venture partners, are the leaders today in vitalizing biotechnology; they are creating the future; they are responsible for the new developments, now advancing at a breakneck pace, which we predict will have impact on a number of industries.

Enough generalization - let's get down to specifics. We come from a company that is now investing millions of dollars of its own money, and comparable amounts on behalf of major company clients, to build profitable, new, biology-based ventures. In the chemical area we are developing new approaches to both highly profitable specialty chemicals as well as commodity products never before produced via microbiology. We are concentrating on technology which utilizes immobilized enzymes, cells and cell fragments - many of which are little known, if at all, and most of which are not now articles of commerce. We're looking, therefore, at novel enzymes - "catalysts" to the

chemical engineer - which can perform tasks formerly left to the often complex pollution - creating, energy-consuming methods of the organic chemist. An example: we have now developed an exciting biological process for the easy conversion of alkenes such as ethylene or propylene to the corresponding oxides. Ambient conditions of temperature and pressure, essentially aqueous conditions, no waste products, no requirement for pure sustrate, and high process versatility characterize the process. With but minor modifications in recovering the oxide produced due to slightly different physical properties, the same plant can be used to produce both propylene and ethylene oxide. Optionally, but not necessarily, attractive co-products can be produced. And, what is the "catalyst"? For now let us say - a consortium of enzymes capable of effecting the transformation without cofactor requirements.
Currently, chemical engineers have the task of bringing a pilot plant on stream. We hope shortly to be free to discuss full details regarding the process.

New processes and technology via the use of immobilized enzymes and cells constitute a most compelling area for study and profit opportunity. Thus, it would be premature for us to drop the subject now; let's just "blue sky" about where enzymology is headed for a few minutes. Enzyme systems are being studied for use in essentially non-aqueous environments. While there should be nothing surprising in this to the microbiologist, it is nevertheless true that industrial applications of enzymes have been restricted to aqueous media for the most part. That dogma must go. Already it has been demonstrated that highly efficient, continuous esterification can be effected in organic solvent between long chain acids (e.g. linoleic acid) and alcohols (e.g. cetyl alcohol) by the use of dried cells (Rhizopus arrhizus), i.e. cells which utilize their own structure as the immobilizing or entrapping matrix for the enzymes they produce. (Ref: Patterson, J. D. E.; Blain, J. A.; Shaw, C. E. L.; Todd, R.; and Bell, G., "Synthesis of Glycerides and Esters by Fungal Cell-Bound Enzymes in Continuous Reactor Systems", Biotechnol. Lett. 1, 211 (1979)). Enzyme conversions effected in organic media containing minimal water open opportunities for performing reactions not otherwise possible or economical because of substrate or product solubilities and/or product recovery considerations.

Furthermore, not only will problems as mundane as increasing yields of enzymes be a fertile research field for molecular biology, but also the more tantalizing and speculative considerations of enzyme "delivery" and modification will be of paramount significance. By enzyme "delivery" is meant the conversion of normally intra-cellular enzyme into an extra-cellular enzyme by incorporation of an appropriate sequence. By enzyme modification is meant the restructuring of enzymes at the then active site and elsewhere by gene synthesis or other applications of molecular biology. Enzyme design will become a most significant area of cooperation between the molecular biologist and the engineer.

In the realm of commonality between biotechnology, enzymology, and recombinant DNA research, a particularly knotty question will be one of balancing new enzyme activities installed in a microorganism with existing activities to allow for a compatible and economically significant multi-enzyme process. Some examples of this kind of problem are the following:

Existing Activity			New Activity		
Cellulose	→	Glucose	Glucose	→	Ethanol
Glucose	→	Ethanol	Ethanol	→	Ethylene
Cellulose	→	Glucose	Glucose	→	Citric Acid

In some cases, such as these, it may be found that a consortium of microorganisms rather than a single, super creation may be a more appropriate solution to particular bioengineering and biotechnological problems. In other instances, it may be necessary to employ more traditional genetic manipulations of the single, super cell to improve the activity of the enzyme component in imbalance. In such cases, in our "shop" at

least, the high volume screening capacity we have developed comes into play.

The conversion of renewable biomass to chemicals and fuels is receiving worldwide attention in view of the "petroleum crisis". Agriculturally-derived starches and sugars, wood-derived cellulose, and various carbohydrate-containing waste products (including cellulose-rich residential garbage) are being considered as fermentation substrates. The objective of most of this work, of course, is to develop the most cost effective processes for converting the products of photosynthesis to storable, transportable, liquid fuels such as ethanol or other useful chemical feedstocks. The role for the biotechnologist is a particularly tough one for he must fight economics all the way. Substrate costs constitute the major (approximately 60%) portion of the final price for ethanol. Thus, his role is to maximize substrate utilization, optimize the biology, and lower processing costs. Alcohol fermentations have been with us almost from the beginning of mankind. Perhaps because of that, little attention has been given over the years to either improving the traditional batch process or the microorganisms used therein. Recently, both have been the subject of concerted efforts. The result, within our company at least, has been a new, genetically improved microorganism tailored to the requirements of a truly continuous, steady state, cell recycle„ minimum cell growth process.

As noted, economic considerations are the key for commercially successful biomass conversion processes. Energy balance studies and comments thereon are unavoidable. A number of detailed analyses of the energy balance involved in the production of ethanol by fermentation have been published. These have attempted to compare the energy yielded in the form of anhydrous alcohol with that expended in its production. Estimates have included the energy required during manufacture, for example: feedstock, preparation, operation of machinery, distillation, and drying of the yeast byproduct, as well as the energy required to grow the crop; for example: fuel to drive farm machinery and to manufacture that machinery, herbicides and fertilizers, and even the energy of human labor. Examining these reports one can find results claiming fermentation alcohol processes to be anywhere from significant consumers of energy to producers of energy in excess of the ratio of 2:1. This compares with a net energy benefit of about 6:1 for gasoline obtained from offshore Texas oil.

Such exercises are laudable in that they attempt to illuminate the "true" energy efficiency of various processes and, therefore, highlights areas where significant energy savings could be made by improved technology. Indeed, such critical energy accounting would be valuable for many industrial processes. However, such calculations often miss or obscure the real case for producing ethanol from renewable carbohydrate resources. Very simply put, and to repeat, the motivation is to convert an indigenous renewable resource to a high quality liquid fuel suitable for transportation use, through the processing of low grade indigenous fuels unsuitable for economical transport, for example, coal and wood or in the case of sugar cane, bagasse, which conveniently happens to be grown as part of the sugar crop.

Viewed in this way, it can be seen that fermentation alcohol should be considered most seriously as a source of liquid fuel which, by upgrading indigenous, low grade forms of energy, would serve to decrease our reliance on imported oil.

Depending on locale, the most economic biomass for alcohol production today appears to be either corn, sugar or cassava. Nevertheless, almost everyone has the objective to use wood or plant-derived cellulose as the long term substrate. Wood and plant pretreatment studies - chemical, biological, thermal, mechanical - are aimed at producing cellulose for use at low cost, in good form for saccharification. Cellulase mixtures or individual cellulase enzymes - derepressed, more thermophilic, with enhanced stability and more reactive - are targets for the genetic engineer. Success, part of which is already achieved, will result in important economic advantages, including improved reaction rates, reduced energy cost for temperature maintenance, and less

chance for microbial contamination. It will hasten the day when biomass conversion becomes highly attractive in economic terms.

An area of enormous proportions and interest is that of tertiary oil recovery - and the possible involvement of microorganisms in this process. In one aspect it might be desirable to have microorganisms migrating through the "solid" matrix of oil fields performing useful metabolic tasks facilitating oil recovery. One major problem is that the bore size of fields limit the usefulness of the procedure since most potentially useful microorganisms may be too large. Decreasing the size of these microorganisms by techniques of molecular biology could have great implications towards the accomplishment of the microbial action. The challenge of designing and using these organisms in the field situation presents many intriguing questions for the biologist and engineer.

Now, how can we possibly forecast a glowing future for biotechnology without discussing genetic engineering and the pharmaceutical industry. As noted above, the "new biology" can develop and refine processes, for example alkene oxide production, which may displace traditional chemical approaches. Each such biological process must compete on several grounds - energy utilization, environmental acceptability - and ultimately, win on economic criteria.

But in the pharmaceutical area, the situation is dramatically different. Here, recombinant DNA, monoclonal antibodies, and other new techniques don't merely threaten to displace other ways of doing things. Far more dramatically, they make possible what was once inconceivable. In so doing, by manufacturing human and other proteins in fermentation processes using microorganisms, they show promise of revolutionizing the pharmaceutical industry, giving rise to a huge new market for novel, safer, and more effective drugs. At the same time, genetic engineering again makes it possible to "design" microorganisms well-suited to meet chemical engineering and plant design requirements.

Interferon can serve as a good example. It is a natural substance made and released in minute quantities in higher organisms in response to viral infection. It has been shown to block the replication of many different pathogenic viruses and to exhibit several complicated hormone-like effects on the immune system. Some combination of these two actions is probably responsible for the impressive anti-cancer activity hinted at by the clinical trials so far conducted. Interferon is emerging as the first broad-spectrum antiviral agent. It could well be for virus diseases what antibiotics have been for bacterial diseases as well as, potentially, the most profoundly effective anti-cancer treatment. Also, we assume, interferon is not toxic in humans - a most important property. The market for it could be in the tens of billions of dollars.

The reason why it is still not known conclusively whether interferon is effective in the treatment of viral diseases ranging from hepatitis to the common cold, or in the treatment of many if not all forms of cancer, is that the only current production method relies on stimulating isolated human cells to make a pitifully small amount of interferon. A large portion of the world's total supply of interferon was purchased last year by the American Cancer Society for two million dollars - enough to treat, at most, 150 people for a very short time. Adequate clinical trials just to determine the efficacy of this compound in the treatment of the most urgent diseases would require at least a hundred times greater quality. Clearly, production costs using current techniques preclude interferon's use in non-debilitating diseases like colds and mild influenza, the treatment of which represents enormous potential markets. Cloning the gene for the production of human interferon into a bacterium, and producing high levels of human interferon in traditional microbial fermentations, will provide virtually unlimited production capability at low enough cost to extend interferon usage into all available markets. The availability of inexpensive interferon will also make possible the development of second-generation modified derivatives, in the same way that new antibiotics have been evolved over the last thirty years. Since cloning techniques are equally applicable to

genes from any species, the same techniques used to clone, produce and develop human interferon can be used to produce interferon for all economically important animals, including cattle, pigs, chickens, horses, and others. There, too, attractive markets are available.

The task of bringing interferon to the point of commercialization requires several significant accomplishments. For example, the production of the protein component of interferon within a microbial factory is already accomplished. In the area of glycosylation interesting problems lie. Will the interferon from microbial sources without glycosylation be usable in human therapy? Will subsequent non-human glycosylation modify the desirable biological activities of the molecule? Will there be immunological difficulties - the patient generating antibodies to neutralize the interferon effects? The very special needs for the large scale, economical production of interferon, assuming a satisfactory answer to the biological questions, looms as a great challenge for the biotechnologist.
Many laboratories throughout the world are addressing such problems as this - many announcements can be expected, undoubtedly with increasing frequency - but the "bottom line" is that the exciting antiviral will be commercialized in the foreseeable future.

The problems of masterminding the proper marriage of genetic, chemical and process engineering in the kinds of research discussed above are, in some cases, unique; in other cases, merely pedestrian. Unique will be the needs of microbial production of human products for human use - the purity requirements, the rigors of retaining molecular integrity, and the exquisite potency of certain products introducing safety precautions par excellence.

Doubtless there will be among these creations of genetic surgery some as demanding and fastidious as a finely bred and trained prima donna offspring, and they will demand a process that cares for their every biosynthetic comfort. The chemical, biological, or process engineer will have the pleasure of housing these sensitive, little genetic creations in his own creation - a properly engineered system.

It is clear that one of the major roles of genetic engineering on the future of biotechnology will be that of the constant and relentless challenger, whose creations for economic and humanitarian good will demand that new horizons of biotechnology be developed to reduce the dreams to practice.

The Social-Political Aspects of Biotechnology

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First I would like to say how very pleased I am to have been invited to speak at this conference on this topic. Not only does it do wonders for my ego, but it indicates that scientists involved in biotechnology are concerned about the political and social implications of their work and moreover are prepared to initiate public discussion. It is also very encouraging that you are willing to discuss the social and political implications of your work with non-scientists such as myself, and I must say that I am now really looking forward to a rational public discussion an debate on biotechnology - a dialogue which accepts the right of the public to be concerned and the need to deal calmly and intelligently with public concerns - a debate which includes give and take on all sides - with the result being a smooth and happy development of biotechnology on lines which everyone accepts as realistic and non-threatening and which therefore has the support of the public. In this way and only in this way will those in this field be able to avoid the conflicts which now seem to be an integral part of other fields such as nuclear physics.

I realise that it may be somewhat over-optimistic to base this scenario on one session of one conference, but I very much hope that this will at least mean a start in the right direction.

Secondly, it now looks as though the social and political implications of biotechnology will be extensive. I certainly don't feel that I can cover them all in depth in this one session. So I'll do my best to cover a few of them in depth and mention a few others and hope that perhaps during the discussion session others will fill in the details of the areas which I will not be able to go into very deeply and also bring in those new areas which I won't have time to cover at all.

I think that probably the best way to approach this problem is to examine the concerns of the groups who will have an interest in this field and then to suggest mechanisms for dealing with them. Biotechnology is only just emerging into the public spotlight. We have time to plot a course of co-operation rather than confrontation between scientists and non-scientists. This involves give and take on all sides; - a process which will only work if we all try to examine and understand each others concerns.

First, naturally, there are the scientists who are doing the research and development work which is laying the base for biotechnology. These scientists of course have a legitimate interest in the work that they are doing, and in what happens to the products of their work. There will be research and development which will be of purely scientific interest. There will also be developments of commercial interest and of course there is no firm dividing line between the two which more often than not, overlap.

In Britain, we have become accustomed to the image of the scientist as mainly an academic, as a person who pursues knowledge and truth for their own sake rather than for the money to be made out of their discoveries. This image is especially strong in the biological sciences. This type of scientific approach is in many ways admirable, but I certainly don't mean to put forward the proposal that all scientists should be totally disinterested in any commercial exploitation of their work. That would be nonsensical, as it would assume that scientists are a group of people different from any other - a sort of superhuman - and we know that that's not true. And it would also be most unjust in that it would single scientists out as a group not entitled to benefit from

their work. But also we have in Britain the example of the fiasco following the development of penicillin by British scientists, a development which was then exploited commercially outside of Britain. Since then it has cost Britain a fortune to buy the product back.

I don't really wish to comment one way or the other on this point, but merely to note that scientists have a very definite interest in the exploitation of their work, albeit for different and diverse reasons. In the field of recombinant DNA for example, we find scientists working in universities and research institutes to solve the mysteries of genetics, and we also find scientists working for private companies to engineer bugs which will produce commercially viable products. The scientific interests will of course have to be represented in any forum for public discussion of biotechnology, but the statements of those with commercial interests may well be viewed with suspicion - especially assertions of safety of production processes without supporting data.

Another interest group in the biotechnology field is the commercial company, the large corporation. With the development of such techniques as genetic manipulation and enzyme or cell immobilisation, biotechnology is looking more and more like a potential gold mine. Private companies, in their pursuit of profits will naturally be interested. The fact that representatives of three major British companies will be speaking at this conference is an indication of that interest, as is the fact of ownership of Cetus, Genertech and Biogen by such companies as Standard Oil and National Distillers. And of course other large companies, such as Upjohn, Eli Lilly and Searle are investing heavily in the field.

So it seems to me that we can count on a significant commercial interest in this area, possibly even a dominating one, with important implications for the directions that research will take.

Companies are out to make profits; they see the potential for very large profits in biotechnology, and pressure will therefore very naturally be put on the scientific community to come up with products of commercial value. Although it might be quite a legitimate objective for a commercial firm, the pursuit of profit doesn't always coincide with social priorities. Where the two areas diverge, choices made by scientists will have important effects on society.

A third interest group in this field could be said to be national governments, for a number of different reasons. Some governments or governments sponsored concerns should be interested in biotechnology for the same reasons that private firms are interested - for the production of useful products and for the money to be made out of it - which they wish to see going into the public purse rather than the profits of private companies. But beyond this there are potentially important effects on national economies and, as a consequence, on world politics. I don't want to go into this in detail, but I think that it is worthwhile to mention and to keep in mind. A couple of examples will perhaps suffice.

First, the U.S. reaction to the recent invasion of Afghanistan highlighted the dependence of the Soviet Union on imported foodstuffs. The Soviets are now I understand, investing substantially in production - through biotechnology - of enough protein to become self sufficient. If and when they accomplish this self sufficiency, a small swing will have taken place in the balance of power in the world.

Another example is energy. Most of the western world depends on oil from Middle Eastern countries. Politically the Middle East is unstable and potentially very explosive. The development of new and renewable sources of energy could lessen dependence on the Middle East. These two examples indicate the tremendous impact on world trade, and thus on the balance of power that biotechnology could well have.

Another group with a definite interest in biotechnology is the trade unions. Biotechnology is a new field, with unknown consequences which include possible dangers, and a major area of the trade unions' concern will be the health and safety of their members working in this field. Over the past few years, trade unions in Britain have shown a growing interest in health and safety. Indeed, my own opinion, A.S.T.M.S., has been particularly involved. There is an increasing awareness that many hazards can be eliminated from workplaces, but that employers will not spend the necessary money to eliminate them unless pushed to do so by trade unions.

Disaster after disaster has shown us that we cannot entrust the health and safety of our members to employers. One of the first cases which brought this to our attention was when Dunlop workers in Britain contracted bladder cancer from a chemical used in rubber processing. A legal case for compensation was fought between union and employers all the way through the courts to the highest British court, the House of Lords, where it was found that not only had the Dunlop Company been negligent, but so had another employer, I.C.I., who had supplied the chemical. I.C.I. actually had information which showed the dangerous qualities of this chemical, but had not passed this information on.

There have been many other examples - thousands in fact - where trade unions have successfully sued companies for damages on behalf of members whose health and safety has been put at risk by company negligence - so many in fact that we have taken a new look at the whole health and safety field, and our concern has shifted to include prevention of these incidents as well as our traditional role of mopping up the mess afterwards. We are now becoming involved at many different levels, from negotiations on the shop floor to participation in government advisory groups which attempt to prevent disasters before they occur. We are now insisting on being involved in all decisions which will affect the health and safety of our members. Given that we can establish this position with employers, the benefits will obviously accrue to all of us - to our members in the form of less accidents and illness arising from their work, and to employers who will undoubtedly save time and money on confrontations, public investigations and law suits, and whose firms will benefit from improved industrial relations.

As trade unions in Britain, we also take a wider interest in the benefits which the products of biotechnology could confer on the population, the economy and on the government. In this sense we would wish to encourage the development of biotechnology in Britain, for the potential benefits to people and for the contribution - albeit small - which could be made by reducing unemployment. Although we wish to encourage this development, we are quite definite that it must be in as safe a way as possible. Those who will be first and foremost at risk from any hazards in this work will be the people employed in the establshments where it takes place, and we shall wish to investigate, and as far as possible eliminate, any possible risks to them.

The largest and potentially the most influential group with an interest in biotechnology is the public. It is a difficult group to define but must nevertheless be taken into account, in the most effective way possible. I am assuming that we are talking in the context of western style democratically governed countries. Democracy, after all, is government by the people. It includes the right of people to make decisions - even when they are wrong. If we acknowledge this, then we accept the right of the people, or their representatives, to be involved in taking decisions about matters which affect them, and there is certainly potential for aspects of biotechnology to affect the lives of everyone.

One of the first concerns of the public will coincide with that of the trade unions - any possible risks to health and safety from research, development and production. This has already been seen in the heated public debates surrounding recombinant DNA work.

As you know, concern about possible hazards was first expressed by scientists them-

selves in the now famous Berg letter and at the subsequent international conference at Asilomar in California. These scientists brought to the public an awareness of potentially very grave risks in genetic engineering work. The public quite naturally reacted with some alarm.

What appeared to happen next - and I am now talking about appearances only as I don't wish to try to analyse the motives of the people concerned - was a counter reaction on the part of many scientists who felt that their work was being threatened, and who then rather unfortunately in my view decided to publicly regret that they had ever raised the alarm in the first place and to assert that genetic engineering research was safe. As the debate grew more heated, the contribution to it from all areas became more emotional, more defensive, and less illuminating. Very little hard evidence was discussed in anything like a rational manner partly because there was very little hard evidence and partly because tempers were high.

Suspicions were raised by meetings such as the one at Falmouth in the U.S. where the "expert" participants disagreed about what conclusions had been reached by the Conference. A letter written to the N.I.H. from one of the Conference organisers said that the Conference had concluded that it was unlikely that E. coli. K. 12 could be converted into an epidemic pathogen. On the one hand some participants denied that this conclusion had been reached, while on the other hand scientists who had not been there and who were anxious to prove their case, extended this statement to the point where it now appeared to say that E. coli. K. 12 could not be converted into a pathogen at all. There is obviously an immense difference between the two statements.

Another such conference was held by Cogene and the British Royal Society at Wye College last year. It was a two day conference during which assertions were made repeatedly that recombinant DNA work after all posed no hazards. In spite of the fact that this position is not universally accepted, there were no speakers invited to put an opposite point of view or to query any of the assertions made. I did in fact raise some queries from the floor and I did so in what I felt to be a fairly mild manner. In spite of this I got no replies to any of my questions, but merely provoked responses which either tried to call into question my motives for asking questions or were somewhat over emotional - such as James Watson's comment on his signature to the Berg letter: he shouted "I was a jackass" - hardly a contribution to a reasonable scientific debate or to calming public fears.

With new information about the action of introns in attempts to get expression of eukaryotic genes in prokaryotic systems, the stringency of guidelines covering recombinant DNA work is now being relaxed in most parts of the world. The potential hazards of industrial production must now be considered when each cell could produce thousands of protein molecules rather than the two or three produced in the research laboratory, and where these proteins may be exported from the bacterial cells.

There is unfortunately however, a bad taste left from the debate, from incidences such as Falmouth and Wye College, which contribute to a growing distrust of science and scientists. It has in the past been centred around such issues as the use of nuclear power, the development of biological and chemical warfare, and technological pollution of the environment. Unfortunately I think we have now added genetic engineering to this list. At least in the United States.

If you believe as I do in democracy and in the right of the people to make decisions, even when they are wrong, then the need to try to ensure that they are right will be obvious. If we extend this concept to biotechnology, then this means that there should be rational and unemotional public debate about all of the issues involved with scientists playing a key role. All evidence should be considered, whether it advances one's case or not, and information should be fairly and clearly put.

It must also be recognised that there are sometimes differences between the actual risks and the public's perception of risk. Neither can be ignored. Intelligible argument and education must be used to allay the public's fears, whether scientists feel that the fears themselves are reasonable or not. Such a process will enable the public to take informed and intelligent decisions and will I am sure enable biotechnology to go forward, perhaps at a slightly slower pace than some would wish, but with the support of the public rather than its antipathy. In addition to the other arguments in support of this process, it must be considered a significant advantage to scientists who wish to get on with their work rather than spend their time in unnecessary combat with an alarmed public.

In addition to the problem of risks, there will be important ethical questions involved in research, especially into human genetics. These questions cannot be left to scientists alone to decide, and I say this as much in the interests of scientists as of non-scientists. The development for example of in vitro fertilisation when seen together with the development of recombinant DNA work poses possibilities for the future of reshaping people. Whether or not we decide to do so depends on decisions which must be taken by the widest possible participation of the community as a whole.

So we now have a list of groups which have an interest in the future of biotechnology - scientists, employers, governments, trade unions and the public. I would now like to tell you about my experience as a member of the British Genetic Manipulation Advisory Group, because I feel that this body could well act as a model forum in which the interests of all of these groups are represented, in which discussion and debate takes place, where conflicts can be resolved, and which enables scientific work to progress with the assent and support of all concerned.

The Genetic Manipulation Advisory Group - or GMAG as it has come to be called - was established as a result of a recommendation of the Williams Working Party in 1976. The working party report also laid down guidelines for physical containment in laboratories doing recombinant DNA work and specified which types of work would be done in which types of laboratory. With the extraordinarily rapid development taking place in genetic engineering research, it was felt after a few years that the Williams guidelines were outdated, and this brings me to one of the strengths of GMAG, its ability to be flexible. Not only have we changed the whole basis on which we assign different pieces of work to different categories of laboratory, but we continue to consider the safety precautions necessary for each experiment individually, which allows us to make each decision on thoroughly up to date knowledge.

The way in which this happens is that once an experiment is first proposed, it is discussed in the laboratory concerned. The key people involved in these discussions will be those who wish to do the experiment, the biological safety officer of the establishment, and a properly constituted and representative local safety committee. In their discussions they will come to the conclusion that the proposed experiment falls within a certain category. There are four categories to choose from, category one being the least potentially hazardous, and category four being the most potentially hazardous. If their conclusion is that the experiment falls into a low category, then the work can be done but they must notify GMAG immediately. It it falls into a higher category, then they must notify GMAG and wait for advice before beginning work.

Laboratory safety committees are at least as important as GMAG for ensuring that this work is done safely. By putting an experiment in a low category they can determine that the work starts immediately, even before GMAG's advice is received. Additionally, because GMAG acts as an advisory body, and not a policing body, it is up to the local safety committees to monitor the work as it goes on and ensure that it continues within the appropriate guidelines. They can call on assistance from government health and safety inspectors and if they need further advice they can easily approach us again. But basically it will be the safety committee, the people on the spot, who will be the ones

to know what is going on in the laboratory and the only ones in a position to give an alarm, should one be necessary. As a result of GMAG's insistence on this type of system, there has been a substantial increase in awareness of safety in genetic engineering laboratories, something that has hardly begun in other biological laboratories and something that must be accounted a major achievement of GMAG. I firmly believe that had such a system been in operation in laboratories using dangerous pathogens then the death from smallpox of one if our members working at the Birmingham Medical School a year and a half ago would not have occurred.

Once a proposed experiment has been approved by the local safety committee the details are sent to GMAG with the comments of that safety committee. The more extensive and knowledgable these comments are, the eassier is GMAG's task, as we will then feel that we can place a great deal of reliance on that safety committee to monitor the situation.

GMAG will then have three choives: (1) we can advise that the work can proceed in the category proposed (2) we can advise that there should be additional precautions taken and/or that the work involved falls into a different category (3) we can advise that the particular experiment cannot be undertaken safely. In the past three years plus we have looked at hundreds of experiments. In the vast majority of cases we have agreed with the proposals as to the containment category. In some cases however we have disagreed and advised either a more stringent category and even in some cases a less stringent category from the one proposed. We have not yet advised that any experiment could not be undertaken at all.

This is the way in which GMAG works, but it's second major area of importance - in addition to creating an enhanced awareness of safety - is as a model of a forum in which different social groups can discuss and resolve potential conflicts. The constitution of GMAG is unique and was initiated as something of an experiment. It is composed of representatives of the scientific community, the public, trade unions and employers. It was constituted in this way, according to the Williams Working Party Report, to command the respect of the public as well as the scientific community. It provided, and continues to provide, an input into policy making for representatives of the public and of workers, a channel which was completely lacking on other countries where the genetic engineering debate instead was channelled into emotional confrontations in the public media.

The different groups in GMAG are not always in agreement; in fact we have many arguments. But we resolve them through debate and through give and take on all sides. As a result, on the whole, genetic engineering work has progressed smoothly and has expanded tremendously, all without significant public opposition. There is excellent scientific work going on in this field in Britain, having been assisted by the Genetic Manipulation Advisory Group.

Shirley Williams, the former Secretary of State for Education and Science, who established GMAG, was recently quoted in "Nature" as saying "as a step into a whole new area of science with a great number of public fears associated with it, you only need to have one accident for the whole genetic engineering to be at great risk, and for some parliamentarians to say ban the lot. Indeed there was quite a strong move in parliament to say that we should not do some of the experiments that fell into the containment categories. And so although I know now that scientists and even more, industrialists would criticise GMAG as being exceptionally restrictive, nevertheless as a way of showing that public fears were being taken seriously it was an important beginning. The risks may be low, but what's at stake is so high that the risk benefit equation does not involve a great deal."

If we extend the GMAG model into other areas of biotechnology then I am sure that we will have a basis for avoiding conflict and preventing antogonisms, for allowing bio-

technology to move forward as quickly and as smoothly as possible and for attracting public support in principle and in money. But it must be accepted that the control of biotechnology must be in the hands of those who work on it and those who are affected by it. No one single social group has all the answers. It is through the acceptance of this principle of co-operation, and mutual respect and regard for the concerns of all interested groups that the shining future of biotechnology will be achieved to the advantage of all.

The Astonishing Synthetic Versatility of Micro-organisms

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Micro-organisms have three important characteristics which have made them indispensible to the world of synthesis: (1) a high ratio of surface to volume which facilitates rapid uptake of nutrients and high rates of metabolism and biosynthesis; (2) a capability of carrying out a tremendous variety of reactions, and (3) a facility to adapt to a large array of different environments. As a result, the microbe has survived in competition with the chemist even in the manufacture of simple molecules such as L-glutamic acid, L-lysine and riboflavin. Microbes, by environmental and genetic manipulations, have been forced into overproducing primary metabolites to an extent exceeding 50,000 times their own requirement. Commercial exploitation of micro-organisms has yielded ethanol, amino acids, antioxidants, flavor nucleotides, organic acids, sugars, vitamins, solvents, enzymes, single cell protein, plant growth factors, antibiotics, coccidiostats, polysaccharides, alkaloids and fermented foods. In a few short years, we shall leave the petrochemical era and enter the microbiological era for energy and chemical feedstocks. Indeed, our future survival depends, to a large extent, on the microbe.

I. Introduction

The versatility of microbial biosynthesis is enormous as indicated by the products commercially produced in 1977. Todays products of the microbe vary from the simplicity of methane and acetic acid to the complexity of vitamin B_{12} and proteins. In the near future, we shall see the development of exciting processes for even simpler molecules (hydrogen) and more complex molecules (human interferon). The amazing versatility of structures of secondary metabolites provides a feast of molecules for future application.
Most of their pharmacological activities have not even been exploited yet. Indeed as we deplete our supplies of liquid fuel, we shall pass from the petroleum century into the era of biotechnology where the microbe will not only be called upon to solve our medical and nutritional problems but also those of energy and food.

For centuries, yeasts, molds and lactic acid bacteria have been used to preserve milk, fruits and vegetables and to enchance the quality of life with the resultant beverages, cheeses, bread, pickled foods, and vinegar. Interest in the mechanisms of these fermentations resulted in the investigations by Pasteur which not only advanced microbiology as a distinct discipline but also led to the development of vaccines and concepts of hygiene which revolutionized the practice of medicine. The field of biochemistry originated in the discovery by the Buchners that cell-free yeast extracts could convert sucrose into ethanol. The butyric acid bacteria, used for centuries for the retting of flax and hemp, were applied by Weizmann for production of acetone and butanol. This fermentation, with its problems of microbial and viral contamination, led the way for the large-scale use of pure cultures for the production of beneficial products. Not too many years later, the discoveries of penicillin and streptomycin and their commercial development heralded the start of the antibiotic era. Countless lives have been saved and tremendous suffering alleviated by these remarkable compounds. Today, about 100 of such agents are used to combat infections of humans, animals, and plants. Development of the penicillin and streptomycin fermentations into industrial processes led to the birth of the field of biochemical engineering.
The use of antibiotics as tools of basic research are responsible for the remarkable advances in the fields of molecular biology and genetic engineering. Following establishment of the antibiotic fermentations was the development of efficient industrial processes for the manufacture of vitamins, plant growth factors, enzymes, amino acids, flavor nucleotides, and polysaccharides. The use of such products in nutrition, agriculture, food processing and industrial processes is well-known. The discovery of the microbial conversions of

the steroid molecule yielded new drugs used for the alleviation of human suffering. Other bioconversions were soon used to devise new methods of manufacture which combined chemical and biological steps such as in the production of vitamin C and the "semi-synthetic" antibiotics.

Another important chapter in the beneficial application of the microbe is the development of single-cell protein for feeding of animals and potentially humans.

The importance of the fermentation industry results from three important characteristics of micro-organisms: (i) a high ratio of surface area to volume, which facilitates the rapid uptake of nutrients required to support high rates of metabolism and biosynthesis; (ii) a tremendous variety of reactions which micro-organisms are capable of carrying out (this especailly applies to secondary metabolism, resulting in an apparent inexhaustible supply of secondary metabolites available for commercial exploitation) and (iii) a facility to adapt to a large array of different environments, allowing a culture to be transplanted from nature to the laboratory flask, where it is capable of growing on inexpensive carbon and nitrogen sources and producing valuable compounds.

The power of the microbial culture in the competitive world of synthesis can be appreciated by the fact that even simple molecules, i.e. L-glutamic acid and L-lysine, are still made by fermentation rather than by chemical synthesis. Although a few products were temporarily lost to chemical synthesis (e.g. industrial alcohol. solvents), it is obvious that most natural products are made by fermentation. Despite the efficiency of the chemical route to riboflavin, production of this compound is still carried out by fermentation as well as by synthesis; almost complete chemical processes to vitamin C and steroids still use microbial bioconversion steps. Most natural products are so complex and contain so many centers of asymmetry that they probably will never be made commercially by chemical synthesis.

II. Regulatory Mechanisms and Industrial Fermentations

A growing micro-organism breaks down high molecular weight carbon and energy sources, brings the smaller derivatives into the cell, degrades them to smaller molecules, converts these to amino acids, nucleotides, vitamins, carbohydrates, and fatty acids, and finally builds these basic materials into proteins, coenzymes, nucleic acids, mucopeptides, polysaccharides, and lipids. Hundreds of enzymes must be made and must act in an integrated manner to avoid total chaos. To do this, regulatory mechanisms have evolved that enable a species to avoid excessive production of its metabolites so that it can compete efficiently with other forms of life and survive in nature. Some of the important control mechanisms are induction, feedback regulation, catabolite regulation, and energy charge regulation. The fermentation microbiologist, on the other hand, desires a wasteful strain, which will overproduce and excrete a particular compound that can then be isolated. Organisms from culture collections or from nature are first screened for their ability to overproduce the desired product.

At this stage, the microbiologist is searching for organisms with the weak regulatory mechanisms. Once the desired strain is found, a development program is begun to improve yields by modification of culture conditions and by mutation.

The microbiologist is actually modifying the regulatory controls remaining in the original culture so that its inefficiency can be further increased. Until very recently, these manipulations were done in total ignorance of the basic factors involved. Due to the increase in our knowledge of microbial biochemistry and genetics, we now have some ideas of these factors, and new fermentation processes are being developed on a more rational basis.

III. Enzyme Fermentations

Enzymes are becoming more and more attractive in manufacturing, analytical chemistry, and medicine. The properties of these proteins that lend themselves to extensive use include their rapidity and efficiency of action at low concentrations and under mild conditions of pH and temperature, their lack of toxicity, and the easy termination of their action by mild treatments. About 200 enzymes are available, but this number only scratches the surface, since about 2,000 enzymes have already been described. Clearly micro-organisms

are the best source of industrial enzymes, and special strains are used to make each particular enzyme.

The main reason for the attractiveness of micro-organisms as potential enzyme sources is the ease with which enzyme levels may be increased by environmental and genetic manipulations. Thousand-fold increases have been recorded for catabolic enzymes, and biosynthetic enzymes have been increased by several hundred-fold. Of course, the higher the specific activity, the simpler will be the job of enzyme isolation. Other reasons for using microbial cells as sources of enzymes are:

1. Enzyme fermentations are economical on a large scale because of short fermentation cycles and inexpensive media.
2. Screening procedures are simple and thousands of cultures can be examined in a reasonably short time.
3. Different species produce somewhat different enzymes catalyzing the same reaction, allowing flexibility with respect to desired operating conditions in the reactor.

In devising an enzyme fermentation it is important to begin with the most active strain available. Known enzyme-producing strains are often obtained from workers in the field or from culture collections. In the absence of such cultures, a screening program is devised in which cultures from nature or from collections are examined for enzyme activity. The major requirement in screening is simplicity, so that rapid examination of a large number of strains is feasible.
Once a good strain is obtained, fermentation parameters are optimized to maximize growth and enzyme production. Of importance here are temperature, pH, and oxygen transfer. Also important is the nutrition of the micro-organism, especially with respect to sources of carbon, nitrogen, phosphorus, sulfur, and mineral salts. Often, especially with extracellular enzymes, the addition of surfactants is important. Non-ionic surfactants are generally preferred over anionic and cationic agents which are usually toxic to the micro-organism.
In addition to the factors outlined above, the genetic regulation of enzyme biosynthesis is exploited in the development of enzyme fermentations. The important factors here are enzyme induction, feedback repression and carbon or nitrogen catabolite repression. Inducers are added which can increase enzyme formation as much as one thousand fold. Alternatively, genetic mutations can be carried out which allow high enzyme production in the absence of inducer. Feedback repression can be combatted by the addition of pathway inhibitors, by limitation of the growth factor supply to auxotrophic mutants, by the use of slowly-utilized derivatives of the required growth factor, or by the slow growth of bradytrophic mutants. Catabolite repression can be avoided by the use of slowly utilized carbon or nitrogen sources. Genetic solutions to the problems of feedback repression and catabolite repression involve the isolation of regulatory mutants which are no longer sensitive to these controls.
Regulatory mutants have often been obtained which produce more enzyme than their parent even under the most favorable condition found for the parent. For example, Bacillus licheniformis mutants produce ten times more x-amylase than their glucose-sensitive parents even in the absence of glucose.
In some cases it has been found that the hyperproducing mutants contain multiple copies of the structural gene coding for the enzyme. Since increasing the number of copies of a gene increases production of its specific enzyme, the ability to do this intentionally is desirable. In micro-organisms, an increase in gene copies by genetic manipulation has been achieved by transferring plasmids (extrachromosomal DNA segments) or by the use of transducing phage. Production of several enzymes have been increased many fold by transfer of plasmids containing the respective structural genes into recipient cultures. In a similar way, large increases in production of enzymes have been achieved by using transducing phage containing the structural gene coding for these enzymes.

IV. Production of Primary Metabolites

Primary metabolites are the small molecules of all living cells that are intermediates or

end products of the pathways of intermediary metabolism or are used as building blocks for essential macromolecules or are converted into co-enzymes. The most industrially important are the amino acids, nucleotides, vitamins, solvents, and organic acids. Organisms producing such products are fantastic in their degree of overproduction. The riboflavin producer makes over 20,000 times more vitamin than it needs for its own growth; with vitamin B_{12}, the figure is 50,000.

In biosynthetic pathways leading to primary metabolites, the main control mechanism is feedback regulation which involves inhibition of an early biosynthetic enzyme and/or repression of one or more of the biosynthetic enzymes by the final product or its derivative. Most processes designed to produce primary metabolites work by limiting the intracellular concentration of feedback inhibitors or repressors. Usually, one employs an auxotrophic (i.e. a nutritional) mutant. Since such an organisme requires the end product for growth, one can limit the intracellular concentration of this inhibitory or repressive end product by feeding it to the culture at growth-limiting levels; feedback regulation is then bypassed, and high levels of the desired intermediate accumulate.
A second way to eliminate feedback regulation and accumulate primary metabilites is to alter the structure of the enzyme subject to inhibition or to modify the regulatory genes so that the system is no longer repressible. This modification is done by selecting mutants that resist the toxic effects of an analogue of the desired product. The plating of a mutagenized population of cells on such an antimetabolite will kill of the cells and select for resistant mutants, some of which overproduce the metabilities.

a. Amino acids

Feedback regulation is bypassed by producing an auxotrophic mutant and partially starving it for its requirement. High levels of amino acids are obtained by such a procedure. A second means to bypass feedback regulation is to produce mutants resistant to a toxic analogue of the desired metabolite. Some of these overproducing resistant mutants are deregulated in amino acid production whereas others are transport mutants which are unable to keep the amino acid in the cell. Successive resistance mutations imposed on a partial auxotroph ("bradytroph") lead to an increase in production of amino acids. Such a combination of auxotrophic and resistance mutations is common in current primary metabolite processes.
Permeability alteration is very important in the production of L-glutamic acid, the major amino acid of commerce. About 550 million pounds of monosodium glutamate, a potent flavor enhancer, are made annually, all by fermentation. The glutamic acid bacteria include many organisms with different names, e.g. species of Micrococcus, Corynebacterium, Brevibacterium and Microbacterium; however all are taxonomically similar and should be included in a single genus.
All glutamate-overproducers have a block in the tricarboxylic acid cycle, i.e. they are deficient in x-ketoglutarate dehydrogenase, thus the carbon flow is shunted to glutamic acid. Normally, glutamic acid overproduction would not occur due to feedback regulation of phosphoenolpyruvate carboxylase, citrate synthase and glutamate dehydrogenase by glutamate. Glutamate dehydrogenase is also inhibited by glutamate. However, due to a decrease in the effectiveness of the barrier to outward passage, glutamate leaves the cell thus allowing its biosynthesis to proceed unabated. The premeability alteration is intentionally effected by various manipulations including biotin limitation (all glutamic acid bacteria are biotin auxotrophs), glycerol limitation of glycerol auxotrophs, oleate limitation of oleate auxotrophs, or addition of penicillin or fatty acid derivatives to exponentially growing cells. Apparently all of these manipulations result in a phospholipid-deficient cytoplasmic membrane which favors exit of glutamate from the cell and extracellular accumulation of over 100 g/liter.
The bulk of the cereals consumed in the world are deficient in the essential amino acid, L-lysine. Lysine supplementation converts such cereals into balanced food or feed. Homoserine-requiring mutants of the glutamate-overproducer, Corynebacterium glutamicum, produce large amounts of lysine when grown under the proper conditions, i.e. 75g lysine/liter at a weight yield of 25% to 30% of consumed glucose. Concentrations of biotin optimal for growth must be provided to the culture. If suboptimal concentrations

are provided, glutamatic rather than lysine is excreted. The reason is that nitrogen assimilation by the glutamate-overproducers occurs only through the reductive animation of α-ketoglutarate to glutamate, the reaction being catalysed by the NADP-linked glutamate dehydrogenase. Thus, the nitrogen of all of the natural amino acids is derived from internal glutamate by transamination. When the permeability mechanisme is altered by limitation of biotin, the glutamate is lost from the cell and is no longer available as an intracellular nitrogen donor for lysine synthesis.
The key to lysine overproduction is the avoidance of feedback inhibition by: (a) limitation of threonine, a feedback inhibitor of aspartokinase; and by (b) possession of a feedback-insenstive dihydrodipicolinate synthase. Lysine belongs to the aspartic acid family, being produced from aspartate in a branched pathway along with threonine, methionine and isoleucine.

b. Flavor nucleotides

The interest in nucleotide fermentations is due to the activity of three purine ribonucleoside 5'-monophosphates, namely, guanylic acid (guanosine 5'-monophosphate; GMP), inosinic acid (inosine 5'-monophosphate; IMP);and xanthylic acid (xanthosine 5'-monophosphate; XMP), as enhancers of flavor (in order of decreasing potency). Some 2500 tons of GMP and IMP are produced annually in Japan alone. Three main processes are used: (1) hydrolysis of yeast RNA by fungal nuclease plus enzymatic deamination of AMP to IMP; (2) fermentative production of nucleosides plus chemical phosphorylation and (3) direct fermentation of sugar to IMP plus conversion of guanine to GMP by salvage synthesis.
The key to effective purine derivative accumulation is the limitation of intracellular AMP and GMP. This limitation is best effected by restricted feeding of purine auxotrophs. Thus, adenine-requiring mutants accumulate inosine that results from breakdown of intracellularly accumulated IMP. Further mutation to purine-analogue resistance has yielded a mutant producing 31 g of inosine per liter. Other mutants produce guanosine at a level of 16 g/l.
Until 1964, no successful direct fermentation leading to production of nucleotides had been reported. An important advance, however, came with the discovery that the glutamate-overproducers could excrete intact nucleotides. When grown in media containing growth-optimal concentrations of biotin, adenine auxotrophs of C. glutamicum produce IMP. GMP was found to be produced best by salvage synthesis using Brevibacterium ammoniagenes. The 'salvage synthesis' reaction:

$$\text{purine} + \text{PRPP} \longrightarrow \text{purine nucleotide} + \text{pyrophosphate}$$

had been known for years to proceed inside cells of microbes that are provided with pre-formed purines; it had been demonstrated with cell-free extracts and with purified enzyme but had not been known to occur extracellularly with intact cells not given PRPP. Investigators soon found that, as long as certain fermentation requirments were met, several purine bases could be added to wild-type cultures of B. ammoniagenes for conversion to their respective nucleotides. Thus GMP, the most potent nucleotide flavoring agent can be produced by adding guanine to cells of B. ammoniagenes.

c. Vitamins

Riboflavin (vitamin B_2) is produced commercially by both fermentation and chemical synthesis. Whereas the chemically synthesized material is generally used for pharmaceuticals and food, riboflavin produced by fermentation is used in animal feed.
The vitamin is generally present in microbial cells as the coenzymes flavin mononucleotide (FMN) and flavin adenine nucleotide (FAD), which are bound to protein. Riboflavin overproducers contain as much FMN and FAD as normal micro-organisms but they also have a large intracellular pool of free riboflavin. Normal micro-organisms excrete less than 10 mg riboflavin/liter. The overproducers can be divided into three groups. The low overproducers include the clostridia, which can produce, at best, about 100 mg/liter. Yeasts, especially Candida species, are moderate overproducers that can be made to yield about 600 mg riboflavin/liter. The high overproducers are two yeastlike molds,

Eremothecium ashbyii and Ashbya gossypii, which synthesize riboflavin in concentrations higher than 10,000 mg/liter.
The biochemical key to riboflavin overproduction appears to involve iron. Ferrous ion severly inhibits riboflavin production by low overproducers; it inhibits formation by the moderate overproducers to a lesser extent, and has no inhibitory action against E. ashbyii and A. gossypii. I favor a hypothesis that an iron-flavo protein is a repressor of riboflavin synthesis. If such an enzyme or a non-enzyme iron-flavo protein were the repressor or riboflavin synthesis, growth in iron-deficient media would produce cells with little or no repressor, and flavin synthesis would be derepressed. Another possibility is that iron represses the riboflavin biosynthetic enzymes whereas riboflavin or a derivative inhibits the first enzyme of the pathway.
Vitamin B_{12} is the other vitamin made by fermentation. Two very different types of bacteria are used by the industry, i.e. Propionibacterium shermanii and Pseudomonas denitrificans. The key to the P. shermanii fermentation is apparently avoidance of feedback repression by vitamin B_{12}. Thus the early stage is conducted under anaerobic conditions in the absence of the precursor, 5,6-dimenthylbenzimidazole. These conditions prevent vitamin B_{12} synthesis and allow for the accumulation of the intermediate, cobinamide. Then the culture is aerated and dimenthylbenzimidazole is added, converting cobinamide to the vitamin. In the P. denitrificans fermentation, the entire process is carried out under conditions of low oxygen transfer but the key to the fermentation is the methyl donor, betaine (or choline). Vitamin B_{12} formation by P. denitrificans is totally dependent upon betaine but the mechanism of control is completely unknown.

d. Organic acids

Filamentous fungi have been widely used for the commercial production of organic acids. With respect to amounts, citric acid is the major product made by mycelial fungi. About 300 million kilograms of citric acid are produced per year. The commercial process employs Aspergillus niger in media deficient in iron and manganese. The decisive role of iron limitation is thought to be due to the need of high iron by aconitase (the enzyme which converts citrate to isocitrate) for its induction, activity, and/or stability. The main features of the fermentation are a high initial concentration of sugar (about 15%), low levels of magnesium and iron and a pH value below 3,5. Phosphate and nitrogen are usually supplied at very low levels. The incubation temperature is around 30°C and highly aerobic conditions are necessary. In approximately 4 to 5 days the major portion (80%) of the sugar is converted to citric acid, titers reaching about 100 g/liter.
Citric acid is widely used in the food and pharmaceutical industry as an acidifying agent. It also serves an antioxidant for inhibiting rancidity in fats and oils. Citric acid and its salts are used as buffers in such foods products as jams and jellies as well as stabilizers in a variety of foods. The pharmaceutical industry uses approximately 16% of the available supply of citric acid.
In recent years, new processes have been developed for the production of citric acid by Candida yeasts, especially from hydrocarbons. Such yeasts are able to convert n-paraffins to citric and isocitric acids in extremely high yields (150-170% on a weight basis). Production of citirc acid over isocitric acid is favored by selecting mutants which are deficient in the enzyme aconitase.

e. Solvents

Ethyl alcohol represents a primary metabolite that can be produced by fermentation of any carbohydrate material containing a fermentable sugar or a polysaccharide that can be depolymerized to a fermentable sugar. Yeasts are preferred for these fermentations but the species to be utilized is determined by the substrate added to the medium. Saccharomyces cerevisiae is generally employed when the alcohol is derived from the fermentation of hexoses, whereas Kluyveromyces fragilis, Candida utilis or Candida pseudotropicalis may be utilized if lactose or pentoses are the substrates.
Under optimum conditions, within a realistic period time, approximately 10 to 12% alcohol by volume is readily obtained. This concentration of alcohol slows down growth

and the fermentation ceases. With special yeasts, the fermentation can be continued to alcohol concentrations in the range of 20% by volume. However, these concentrations are attained only after months or years of fermentation, such as in the case of wines. In general the commercial production of alcohol by fermentation is completed within a five day period, alcohol concentrations approximating 12% by volume. At present alcohol is mainly manufactured by the petrochemical industry from ethylene. However, with the prices of petroleum increasing and an expected abundance of grains, it appears likely that the fermentation process for ethyl alcohol will be reinstituted. The prospects are that the fermentation process will be competitive with those of the chemical process industries. In this regard, organisms such as clostridia are being reexamined after years of neglect. Clostridium thermocellum, an anaerobic thermophile, can convert waste cellulose directly to ethanol. Other clostridia produce acetate, lactate, acetone and butanol and will be utilized more and more as the liquid fuel crisis deepens.

V. Bioconversions

In addition to the multireaction sequences of fermentations, micro-organisms are extremely useful in carrying out processes in which a compound is converted into a structurally related product by the use of one or a small number of enzymes contained in cells. Such bioconversions (or microbial transformations) may be carried out with growing cells, resting cells, spores or dried cells.
One of the earliest bioconversions known was the quantitative conversion of ethanol to vinegar by the acetic acid bacteria. This group of bacteria is especially useful in carrying out incomplete oxidations of organic compounds and is used commercially in the oxidation of sorbitol to sorbose, the single biological step in the otherwise chemical production of vitamin C (ascorbic acid).
Bioconverting organisms are known for practically all types of chemical reactions. The reactions are stereospecific, the ultimate in specificity being exemplified by the steroid bioconversions. This specificity is exploited in the resolution of racemic mixtures of amino acids and intermediates of prostaglandin synthesis. In many cases, the bioconversion reaction is preferred over a chemical step when a specific isomer rather than a racemic mixture is desired. Bioconversions are characterized by extremely high yields, i.e. from 90 to 100%.
Other attributes include mild reaction conditions and the coupling of reactions using a micro-organism containing several enzymes working in series.
In developing bioconversions, it is important to examine the regulation of enzyme synthesis during growth since the "quality" of a bioconverting cell population depends on the concentration of enzyme in those cells. Often inducers are useful and it is imperative to avoid catabolite repression. Mutation can be used to eliminate further catabolism of the desired product. Permeability is often a problem with respect to contact of the substrate with the enzyme in the cell. In certain processes, Mn^{++} deficiency or addition of surface active agents has been used to decrease the effect of the permeability barriers. It is sometimes desirable to grow cells on one substrate and convert a different substrate; this is known as "cometabolism". Problems of product inhibition of bioconversions can be solved by addition of ion exchange resins or by dialysis culture. Mixed cultures or sequential addition of cells have been used to carry out bioconversions involving several steps in series catalyzed by different cultures. The problem of insoluble substrates, especially prevalent in the steroid field, can be resolved by using finely divided suspensions of substrates, suspensions in suface-active agents such as Tweens, or soluble complexes or esters of substrates. In recent years, there has developed a tremendous interest in immobilized cells to carry out such processes. These are usually much more stable than either free cells or enzymes and more economical than immobilized enzymes.
Gluconic acid is produced via bioconversion of glucose by a large variety of fungy including the A. niger group as well as many species of Penicillium. The medium often contains glucose concentrations approaching 30%. Gluconic acid in the form of its calcium salt is used pharmaceutically for calcium deficiency. Sodium gluconate is employed as a sequestering agent to prevent the deposition of soap scums on cleaned surfaces. The free acid finds use as a mild acidulant in a variety of industrial processes (metal processing, leather tanning, and foods).

VI. Secondary Metabolites ("Idiolites")

Microbially produced secondary metabolites are extremely important to our health and nutrition. As a group that includes antibiotics, toxins, alkaloids, and plant growth factors, they have tremendous economic importance.

Idiolites are produced in batch culture during the idiophase (a phase which may overlap but often follows the growth phase or trophophase), have no function in growth (although they probably contribute to survival of a particular producing organism in nature), are produced by certain restricted taxonomic groups of organisms, and are usually formed as a mixture of closely related members of a chemical family. Production ability is easily lost by mutation or plasmid loss (strain degeneration).

The best known of the secondary metabilites are the antibiotics. About 3500 antibiotics have been described, 2500 from actinomycetes alone, and they still are being discovered at a rate of about 50-100 per year. Some species are remarkable in their ability to produce antibiotics. For example, Streptomyces griseus produces over 40 different antibiotics and Bacillus subtilis over 60.

Environmental manipulation of culture media in any development program often involves the testing of hundreds of additives as possible precursors of the desired product. Occasionally, a precursor that increases production of the secondary metabolite is found. The precursor may also direct the fermentation towards the formation of one specific desirable product; this is known as "directed biosynthesis". An example is the use of phenylacetic acid in the benzylpenicillin (penicillin G) fermentation. In many fermentations, however, precursors show no activity because their syntheses are not rate-limiting. In such cases, screening of additives has often revealed dramatic effects, both stimulatory and inhibitory, of nonprecursor molecules which are due to interaction of these compounds with the regulatory mechanisms existing in the fermentation organism.

The regulatory mechanisms prevalent in secondary metabolism are essentially the same as found in primary metabolism. Thus chloramphenicol limits its own synthesis by repressing arylamine synthetase and ergot alkaloids limit their production by inhibiting dimethylallyl transferase. In both cases, these are the initital enzymes unique to the secondary pathway.Glucose, a sugar usually used rapidly for growth, exerts carbon catabolite repression and inhibition of idiolite synthesis. Similarly ammonium ion or rapidly-used amino acids exert nitrogen metabolite regulation on secondary metabolism. Enzyme induction is also evident in the production of alkaloids and antibiotics. Inorganic phosphate exerts a strong negative effect in secondary metabolite fermentations by an unknown mechanism which may involve ATP or some other nucleotide.

Since the production of secondary metabolites appears to be affected by the same regulatory mechanisms that control primary metabolism, and because these mechanisms are genetically determined, mutation has had a major effect on the production of secondary metabolites. Indeed, it is the chief factor reponsible for the hundred- to thousand-fold increases obtained in production of antibiotics from the time of their initial discovery to the present. These dramatic improvements essentially resulted from procedures involving mutagenesis followed by the testing of random survivors and of morphological (and color) mutants. Although the basic rationales are still unclear, the following additional mutant types have shown to include improved producers of secondary metabilites: auxotrophs, "revertants" of auxotrophs, "revertants" of non-producing mutants, amino acid analogue-resistant mutants and mutants resistant to the secondary metabolites which they produce.

Mutation can be used to create new antibiotics. Mutation of Streptomyces aureofaciens led to the commercial production of 6-demethylchlortetracycline and of 6-demethyltetracycline. These antibiotics have greater stability to acid and alkali than the original methylated tetracyclines and are now commercial antibiotics. Another example is the color mutant of Streptomyces peucetius which produces 14-hydroxydaunomycin (adriamycin) instead of the parental product, daunomycin.

A further development in the use of mutation for the production of new antibiotics is called "mutational biosynthesis". In this procedure, a mutant is isolated which cannot make a part of the antibiotic; thus antibiotic production depends on supplementation of the medium with the missing moiety. Such a mutant is called an "idiotroph". When the mutant is fed analogues of the missing part, new antibiotics are produced. Use of mutational biosynthesis

had led to the discovery of many new antibiotic derivatives especially aminocyclitol antibiotics.

VII. How Antibiotic-producing Micro-organisms Avoid Suicide

Because antibiotics are among the most potent compounds made by living organisms, it is remarkable that producing strains can remain metabolically active and viable in their own environment, which in some cases contain a concentration of antibiotic as high as 50 mM. Despite this apparent insensitivity, when a producing strain is inoculated into a fresh medium that contains an even lower concentration of antibiotic, adverse effects on growth are observed. For example, Streptomyces niveus fails to grow when inoculated into a medium that contains 50-75 ug/ml of novobiocin, its own antibiotic; yet this strain is capable of producing more than 500 μ g/ml.

Micro-organisms grow and synthesize antibiotics to which they are sensitive by elaborating their secondary products only after having passed through most of their rapid growth phase. Antibiotic is not formed until after growth in batch culture because enzymes specifically involved in antibiotic biosynthesis are respressed or inhibited during growth. Then, the synthesizing organism physiologically develops resistance during the early idiophase. The mechanisms of resistance are no different than those evolved by target micro-organisms in the clinical setting. Permeability modifications are involved in many instances. Antibiotics are pumped out of cells against a concentration gradient. A decrease in inward permeability during the idiophase protects the organism from high extracellular concentrations of its own antibiotic.

Additional mechanisms protecting cells from internal antibiotic include the synthesis of enzymes that modify the antibiotic into active or less active derivatives. Another mechanism involves a modification in the machinery of the producer, e.g. the ribosome, which serves as target of the particular antibiotic. Another means by which antibiotic producers protect themselves is by feedback inhibition and/or repression of antibiotic production.

VIII. The Future of Applied Microbiology

As long as people, animals, and plants suffer from diseases thought to be related to micro-organisms, challenges and opportunities exist. Better and safer antibiotics are needed to combat Gram-negative infections. Truly nontoxic antifungal agents for systemic use have yet to be discovered. Agents active against protozoal infections are required. In the area of viral diseases and cancer, a real need exists for effective antiviral and antitumor agents.

Many other areas are ripe for development. Economical fermentations for essential amino acids such as tryptophan and methionine are yet to be devised. The field of bacterial and viral insecticides is sure to be expanded. Human, agricultural, and industrial wastes will be transformed from a pollution problem into a resource with development of cellulose and lignin fermentation organisms.

The area of enzyme application is certainly going to increase in the next few years, especially in the areas of analytical chemistry (e.g., detection of pesticide residues), waste disposal (e.g., destruction of chemical wastes), food technology, and medicine. Hundreds of untested intracellular enzymes, particularly those with biosynthetic activity, await exploitation by future applied biologists and engineers.

For years, the major drugs (such as hypertensive and anti-inflammatory agents) used for noninfectious diseases have been strictly synthetic products. Similarly major therapeutics for parasitic diseases in animals (e.g. coccidiostats and antihelmintics) resulted from screens of synthesized compounds followed by molecular modification. Despite the testing of thousands of compounds, only a few promising structures have been uncovered. As new lead compounds become more and more difficult to find, microbial broths will have to fill the void. It is not wishful thinking to predict that microbial products will gain importance in therapy of nonmicrobial diseases. Already products such as monensin and lasalocid dominate the coccidiostat market. New opportunities exist for applied scientists who can devise simple tests for screening microbial broths for such activities. Indeed, workers

in Japan have isolated many microbial products with important pharmacological activities by using simple enzymatic assays. The field has a bright future indeed.
The birth of recombinant DNA technology in 1973 has already made an impact on the field of microbial technology. This ability to recombine the genetic material of widely-differing species has yielded Escherichia coli strains which can make mammalian proteins including human insulin and human interferon.
Thus a new facet has been added to make even more astonishing the synthetic versatility of micro-organisms.

Table 1 Microbial Products

Antibiotics
Adriamycin
Amphomycin
Amphotericin B
Avoparcin
Azalomycin F

Bacitracin
Bambermycins
Bicyclomycin
Blasticidin S
Bleomycin

Cactinomycin
Candicidin B
Candidin
Capreomycin
Cephalosporins
Chromomycin A_3
Colistin
Cycloheximide
Cycloserine
Chlortetracycline

Dactinomycin
Daunorubicin
Destomycin
Demeclocycline

Enduracidin
Erythromycin

Fortimicins
Fumagillin
Fungimycin
Fusidic acid

Gentamicins
Gramicidin A
Gramicidin J (S)
Griseofulvin

Hygromycin B

Josamycin

Kanamycins
Kasugamycin
Kitasatamycin

Lasalocid
Lincomycin
Lividomycin

Mepartricin
Midecamycin
Mikamycins
Mithramycin
Mitomycin C
Mocimycin
Monensin
Myxin

Neomycins
Novobiocin
Nystatin

Oleandomycin
Oligomycin
Oxytetracycline

Paromomycins
Penicillin G
Penicillin V
Peniciliins (semisynthetic)
Pentamycin
Pimaricin
Polymyxins
Polyoxins
Pristinamycins

Quebemycin

Ribostamycin
Rifamycins

Sagamicin
Salinomycin
Siccanin
Siomycin
Sisomicin
Spectinomycin
Streptomycins

Tetracycline
Tetranactin
Thiopeptin
Thiostrepton
Tobramycin
Trichomycin
Tylosin
Tyronthricin
Tyrocidine

Uromycin

Validamycin
Vancomycin
Variotin
Viomycin
Virginiamycin

Enzymes
Amylases
Amyloglucosidase
Anticyanase
L-Asparaginase

Catalase
Cellulase

Dextranase
'Diagnostic enzymes'

Esterase-Lipase

Glucanase
Glucose dehydrogenase
Glucose isomerase
Glucose oxidase
Glutamic decarboxylase
Gumase

Hemi-cellulase
Hespiriginase

Invertase

Lactase
Lipase

Microbial rennet

Naringinase

Pectinase

Pentosanase
Proteases

Streptokinase-streptodornase

Uricase

Organic Acids
Citric acid
Comenic acid

Erythorbic acid

Gluconic acid

Itaconic acid

2-Keto-D-gluconic acid
α-Ketoglutaric acid

Lactic acid

Malic acid

Urocanic acid

Solvents
Ethanol

2,3-Butanediol

Vitamins and Growth Factors
Gibberellins

Riboflavin

Vitamin B_{12}

Zearalanol

Nucleosides and Nucleotides
5'-Ribonucleotides and nucleosides

Orotic acid

Ara-A-(9-(β-D-arabinofuranosyl) adenine
6-Azauridine

Amino acids
L-Alanine
L-Arginine
L-Aspartic acid
L-Citrulline
L-Glutamic acid
L-Glutamine
L-Glutathione
L-Histidine
L-Homoserine
L-Isoleucine
L-Leucine
L-Lysine
L-Methionine
L-Ornithine

L-Phenylalanine
L-Proline
L-Serine
L-Threonine
L-Tryptophan
L-Tyrosine
L-Valine

Miscellaneous Products and Processes
Acetoin
Acyloin
Anka-pigment (red)

Blue cheese flavor

Desferrioxamine
Dihydroxyacetone
Dextran
Diacetyl (from acetoin)

Ergocornine
Ergocristine
Ergocryptine
Ergometrine
Ergotamine

Bacillus thuringiensis insecticide

Lysergic acid

Paspalic acid
Picibanil

Ribose

Scleroglucan
Sorbose (from sorbitol)
Starter cultures
Sterol oxidations
Steroid oxidations

Xanthan

Table 2 Examples of Classes of Organic Compounds in which Secondary Metabolites are Found.

Amino sugars	Lactones	Pyridines
Anthocyanins	Macrolides	Pyrones
Anthraquinones	Naphthalenes	Pyrroles
Aziridines	Naphtaquinones	Pyrrolidones
Benzoquinones	Nitriles	Pyrrolines
Coumarins	Nucleosides	Pyrrolizines
Diazines	Oligopeptides	Quinolines
Epoxides	Perylenes	Quinolinois
Ergoline alkaloids	Phenazines	Quinones
Flavonoids	Phenoxazinones	Salicylates
Furans	Phtaldehydes	Terpenoids
Glutaramides	Piperazines	Tetracyclines
Glycopeptides	Polyacetylenes	Tetronic acids
Glycosides	Polyenes	Triazines
Hydroxyamines	Polypeptides	Tropolones
Indole derivatives	Pyrazines	

Table 3 Examples of Pharmacological Activities of Microbial Secondary Metabolites

Anabolic	Estrogenic	Nephrotoxic
Anesthetic	Hallucinogenic	Neurotoxic
Analeptic	Hemolytic	Paralytic
Anorectic	Hemostatic	Parasympathomimetic
Anticoagulant	Hepatotoxic	Photosensitizing
Antiinfective	Herbicidal	Pyrogenic
Antiinflammatory	Hypocholesterolemic	Sedative
Antilipimic	Hypoglycemic	Spasmolytic
Cardiotoxic	Hypotensive	Teleocidal
Convulsant	Hypersensitizing	Teratogenic
Dermonecrotic	Inflammatory	Tremorigenic
Edematous	Insecticidal	Ulcerative
Emetic	Leukemogenic	Vasodilatory
Erythematous		

Enzyme technology and enzyme production

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Introduction

The technology area normally designated 'Enzyme Technology' or 'Enzyme Engineering' is only a part of what to day is called 'Biotechnology'. A precise definition of the term enzyme technology has not yet been agreed upon, but there is a tendency to limit it to non-growth associated practices and to the use of modified or immobilised enzymes. The enzymes used can vary from highly purified preparations to homogenised microorganisms or just centrifuged cell slurries, and the unit operations from simple batch reactions to sophisticated continuous systems.

Enzyme technology as a part of biotechnology may contribute to the solution of some of the most vital problems our society of to day is confronted with:

- Food production, reduction in costs and improved quality,
- Energy supplies,
- Preservation and improvement of environment,
- Health of man.

This listing of activity areas for biotechnology has been elaborated by the Commission of the European Communities and illustrates the vast potential for the technology, which is the topic of this TNO Conference.

It is impossible in the time available to give a comprehensive review of the present state of enzyme technology; instead it will be attempted to illustrate the research and development going on in the field with a few examples taken from activities in Novo's enzyme laboratories.

Production of Soy Bean Protein

In the 1960's and the early 1970's a huge effort was made by many companies and scientific institutions to develop production methods for single cell protein by fermentation using oil fractions or methanol as the raw material. The background for this work was the fear of not being able to supply the fast growing population of the world with adequate protein nutrient, and many successful production methods were developed. However, the rising oil prices after the Middle East War in 1973 eroded the economic basis for the methods developed and to day only a limited production of single cell protein is taking place.

The major concern of the public has in the last years been concentrated upon the energy issue, and this has reduced the interest for the food supply problem. However, it must be remembered that the world population is still expanding at an alarming rate; every day 400,000 children are born, 350,000 of them in developing countries and by the year 2000 it is expected that the world population will be at least 6 billion, 50 per cent higher than to day's population. These figures emphasize the need for allocation of large resources in order to solve the food supply problem, and better utilisation of vegetable protein sources is one of the most realistic ways.

In Asia the soy bean has for thousands of years been a very important nutritious food, but the raw bean has only found limited human consumption because of its

- indigestibility due to trypsin inhibitors,

- flatulence inducing carbohydrates, and
- beany taste.

In order to compensate for these disadvantages many different techniques have been applied for the production of nourishing and palatable foods from soy beans. Some of these foods are produced by fermentation with fungi such as soy sauce, tempeh and miso, while others are based on physical or chemical treatments.

These developments, stretching over hundreds of years, have resulted in a very effective utilisation of the vegetable proteins, while the main protein nutrient in the Western World has been animal protein produced by feeding animals with vegetable proteins, e.g. soy flakes. This of course gives a much poorer utilisation of the nutritive value of the soy protein and it is doubtful if this luxury can be continued in the long run when taking into consideration the growing global need for nutrient protein. To day more than 90% of the soy bean is used for cattle feed and only a few per cent in food products such as luncheon meat, sausages, gravies, etc., where its use is advantageous due to its functional properties and low price.

The soy processing industry produces two special products for a variety of food applications, i.e. soy concentrate and soy isolate, the first having a protein content of approximately 70% and the isolate a content of 90% or above. The protein yield by the production of concentrate is high, approximately 90% while the yield in the isolate process is as low as 60%. It would, therefore, be of great economical importance if the yield in the isolate process could be increased to that of the concentrate process while maintaining the purity at minimum 90%.

It appears from Table I that dehulled and defatted soy beans contain about 50% protein and 33% carbohydrates, of which approximately 50% are water soluble and 50% insoluble. It is the specific removal of the insoluble carbohydrate fraction consisting of pentosans and galactans which is the crucial problem as they can only be removed by using the isolate process, which - as mentioned - is characterised by a low yield. Furthermore, the isolate process results in a product with a decreased protein efficiency ratio (PER value) because the protein fractions lost have a high content of methionine. The goal must, therefore, be to produce an isolate with the same PER value as the starting material and in the same yield as in the concentrate process.

The starting material in the concentrate process (Fig. 1) is defatted soy flakes, and the soluble sugars, ash and minor components are removed by extraction with a dilute acid at pH 4.5 or dilute ethanol leaving the bulk of protein and the insoluble carbohydrates. After separation and neutralisation the product is dried.

In the isolate process (Fig. 2) defatted soy flakes are also the starting material. The soy protein is brought into solution by extraction with alkaline water, and after separation the protein is precipitated by adjusting the pH to 4.5, the isoelectric point. After washing, the product may be dried in the isoelectric form or it may be neutralised before drying.

The raw materials to be used for the two processes should be minimum heat-treated and generally possess high protein solubility in order to ensure high yields and good functionality. Using such raw materials the separation procedures must be so efficient that the main part of trypsin inhibitors soluble at pH 4.5 are removed. The idea behind the new method, which is under development, is to modify the concentrate process by including an enzymatic hydrolysis of the insoluble carbohydrates in the extraction step. An enzyme system meeting these requirements should have the following properties:

- The solubilisation of the insoluble polysaccharides shall be nearly quantitative in order to obtain a sufficiently pure product.
- Only a negligible hydrolysis of the protein must take place.

- The enzyme system shall have a high activity at the isoelectric point of soy protein.
- The processing costs shall be so low that it gives economic incentive to the soy processing industry.

Development of such an enzyme system has been under way for several years, and in Table II the expected results are compared with the corresponding results obtained when using the classical concentrate process. It appears that with a solubilisation of more than 85% of the carbohydrate fraction the desired protein purity of 90% can be attained. If no protein is solubilised by the enzyme system it should be possible to reach a protein yield of approximately 87%, which is more than 20% higher than attainable by the classical isolate process.
In Fig. 3 the results of an experimental run with one of the developed enzyme systems are shown. The pH has been kept at 4.5 and reaction time and temperature have been chosen to 120 minutes and 50° C, respectively, conditions which easily can be maintained in large scale production. It appears that with a suitable enzyme dosage it is possible to obtain nearly the desired solubilisation of the carbohydrates. Fig. 4 shows the protein purity obtained in the same experiments, and it is seen that already at a moderate enzyme dosage a purity of approximately 84% is obtained, which is rather close to the goal of 90%.

The cause of the insufficient purity is the loss of protein substance by enzymatic hydrolysis and the enzyme has to be further purified with regard to protease before a satisfactory industrial process is finally developed. Of course this will be time-consuming but the presented results justify a certain optimism with regard to the industrial future of the process.

Enzyme Technology in the Chemical Process Industry

With the exceptions of the classical fermentations such as alcohol, acetone-butanol, citric acid, acetic acid etc. biotechnology has until now had little importance for the chemical process industry. However, an increasing biotechnological effort can be expected within this area as there is a real technological need for new synthetic methods, where extreme reaction conditions such as high pressures and temperatures, high concentrations of acids or alkali, toxic intermediates, etc. are avoided and substituted by enzymatic catalysis. Many of the present processes can be hazardous to the factory personnel and are sometimes highly polluting, giving rise to concern among the public and the regulatory bodies in the different countries. In the last years the situation has been aggravated by some very serious accidents involving several casualties and a severe pollution of the environment.

Another reason for the interest in enzyme technology from the side of the chemical process industry is the need for processes which are more specific than the present ones based on classical organic chemistry. The goal is the reduction of the number of synthetic steps and a reduction of by-product formation giving a better overall yield and economy. Examples of this development are steroid oxidations, resolution of racemic amino acid mixtures and production of 6-amino penicillanic acid (6-APA) from Penicillin G and V.

At present a vast effort is being made at universities and other academic institutions to produce immobilised cofactor dependent enzyme systems capable of carrying out oxido-reductive reactions on an industrial scale. If this effort succeeds a real breakthrough for enzyme technology will take place in the chemical process industry.

In this paper the area discussed will be illustrated by a new process for production of D-phenyl glycine, jointly developed by the Dutch company DSM, and my own company. D-phenyl glycine is used in the synthesis of the semi-synthetic antibiotic, ampicillin,

by coupling to 6-APA. The starting material for the production of D-phenyl glycine is the racemic D, L-phenyl glycine amide, see Fig. 5.

The method normally used for resolution of this compound is to couple it with another stereoisomer, e.g. camphor sulphonic acid, so that two diastereoisomers with different solubilities are formed. These can then be separated by fractional crystallisation. This method was originally devised more than 100 years ago by Louis Pasteur, who further originated two other methods for the resolution of racemic forms:

- mechanical separation of crystals, and
- microbiological separation.

To day the first method is only of interest academically, but the other is based on the same principle as the method to be discussed here. Pasteur found that the mold, Penicillium glaucum, metabolises D-ammonium tartrate more rapidly than the L-form in a solution of the racemic form, thus allowing a separation of the two isomers.

The enzyme used for the resolution of D, L-phenyl glycine amide is produced by a strain of Pseudomonas putida and it hydrolyses specifically L-phenyl glycine amide to L-phenyl glycine and ammonia leaving the D-form unchanged. As the free amino acid has a very low solubility most of it precipitates from the reaction mixture leaving the D-phenyl glycine amide in the supernatant. This is precipitated chemically and finally hydrolysed yielding D-phenyl glycine with an optical purity over 99.99%. The L-phenyl glycine is racemised and recycled. The extreme optical purity of the product is made possible by the absolute stereospecificity of the enzymatic hydrolysis.
Finally, it should be mentioned that the same enzyme can be used for production of most amino acids optically pure form.

Conversion of Renewable Resources to Motor Fuel

Since 1973 the energy shortage has been in focus of the public discussion, and even though new discoverties of oil deposits have made it feasible that sufficient reserves are available until fusion energy is ready at the turn of this millenium, the situation is aggravated due to political and economic conditions. Many countries in the world face the risk that their oil supplies can be cut off from one day to the next, and naturally this has stimulated the search for alternatives to gasoline as motor fuel.

The first big project in this field was the Brazilian Gasohol programme aiming in the first phase at the admixture of 20% ethanol to normal motor fuel. For Brazil, which has a warm climate and large reserves of uncultivated land, the production of ethanol offered good opportunities for reduction of the deficit of payment by cutting down on oil imports. Until now the Brazilian project has proceeded according to plan, the main raw material in the first phase being cane sugar. Within one to two years a production on the basis of cheap starch from manioc will be started.

The interest for ethanol in countries with a more temperate climate and a denser population has been moderate, but some production has been carried out in the US using starch from corn, and with the present embargo on the export of grain to USSR the interest for the Gasohol concept has risen dramatically.

One of the problems in connection with ethanol production is the poor energy balance. The combustion energy of dehydrated ethanol is approximately 7,000 kcal/kg. 90% of this energy is needed for the production so the net energy gain is only 10% and further, in this calculation the energy needed for the production of fertilisers is neglected. One of the big posts on the debit side of the energy balance is the calories used for the destillation of the ethanol, approximately 2,500 kcal/kg ethanol, another being the heat treatment of the starchy raw material before fermentation. This process requires

approximately 1,000 kcal/kg ethanol. Thus, if the heat treatment of the starch could be avoided a saving of up to 15% of the combustion energy would be obtained. My company is at present developing a process where the saccharification and fermentation are carried out simultaneously at 30-35° C, thereby avoiding the energy consumption in the starch cooking process before fermentation.

In the normal US-procedure for ethanol production ground corn is used as the raw material (see Fig. 6). A slurry of the ground corn is heated to 140° C by steam injection. Prior to and after this pressure cooking a thermostable a-amylase e.g. Termamy® is added in order to reduce viscosity and hydrolyse the starch. After cooling to approximately 30° C the mash is transferred to the fermentor and glucoamylase and yeast are added. The fermentation is finished after 60-100 hours and the ethanol is stripped off from the mash by steam whereupon further rectification and dehydration takes place.

It is known from the literature that it is possible to solubilise granular starch with an α-amylase at temperatures below the gelatinisation point, which for corn starch is above 62° C. This procedure has never found industrial application due to a low reaction rate and a low concentration of granular starch in the reaction mixture. From an energy point of view the ideal process for ethanol production from starch would be a simultaneous liquefaction, saccharification and fermentation in one process step, performed at the highest temperature tolerable to the yeast applied.
With the yeast strains available to day this temperature lies in the range 30-35° C, but more thermostable strains are under development. Some preliminary experiments with the aim of developing such an ideal process will be discussed in the following.

Fig. 7 shows the results of an experiment where a procedure with separate liquefaction, saccharification and fermentation is compared with the new one which conveniently is called the "cold saccharification procedure". It appears that the ethanol concentration attained in the cold saccharification experiment is somewhat lower than that obtained in the control experiment but more important, the fermentation rate is considerably lower. This and similar experiments have led to reflections concerning the rate-limiting steps in the twoo processes. The concentrations of sugars and dextrins during the cold saccharfication are very low, which indicates that the rate-limiting step is the conversion of granular starch to dextrins. In the conventional process the rate-limiting step is the conversion of dextrin to glucose, see Fig. 8. This indicates that the fermentation rate may be accelerated by increasing the concentration of granular starch. Laboratory experiments have verified this hypothesis as shown in Fig. 9.

It appears that the highest rate is obtained at a starch concentration above 25%. Such a high concentration is economically advantageous as it reduces the amount of water used and thereby improves the economy. Of course, the granular starch can only be partly converted to ethanol when present in such high concentrations as normal yeast can only tolerate ethanol concentrations up to about 10%. Therefore, in order to utilise the raw material fully it is necessary to remove the unconverted starch and the yeast by mechanical means and repeat the fermentation. One can envisage a continuous process according to the scheme illustrated in Fig. 10.
Granular starch, α-amylase, glucoamylase and yeast nutrients are continuously fed into the fermentor and yeast and starch are recycled to the fermentor after centrifugal separation. It must be emphasized that the economical and technical feasibility of the process has not yet been demonstrated in pilot plant and production scale.

One of the problems is the enzyme consumption which might be too big, but in this context it should be mentioned that the α-amylase in the fermentor to a high degree is adsorbed to the granular starch so it can be recycled. This is demonstrated in Fig. 11 which shows the concentrations of free α-amylase and glucoamylase during the fermentation. Only 20% of the α-amylase is present in the liquid phase while the rest is absorbed to the granular starch. Unfortunately the same adsorption phenomenon is not observed with the glucoamylase.

As mentioned above the development work upon the process has not yet passed the feasibility stage, but by comparing the pros and cons listed below we think that further investigations are justified:

Pros

- Saving of energy
- Reduction of amount of circulating water
- Reduction of enzyme consumption.

Cons

- Higher infection risk
- A possibly slower fermentation rate.

Enzyme Production

The first microbial enzyme produced in industrial scale was "Takadiastase" based on Aspergillus oryzae. The inventor was the Japanese scientist, Takamine, who in 1890 went to the US and started a production of the enzyme by surface fermentation. However, the history of enzyme production goes back even further as the Danish pharmaceutical chemist, I. Christian Hansen, in 1874 founded a factory in Copenhagen for the production of the milk-coagulating enzyme, rennet. His achievement was the development of methods for extraction, stabilisation and standardisation of the enzyme from calf stomachs so that the dairies had a reliable enzyme for the coagulation of milk. Before that dried stomachs had been used for the purpose and gave rise to many problems due to varying enzyme activity.
Hansen's standardised preparation secured a uniform coagulation from one batch of cheese to the other, and, therefore, meant better cheese quality and better economy - a good example of enzyme technology at its best.

The enzyme industry benefited greatly from fermentation techniques developed during and after the last world war in connection with the production of antibiotics, but the industry remained small and the techniques used were to a high degree based on experience lacking a theoretical background, which is essential for further development. A dramatic change in enzyme production took place in the years 1965 to 1970, where the development in detergent enzymes forced the enzyme manufacturers to expand their production facilities at a rapid rate and allocate great resources to product and production development. In the 1970's the development continued and many techniques, until then only used in laboratory and pilot plant scale, were transferred to production scale, examples being continuous fermentation, enzyme immobilisation, reverse osmosis, etc. In particular, the production of immobilised glucose isomerase was a unique achievement - the summit of 20 years of academic research upon enzyme immobilisation.

Here is not the place to give an account of the present state of the art of enzyme production. Much has already been described in both articles and books, and the request to talk about enzyme production always places the industrial chemist in a crucial dilemma. What is known is of limited interest to the audience, and the knowhow which is of real interest and importance is considered as strictly confidential by the industry.

As mentioned above, development in enzyme production gained speed from 1965 and changed from a handicraft or art into a technology using the most sophisticated techniques. Like biotechnology enzyme production can be characterised as a multi-disciplinary technology comprising at least the following areas:

- Bacterial and fungal genetics.
- Bacterial and fungal physiology.

- General microbiology and fermentation technology.
- Protein and enzyme chemistry.
- Recovery unit operations.
- Analytical chemistry.
- Enzyme immobilisation and derivation.
- Electronic data processing and automation.

Only by employing all these skills is it possible to develop quite new production methods and improve existing ones. The multitude of necessary disciplines make the matrix organisation of research and development the ideal one, and at Novo the production development is carried out in project groups comprising scientists and technologists with widely different academic and industrial backgrounds. Only by such a coordinated effort significant progress can be made, and the industry really needs progress in production economy and also with regard to product quality. With the rapidly increasing prices of raw materials and energy it is mandatory to increase the overall yields and reduce energy consumption, and with the more and more sophisticated methods in enzyme technology it is just as important to improve product quality. Further, the regulatory procedures, requested by the governmental bodies in order to increase the safety for factory personnel and consumers, weigh heavily upon the production costs. Therefore, in order to cope with competition and keep the enzyme prices at such a level that it stimulates the enzyme-consuming industry to invest in further applications of enzyme technology, it is necessary for the enzyme producers to invest large resources in production development.
Undoubtedly many enzyme producers are investing just as much in the development of existing products as in research and development of quite new products.

The importance of production development for an enzyme producer can be demonstrated by plotting in a log-log coordinate system the accumulated R & D expenditures over a period of years against the reciprocal of batch yield which is proportional to a major part of the production cost per enzyme unit. Such an analysis is shown in Fig. 12 for two large volume microbial enzymes, for convenience called enzyme X and Y. The curves are not linear, but it is possible to draw a straight line through the points, giving a characteristic slope for the enzyme product in question. From the curve of enzyme X it can be seen that in the beginning the cost decline is small but when a certain accumulated R & D cost is reached there is a sharp decline. This is attributable to the introduction of a new mutant strain and after some time the batch yield is stabilised at the higher level. Such an analysis can be helpful in making decisions with regard to allocation of R & D resources.

Production of Enzyme Granulates

In the literature on enzyme production much emphasis is laid upon the fermentation aspects while the recovery and finishing operations are not discussed in great detail, the reason for this undoubtedly being that the main part of fermentation research and development carried out has been done in universities and other academic institutions where no hindrance for publication exists. With regard to recovery and finishing operations the situation is quite different as the main effort undoubtedly has been made by industry which in most cases prefers to keep its research confidential, as patents only give a weak protection. A most fascinating chapter in the last 10 years of enzyme production development is the work carried out to reduce the dustiness of detergent enzymes.

The use of proteolytic enzymes for detergents created problems in the late 1960's in detergent factories where workers were exposed to proteolytic enzymes which at that time normally were supplied as a fine dusty powder. Some workers suffered attacks from the proteolytic enzymes especially on the skin around the eyes and in the nose,

and allergic reactions were also observed. These observations led to many detergent manufacturers ceasing to use enzymes in detergent formulations, and the enzyme manufacturing industry had to make an extraordinary effort to develop enzyme granulates with as low dust generation as possible. This work has been a success and to day the use of enzymes in detergent formulations is growing steadily. The development in dust content of detergent enzymes is seen in Table III where the development from 1968 until to day is shown, and the dramatic decrease in dusting properties can be seen. In spite of numerous patents on the production of granulated and dust-free enzymes only two methods are used generally to day in an industrial scale: one being the so-called prilling method and the other the marumeriser method, the principle of which is shown in Fig. 13. The powdered enzyme is mixed with a filler, e.g. a salt, a binder and water to make a paste which is extruded and shaped into spheres. After drying they are coated with a waxy material.

The two methods give sufficiently low dust levels and the safety problems in the detergent industry have thereby nearly been solved. However, both methods have apparent drawbacks. In the prill, 50% of the product must be a waxy material which is expensive and apparently not of great benefit in the detergent formulation. The marume process is difficult to control on an industrial scale due to the rather complicated machinery comprising, mixer, kneader, extruder, marumeriser and drier. In this context it must be remembered that detergent enzymes are produced in an amount of many thousands of tons annually and they have to meet product specifications as strict as those normally demanded for pharmaceutical products, which are only produced on a small scale. This illustrates the problems the enzyme manufacturing industry has been faced with over the last decade.

Bearing in mind the mentioned drawbacks of the two dominating granulation methods it is natural that the search for new simpler and more effective processes has continued, and the latest development is a granulate produced in a pelletising drum using water as the granulation liquid. Granulation in a pelletising drum is one of the most convenient methods but has never found application in the production of enzyme granulates, probably due to the fact that the process is extremely difficult to control. During the production of an enzyme granulate in the drum granulator a thick and sticky layer of the material tends to build up on the walls of the granulator. This problem has now been overcome by an admixture of cellulose to the powder mixture. The new process is illustrated in Fig. 14.

Addition of cellulose fibres has also the advantage that it reinforces the core and gives the granulate some elasticity, which is of importance to the detergent manufacturer. In fast-operating filling machines the enzyme granulates can be subjected to a mechanical action and if the granulate core is brittle it will disintegrate, so that enzyme dust is generated. In the last column of Table III is shown the dust generation of some different granulates after mechanical treatment in a ball mill. It is evident that the addition of the cellulose fibres has given the desired effect.

It is my hope that the different examples given in this paper give some indication of the many possibilities for enzyme technology in the future and the extensive work carried out to improve enzyme production methods. I think that I share the feelings of many industrial chemists working in this field, that the future of biotechnology will be a bright one as indicated in the title of this symposium.

GLOBULIN PROTEIN (N x 6.25)	42.5 %
WHEY PROTEIN (N x 6.25)	7.5 %
SOLUBLE CARBOHYDRATES	15.0 %
INSOLUBLE CARBOHYDRATES	18.0 %
ASH	6.0 %
FAT (ETHER EXTRACT)	1.0 %
MOISTURE	9.0 %
UNDEFINED COMPOUNDS	1.0 %

G~0914

Table I. Proximate composition of dehulled and defatted soybeans.

G~0940	% SOLU-BILIZED PROTEIN	% SOLUBI-LIZED POLYSACCH.	% PROTEIN YIELD	% PROTEIN PURITY
WITHOUT ENZYMES	13	0	87	72
ENZYME PROCESS, TECHNICALLY ACCEPTABLE	13	> 85	87	90
ENZYME PROCESS, IDEAL	13	100	87	100

Table II. Extraction of insoluble carbohydrates with or without enzymes.

YEAR	PHYSICAL FORM		DIAMETER MEAN µ	DUST "ELUTRIATION METHOD" (60g, 40 min, 0.8m/sek µg ALCALASE pr.60g	
				WITHOUT MECHANICAL TREATMENT	WITH MECHANICAL TREATMENT
BEFORE 1968/69	POWDER		APPROX 30	∞	∞
1970	PRILL		450	1,500	210,000
1972	MARUMES		600-700	1,000	13,000
1974	MARUMES, FILM-COATED		600-700	150	3,000
1980/81	GRANULATE T. (CELLULOSE FIBRES IN THE CORE)		600-700	150	600

Table III. Survey of different forms of dedusted enzyme preparations.

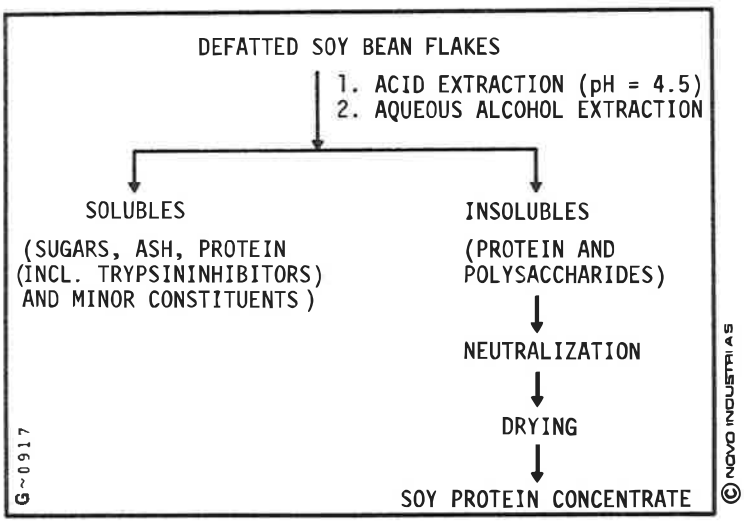

Figure 1. Flow diagram of the soy protein concentrate process.

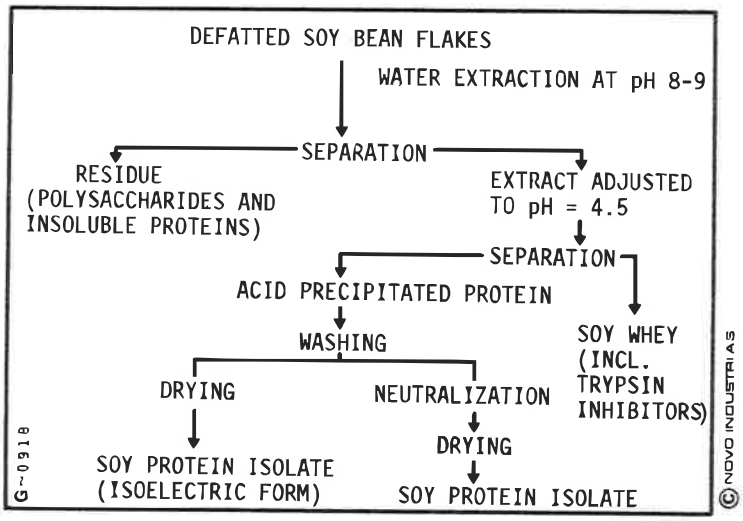

Figure 2. Flow diagram of the soy protein isolate process.

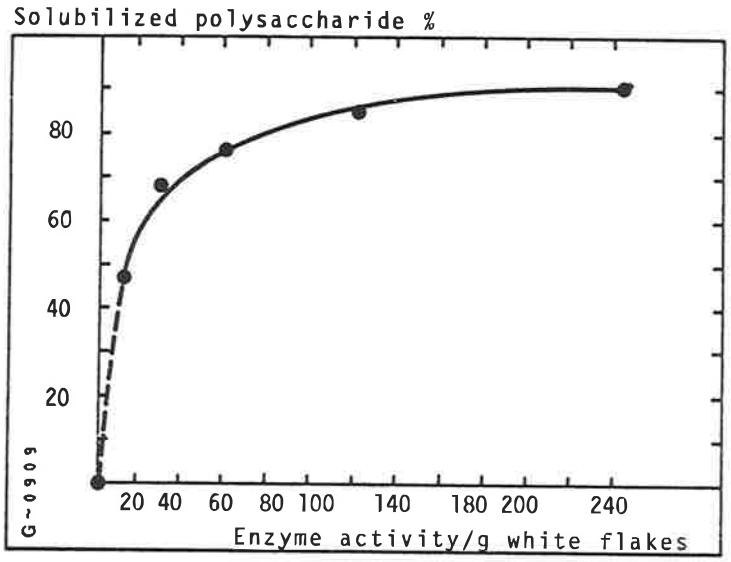

Figure 3. Solubilized carbohydrate at different enzyme concentrations.
Reaction conditions:
Substrate concentration: 10% dehulled white flakes
pH: 4.5
Temperature: 50° C
Reaction time: 120 minutes

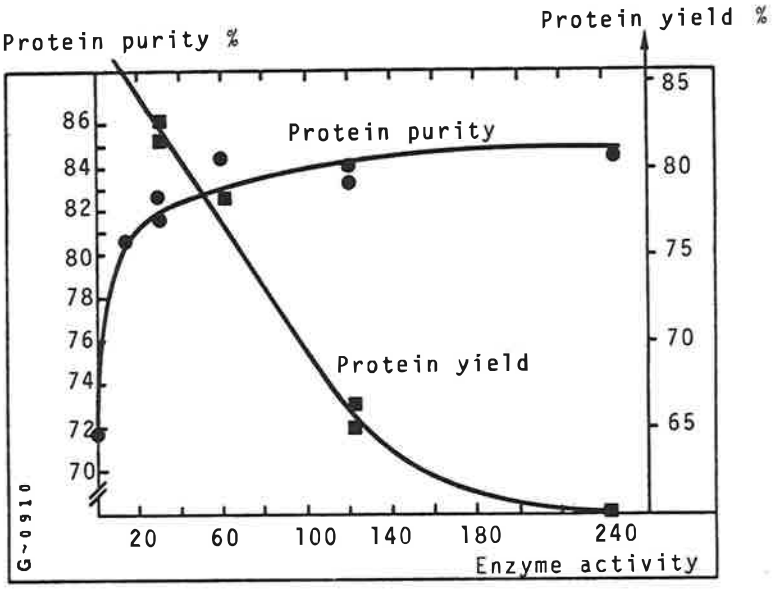

Figure 4. Protein purity (protein/total dry matter) and protein yield at different enzyme concentrations.

Reaction conditions:

Substrate concentration: 10% dehulled white flakes

pH: 4.5

Temperature: 50°C

Reaction time: 120 minutes

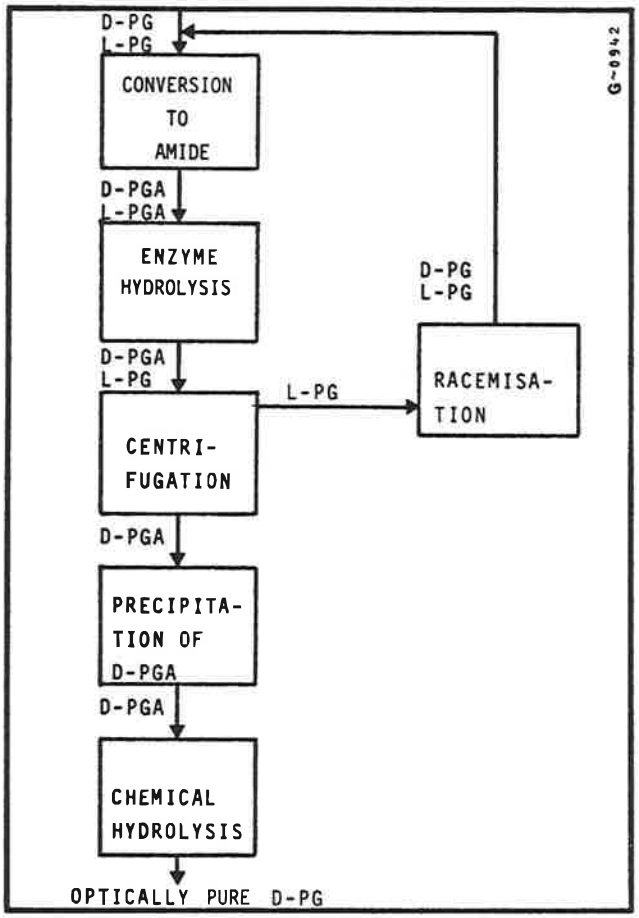

Figure 5. Flow sheet for optical resolution of phenyl glycine.

PGA = Phenyl glycine amide
PG = Phenyl glycine

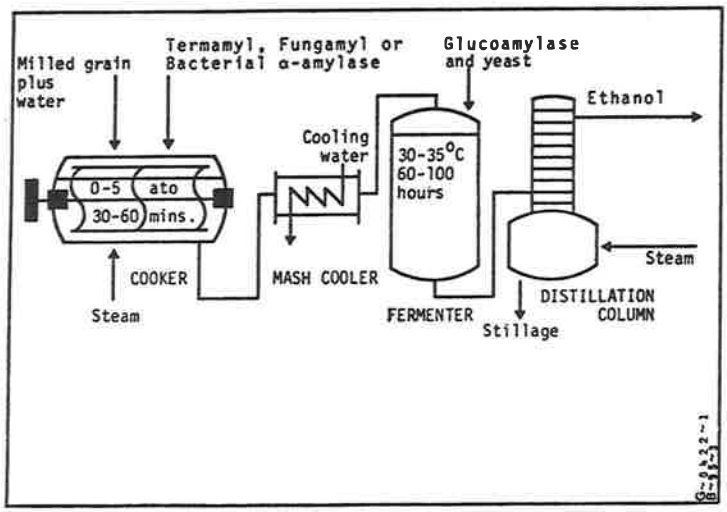

Figure 6. Flow sheet for normal US-procedure for ethanol production.

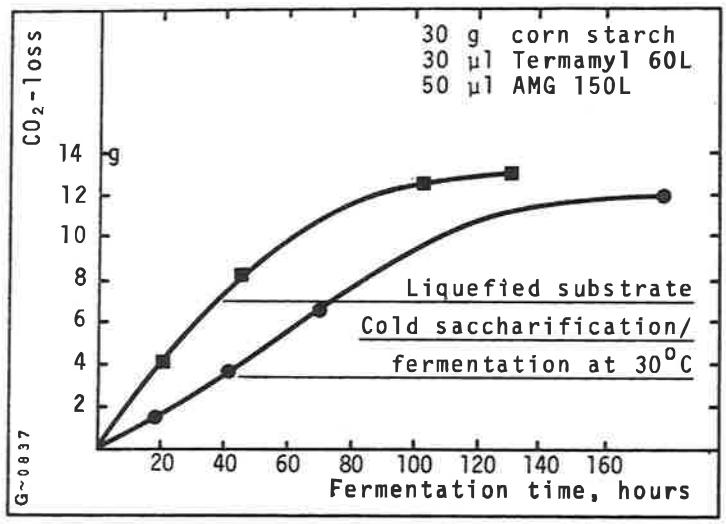

Figure 7. Fermentation rate using liquefied starch as a substrate compared with the rate obtained in the "cold saccharification procedure".

Reaction conditions:

Starch concentration: 200 g corn starch/l

Enzyme concentrations: 200 µl Termamyl 60L/l and 330 µl AMG 160L/l

Temperature : 30° C

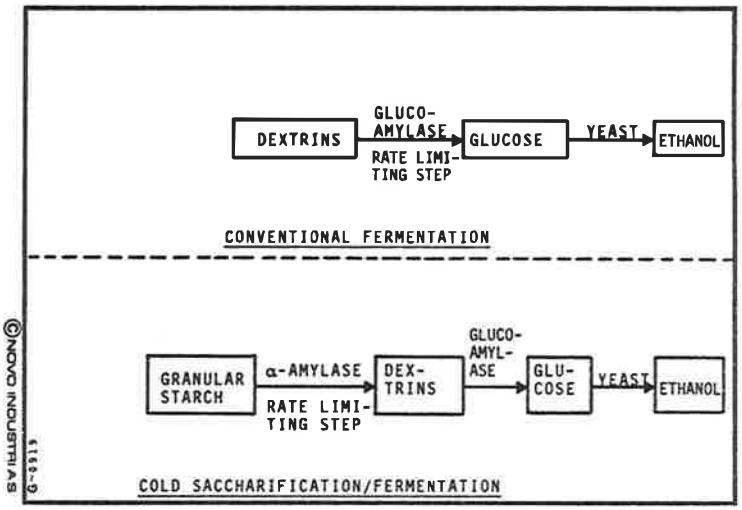

Figure 8. Rate limiting steps in conventional and "cold saccharification" ethanol fermentation.

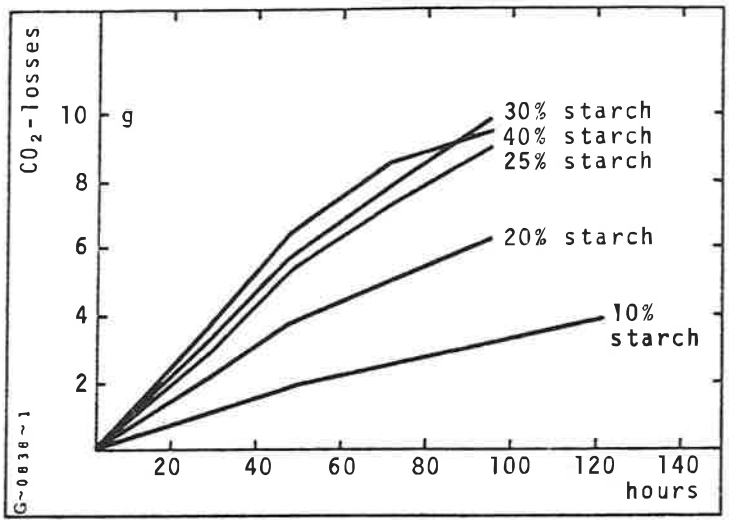

Figure 9. Influence of granular starch concentration upon fermentation rate.

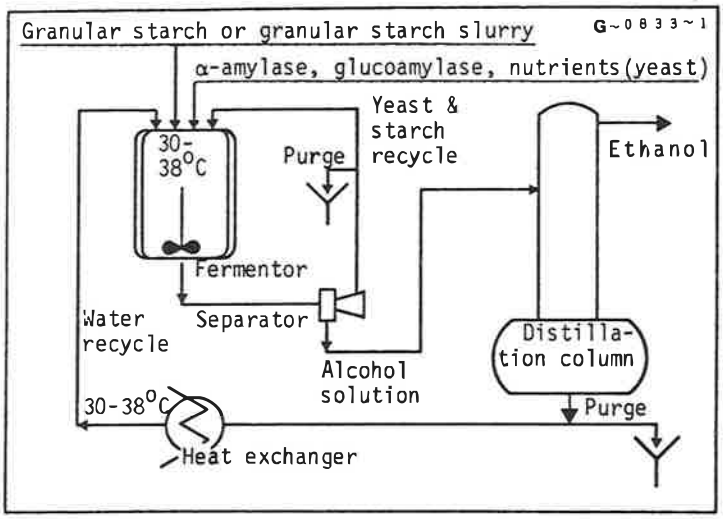

Figure 10. Continuous ethanol fermentation based on the "cold saccharification" principle.

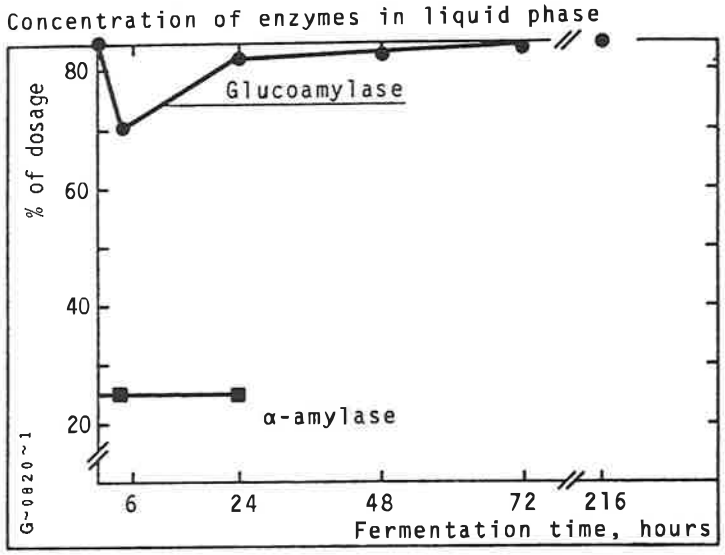

Figure 11. Enzyme concentrations during fermentation.

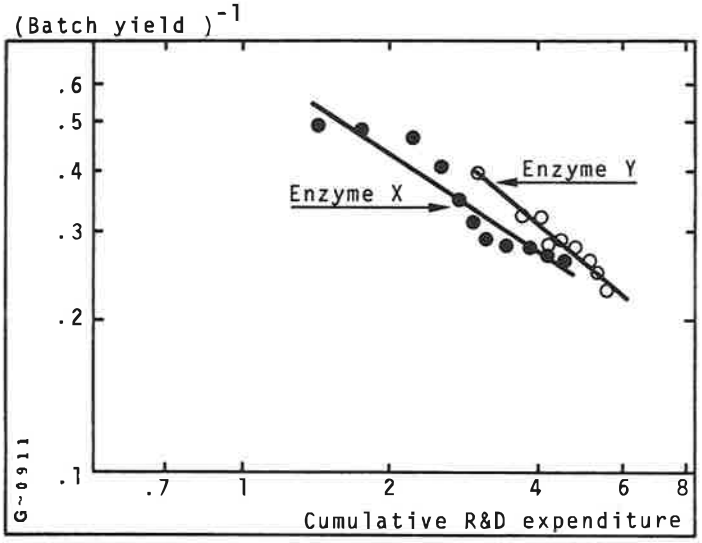

Figure 12. Log-log plot of the reciprocal of batch yield versus cumulative R&D expenditure.

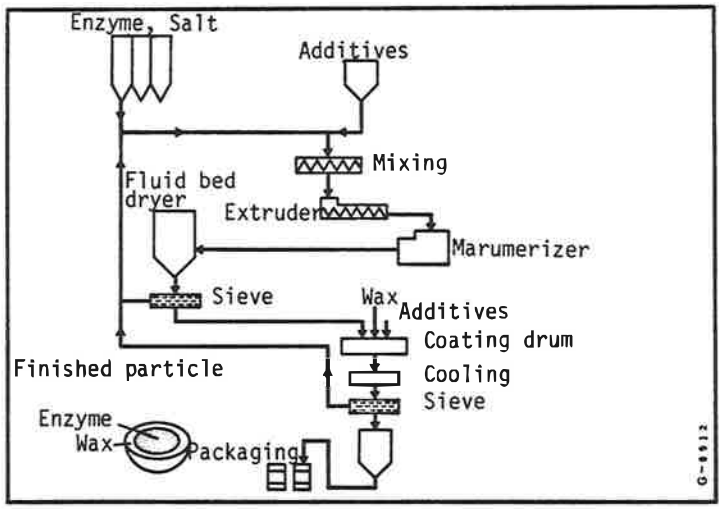

Figure 13. Typical flow sheet for the preparation of a dust-free enzyme product: Marumes.

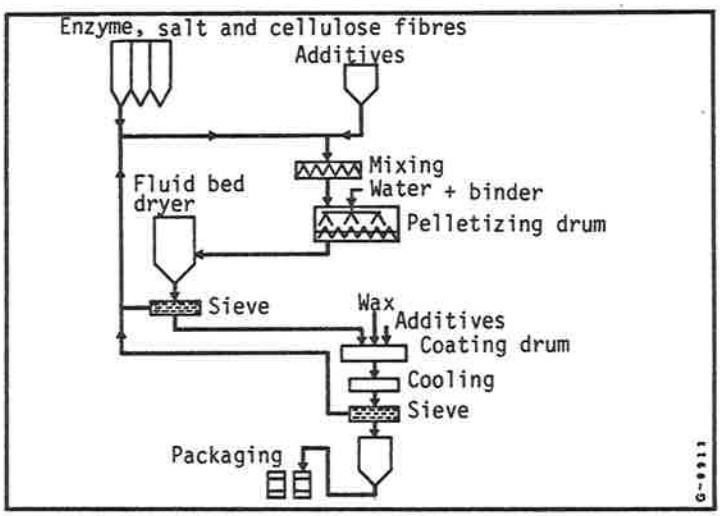

Figure 14. Typical flow sheet for the preparation of a dust-free enzyme product: Granulate T.

Immobilized Biocatalysts

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General Introduction

The lectures held earlier today provided you with many data about the catalytic potentials of micro-organisms and enzymes. To what extent and in what manner these biocatalysts can be used in practice are basic questions to be answered by that domain of the applied sciences which is defined by the word biotechnology. Through this contribution I would like to invite your attention to a very specialized use of biocatalysts, namely that of immobilized enzymes and immobilized cells, which are collectively referred to as immobilized biocatalysts.
The greater part of my lecture will deal with immobilized enzymes. They have been studied for more than 25 years, while the immobilized cells (by which I mean immobilized living cells) are still a rather unexplored area.

What are immobilized enzymes?
To begin with, I will give you the definition as drafted at the First Enzyme Engineering Conference held in 1971. This is a biennal event where immobilized enzymes have remained to be the main topic. Immobilized enzymes are enzymes which are physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously, For a definition of immobilized cells it may be helpful to replace the term "enzyme" by the designation "cell".

First, I will give you a classification of the various types of biocatalysts according to their complexity and I will pause on the why of immobilization.
Next, I will give you an idea of the technique of preparing immobilized biocatalysts and summarize the respective areas of application.
Finally, I will illustrate the application of immobilized biocatalysts with 4 examples which I hope will provide an outline of the promising potentials of immobilized biocatalyst technology.

Classification of immobilized biocatalysts

Since the term "immobilized biocatalyst" is used for a rather simple system as the immobilized enzyme as well as for a complicated one as the immobilized cell, it would be wise to try and structure the immense field of immobilized biocatalysts and to divide them into a few classes. This is best achieved on the basis of their complexity and that of the reaction or reactions they catalyze.
Going from "simple" to "complex", we may start with the "single enzyme", the immobilized biocatalyst involved in just a single reaction (Figure 1).

The majority of the immobilized enzymes that are being utilized commercially belong to this type, catalyzing degradative hydrolysis reactions or conversion reactions. Only these classes do not require co-factors, by which are understood compounds which an enzyme needs to display its activity.

The multi-enzyme systems (Figure 2), in which at least two enzymes act as catalysts, are somewhat more complex.

As long as no co-factors are involved, these reactions are, in fact, hydrolysis and/or

conversion reactions.

The next step to complexity is formed by the system in which the immobilized enzyme or enzymes are used together with co-factors, which can similarly be given an immobilized form (Firgure 3).
Such a system makes it possible to catalyze more complex reactions for the synthesis of complex molecules. These reactions require energy and electron transfer and hence co-factors. Currently, the use of this type of enzymes for large-scale synthesis is restricted by the fact that the co-factors required such as ATP, NAD and NADP are very expensive. In addition, they are converted in the course of the reaction, and these two features have prompted enzyme technologists to search for a means of regenerating co-factors.

The subsequent step in the direction to complexity lies in the system where the microbial cell in its entirely is immobilized. In principle, the immobilized cell can be used for various purposes, ranging from simple one-step to extremely complex reactions. At this point there may be some confusion about the term "immobilized cells", because they may be involved in the catalysis of one single enzyme reactions and could thus be conceived as immobilized enzymes. In my opinion, the term "immobilized cell" had better be reserved for immobilized living cells regardless what type of reactions they catalyze. So far the classification in terms of complexity.

Reasons of enzyme and cell immobilization

Enzymes are produced by organisms to satisfy their specific requirements, and although they are efficient and effective catalysts, they are not always ideally suited to biotechnical application. They are, for instance, generally unstable and are frequently unsuitable for use in organic solvents or at elevated temperatures. Conventionally, enzyme reactions have been carried out in batch processes by incubating a mixture of substrate and soluble enzyme. In such a process it is technically very difficult to recover active enzyme from the reaction mixture for re-use. As a result, there is a loss of active enzyme during each reaction cycle, and as most enzymes are expensive this may be regarded as an undesirable feature.
These disadvantages of soluble enzymes can be overcome by the use of immobilized enzymes. The can be re-used and are normally much more stable than their soluble counterparts with the added advantage of making a continuous process possible. In other words, enzymes can thus be applied in an immobilized form in the same way as heterogeneous catalysts are ordinarily used in chemical reactions. Since enzymes can catalyze specific reactions under mild conditions including a normal temperature and pressure, the application of immobilized enzymes in the chemical industry may reduce energy requirements.
Although enzymes are produced by all living organisms, those from microbial sources have remained most suitable for industrial purposes. They can be classified in two groups - the extracellular enzymes, which are secreted into the growth medium and the intracellular enzymes, which are retained in the microbial cell.
Extraction from microbial cells cannot be dispensed with if intracellular enzymes are to be used. However, it may well be that the immobilization of intact micro-organisms (whole cells) provides a more practical means. Preserving enzymes and co-factors within their natural environment offers several advantages. Costly and tedious purification processes can be avoided and the loss of enzyme activity is often less than in the case of the isolated enzyme. Such a process might be easier to achieve, since in the immobilized cell the various enzymes and co-factors occur together naturally.

Immobilization techniques

Methods of enzyme immobilization can be put into three categories as follows
1) binding of enzymes to water-insoluble carriers by
   physical adsorption

ionic binding
covalent binding

2) intermolecular cross-linking of enzyme molecules by means of bi- or multifunctional reagents;
3) entrapment of enzymes in the lattice of a semi-permeable gel or enclosing them in a semi-permeable membrane.

Broadly, there are two main principles in immobilization methodology, a physical and a chemical one, each having its specific advantages and disadvantages. A nearly endless variety of materials have been used to achieve enzyme and cell immobilization. To name them all would be far beyond the scope of this lecture but to give you some idea, I have made the following enumeration (Figure 4).

A great variety of natural and synthetic polymers plus their chemical modifications have been applied to obtain enzyme immobilization.
Figure 5 visualizes the difference between a soluble and an immobilized enzyme. In the left reaction vessel (reactor) the enzyme is invisible - it is dissolved in water, as are the substrate and the reaction products. In the other reactor, the enzyme has become visible - it is not dissolved in water but the substrate and the reaction products still are. In this example, the immobilized enzymes are manufactured as spherical beads but immobilized enzymes also occur as membranes, tubes and fibers. Furthermore, the particles may differ significantly in shape.

Areas of application

In the following part of my lecture I wish to reflect on the various areas where immobilized biocatalysts are already in use and give examples of further applications. First, I will sketch the situation in a few words, and in the last part of my paper I will discuss four representative examples in somewhat more detail.
So far the use in chemical processes has been most prominent. Here the advantages of immobilized enzyme technology such as automated and continuous operation, increase in yield per enzyme unit, the relatively simple product purifications, etc. are utilized. For any immobilized enzyme process on a large scale to be successful, aspects of basic chemical engineering have to be taken into consideration (Figure 6).

From an industrial point of view the column reactor is the most suitable type with its pressure drop and, related to this, its dimensions as the major features.
What counts most in economic terms during the use of immobilized enzymes is the overall productivity as compared to the decline in activity. The former may be represented as being the total amount of product that can be obtained through the action of biocatalysts during their useful life. In other words:
what is the enzyme cost per product unit?
There is already a long list of chemical processes where immobilized enzymes find application but large-scale operations are limited to only a few (Figure 7).

The process denoted by an asterisk will be given further attention in the last part of my lecture.

Analytical applications are another area where immobilized enzymes play an important role. Being specific and sensitive tools, they find widespread application, especially at biochemical and chemical laboratories,
- in automated analyses
- as enzyme electrodes.

Formerly, the use of enzymes for analytical purposes was limited because of certain drawbacks such as instability in water, and lack of precision and availability.
These have been overcome by the development of immobilization techniques. In the case of automated analytical procedures the specificity of the enzyme is combined with the advantages of the various analytical instruments.

These methods make it possible to analyze a larger number of usually simple compounds. Enzyme or biochemical electrodes represent another advance in analytical chemistry, where their advantages are combined with those of the high rate and simplicity of ion-selective electrode measurements. The result is a means of determining quickly the concentration of any particular compound in solution with a minimum of sample preparation.
Enzyme electrodes have, for instance, been used for the quantitative determination of glucose in serum, plasma and blood.
The potential value of immobilized enzymes in medicine has also captured imagination. In particular, their therapeutic possibilities have been studied and developed. Due to antigen-antibody reactions, a prolonged enzyme therapy presents problems or is even impossible. Encapsulation of enzymes may overcome difficulties. A case in point is the immobilization of the enzyme asparaginase in the therapy of leukemia.
Another medical application of immobilized enzymes lies in artificial organs. A good example of this application has been given by Dr. Chang, who is working on the development of a new kind of artificial kidney.
The fourth field of application is formed by food-manufacturing and -processing. Its use in that domain has recently been increasing rapidly and is expected to continue.
A few instances of the various processes here and of the immobilized enzymes involved may suffice (Figure 8).

Uses of immobilized biocatalysts for waste water treatment are being developed. For example, cyanide in waste water is usually decomposed by the action of microorganisms in a process called the active sludge method. This system has been improved by immobilization. Other examples of this type of application are:
- the reduction of the phenol content, the urea content or both of waste water;
- the reduction of the urea content of waste water.

Though many kinds of harmful substances in waste water cannot be decomposed by a single process, these applications facilitate the breakdown and removal of specific contaminants. Further developments in this area may be expected.

In conclusion, I would like to mention the application of immobilized biocatalysts for energy production.
This is a very difficult area but also a very interesting one, related as it is to the conversion of biological energy to chemical and electric energy. A good deal of biological energy is broken down slowly by oxidations. Organisms regulate these reactions in such a manner that the energy produced can be utilized by the living system. Essentially, oxidations involve the conversion of electron-rich (the nutrients) to electron-poor (metabolites) substances. If part of the electron-transfer system can be used for an electrode reaction and the chemical energy can thus be transformed to electric energy, fuel cells or, more specifically, biochemical fuel cells are within the range of possibility.
So far about the classification of immobilized biocatalysts according to the field of application.

At this point I wish to bring together in a matrix earlier classifications in accordance with complexity and area application (Figure 9).

In the next part of my lecture, I will discuss in somewhat more detail a few immobilized biocatalyst systems, which are indicated in the scheme.

Some examples of the application of immobilized biocatalysts

a) Immobilized amino acid acylase for the production of L-amino acids; a single enzyme system
L-amino acids are used extensively in the pharmaceutical industry and in the food and animal feed manufacture. Chemically synthesized amino acids are what is called racemic mixtures of L- and D-isomers, the former being only the physiological-

ly active form. Several methods are available for their separation. The Japanese company Tanabe developed an enzymic method using an acylase enzyme from mold. In this context, the name of Dr. Chibata must be mentioned. The system operates as follows (Figure 10).
The mixture of D- and L-amino acids is first derivatized to the acyl compound, which is then split off from the free amino acid, while leaving the acyl-D-amino acid unchanged. The two reaction products can then be separated easily.
This method has been used by Tanabe for the industrial production of several L-amino acids. To overcome the disadvantages of soluble enzymes as I summed up before, a continuous process using the enzyme in an immobilized form in a packed bed reactor was developed. This novel process for industrial application was introduced in 1969 and has since been perfected.
Examples of L-amino acids currently manufactured are presented in the following table showing the yield of each in a 1000-litre amino-acylase column (Figure 11).

In fact this was the first industrial application of immobilized enzymes. A comparison of the costs of producing L-amino acids by the conventional batch method using soluble enzymes and by the continuous process using immobilized enzymes, as published by Tanake, is presented in Figure 12.
The overall production costs are more than 40 percent lower, the main reason being that a great saving can be effected in labor and enzyme costs. It is true that the enzyme carrier employed (DEAE-Sephadex) is rather expensive but this does not play an important role because the re-usability of both carrier and catalyst over a prolonged period of time.

b) Enzymic synthesis of gramicidin S; a multi-enzyme system + co-factors
A major objective in enzyme technology is the assembly or synthesis of complex molecules. The reactions often require energy transfer, by which electron transfer is meant. In biological systems the presence of co-factors is needed. Although still on a small scale, this kind of synthesis has already been achieved by, for example, the group of Dr. Daniel Wang at Massachusetts Institute of Technology, which looks to the enzymic synthesis of the drug gramicidin A as a way of expanding the potential of the system.
Gramicidin S is made up by two pentapeptides (Figure 13), each consisting of the same set of 5 different amino acids. To synthesize its molecule, several steps are needed involving various enzymes and co-factors. From the bacterium Bacillus brevis the MIT group has prepared two enzyme fractions responsible for the synthesis of the antibiotic. When the two fractions are immobilized and brought together with ATP (the co-factor) and Mg^{2+}-ions, the synthesis of gramicidin S occurs.
The overall reaction is given in Figure 14.

However, the synthesis of gramicidin S is not the only aspect. As one can see, ATP is degraded to AMP and PP_i (inorganic phosphate) in these reactions. To make a continuous process possible, there should be a continuous ATP supply or better still an ATP regenerating system. This is accomplished by two reaction steps using two different enzyme systems.
In the first reaction use is made of the enzyme adenylate kinase (from S. cerevisiae or bakers' yeast), which catalyzes the following reaction: AMP + ATP $\rightarrow$ 2 ADP.
The second needs acetate kinase, an enzyme which can be isolated from E. coli and catalyzes the following reaction: Acetyl-phosphate + ADP $\rightarrow$ Acetate + ATP (Figure 15).
All four enzyme systems have been immobilized and it has been demonstrated on a laboratory scale that they make a good job of synthesizing gramicidin S from simple compounds.

c) Artificial kidney, a single enzyme system
At present, the therapy of uremic disorders is extracorporeal, removing metabolites such as urea and uric acid from the blood through a dialysis membrane (Figure 16).

A major drawback is the large volume of dialysate.
The use of immobilized enzymes in this process has been investigated by, for example, Dr. Chang in Canada.
Urease, which breaks down urea to NH_3 and CO_2, ion exchange resin and activated carbon are encapsulated and packed in a column reactor. The dialysate, which has taken up the metabilites, is passed through the column, where the metabolites are degraded and adsorbed. Next, the dialysate can take up metabilites again. This system requires a far smaller volume of dialysate than the conventional set-up, as the circulated dialysate can be re-used (Figure 17).
The NH_3 is removed by the ion-exchange resin and the other metabolites by the activated carbon. The carbon dioxyde is exhaled via the lungs. Chang's group did experiments with dogs, showing that after the artificial kidney has been installed the ammonia content of blood increased as the urea content of blood decreased.
Chang also is seeking an alternative for the adsorption process.

d) Production of H_2; immobilized living cells
The sun is, in fact, the ultimate source of all energy to sustain life here on earth. Sunlight in discrete quantities, called photons, have always been absorbed by plants. Their photosynthetic systems transform the energy into energy-rich organic compounds, that is plant materials. With time chemically fixed energy has been converted into well-known fuels like oil and gas, which are now largely used for conversion into mechanical and electric energy. The latter kind is not obtained by direct conversion but through oxidative processes at power plants. In case chemical energy is transformed directly into electric energy by means of electrode reactions, the designation fuel cell is used.
Immobilized enzyme technology is involved in the field of energy production where biotechnological alternatives to two kinds of conversions - namely from sunlight to chemical energy and from chemical to electric energy - are being sought.
The aim of the latter process is to achieve an efficient conversion of chemical into electric energy. In the fuel cell electrons are transferred from fuel to electrodes with the release of electric energy. Normally, this process has a rather low efficiency and it is hoped that the use of immobilized enzymes, for instance, bound to the electrode, will improve efficiency (the term biochemical fuel cells would then be appropriate).
The former process attempts to utilize immobilized catalysts for the conversion of solar into chemical energy. A case in point is the work of Krampitz, who has studied the possibilities of photosynthetic plant systems for the photolysis of water involving the production of the fuel H_2. In fact, this is a two-step system, first the degradation of water and then the formation of the fuel: hydrogen (Figure 18).

In principle, many compounds other than H_2 can serve as fuels. However, H_2 is an important energy carrier. It can be used in fuel cells, producing water and electric energy by reaction with oxygen. In the same category should be mentioned H_2 production with micro-organisms under investigation by, amongst others, a few Dutch groups:
Prof. Stouthamer (Amsterdam) - Proteus mirabilis
Prof. Slater (Amsterdam) - Chromatium vinosum
Prof. Veeger (Wageningen) - Desulfovibrio vulgaris.
The reaction in which H_2 is formed from the two H^+- ions and two electrons is catalyzed by the enzyme hydrogenase. But the main question is: "Where does the electron (= the energy) come from?" The problem is to find cheap electron donating compounds.

The biological H_2 generation is as yet far more expensive than the production with electrochemical means. Ultimately, upgrading waste materials into a useful product like H_2 will have to be attempted. It may be clear that the application of immobilized biocatalysts for the production of alternative fuels or for use in biochemical fuel cells to solve energy problems is of great interest but, although it certainly offers prospects of success, much work remains to be done.

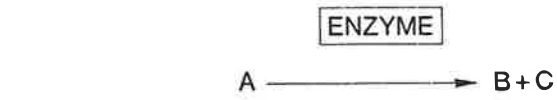
Ladies and gentlemen, I am almost at the end of my lecture. I hope I have given you some idea about the usage and further potentials of immobilized biocatalysts and the vast volume of work that has to be dealt with yet.

Most of the present applications of immobilized biocatalysts are confined to industrial processes, of which only 5 take place on a large scale (Figure 19).

But, as we have seen, immobilized biocatalysts have many advantages and in combination with an energy-regenerating system their opportunities can be extended. It may, therefore, be expected that direct immobilization of complete microbial cells will gain in importance.

In my opinion, it is not an essential objective to develop processes on the basis of immobilized biocatalysts as an alternative to those based on soluble counterparts.

The chief potentials of immobilized biocatalysts lie in novel applications and development of new products.



SINGLE ENZYME

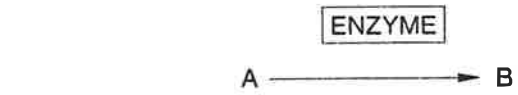
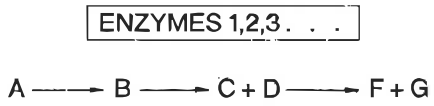


Figure 1



single enzyme MULTI ENZYME

Figure 2

single enzyme multi enzyme (MULTI)-ENZYME + COFACTORS

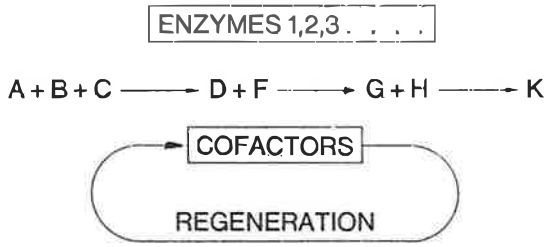


Figure 3

Starch	Synthetic polymers
Clay	Titania
Carrageenan	Gelatine
Cellophane	Agar
Activated carbon	Alumnia
Glass	Cellulose
Polyacrylamide	Nylon
Alginate	Collagen
	etc. etc.

Figure 4

- enzyme reactor type
- pressure drop in column reactors
- column (reactor) dimensions
- decay of activity and productivity

Figure 6

	PROCESS	PRODUCT
	Hydrolysis of penicillin	6-Aminopenicillanic acid
	Isomerisation of glucose	High fructose syrup
*	Optical resolution of D- and L-amino acids	L-amino acids
	Synthesis of amino acids	L-aspartic acid

Figure 7

IMMOBILIZED BIOCATALYST	PROCESS
Immobilized lactase	Hydrolysis of lactose in milk and whey
Immobilized yeast	Production of beer
Immobilized glucose oxidase	Removal of O_2 from fruit juice

Figure 8

COMPLEXITY / APPLICATIONS	single enzyme	multi enzyme	(multi)-enzyme + cofactors	cell
chemical processes	D,L-amino acid acylase	–	Gramicidin S synthesis	–
analytical applications	–	–	–	–
medical treatment	Artificial kidney	–	–	–
food processing	–	–	–	–
waste water treatment	–	–	–	–
energy production	–	–	–	Hydrogen production

Figure 9

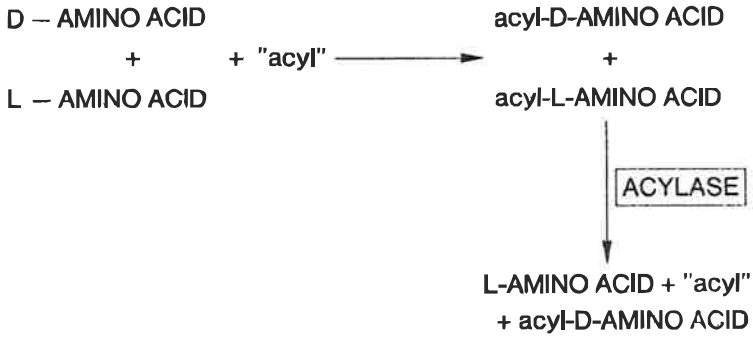


Figure 10

	Yield of L-amino acid per 24 h (Kg)	20 days (Kg)
L-alanine	214	6,420
L-methionine	715	21,450
L-phenylalanine	594	17,820
L-tryptophan	441	13,320
L-valine	505	15,150

Figures from Chibata, Tanabe

Figure 11

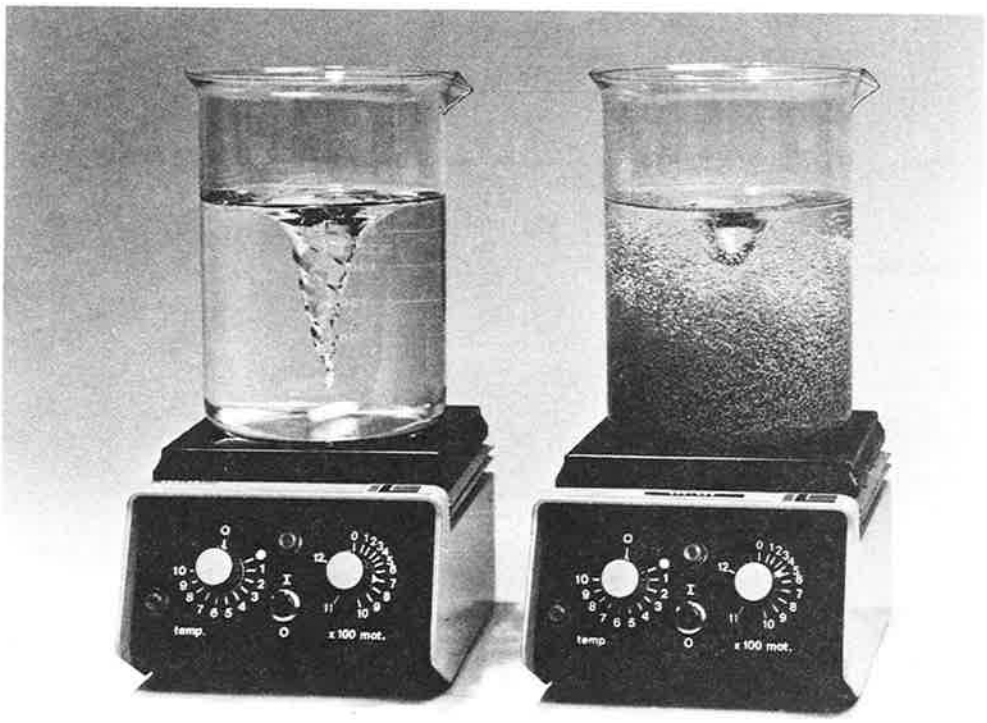


Figure 12

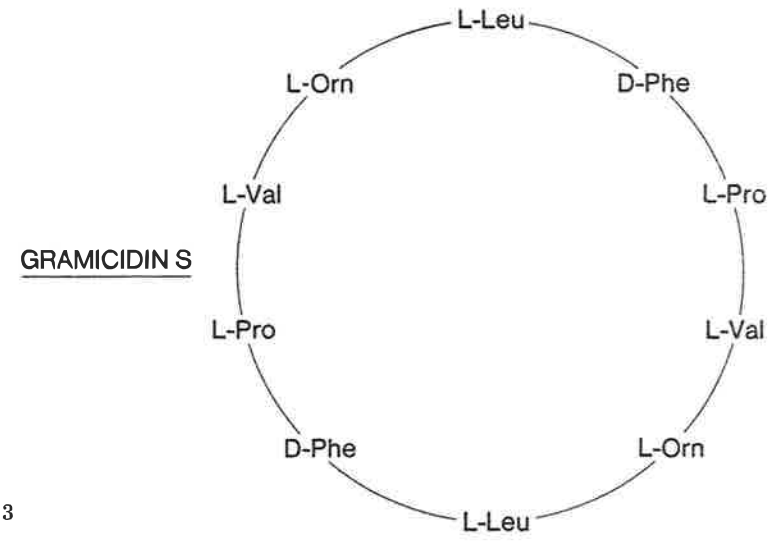


Figure 13

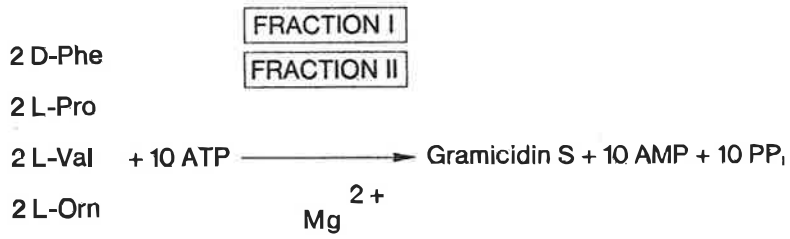


Figure 14

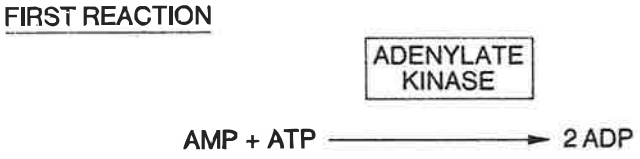


Figure 15

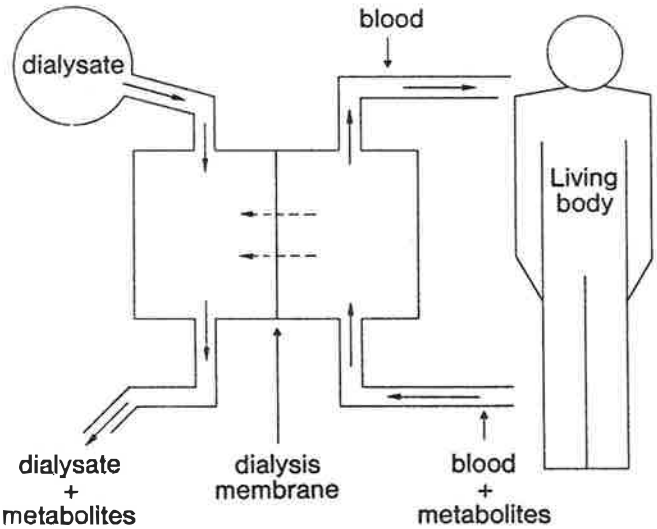


Figure 16

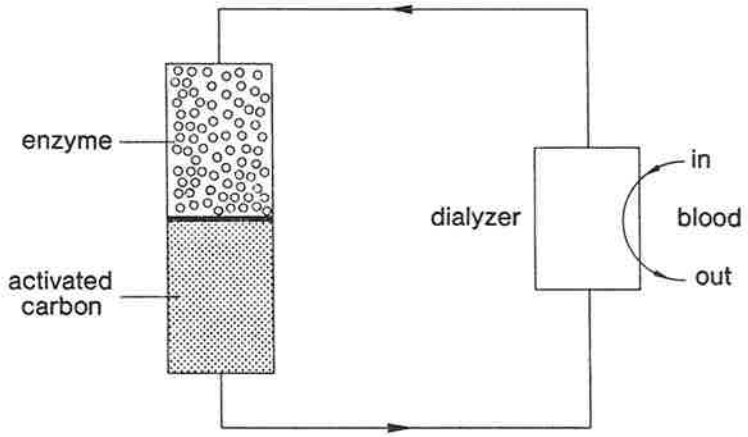


Figure 17

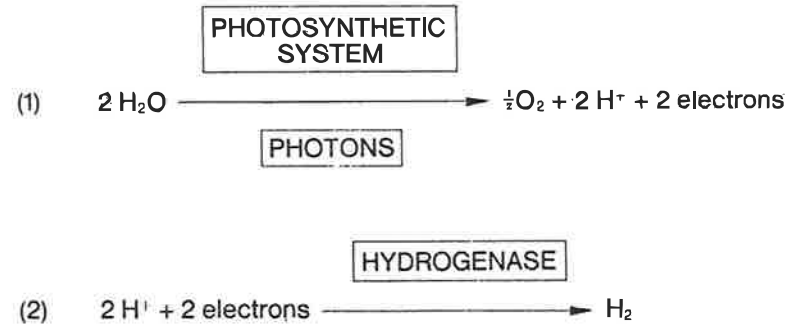


Figure 18

PRODUCTION OF	BY
L-amino acids	immobilized enzyme
6-aminopenicillanic acid	,, enzyme
Fructose syrup	,, enzyme
L-aspartic acid	immobilized cell
L-malic acid	,, cell

Figure 19

Proposal for a community programme of research
and development in biomolecular engineering (1981-1985)

Dr. G. Holt and Dr. A. Goffeau
Commission of the European Communities

In January 1980, the Commission of the European Communities approved and transmitted to the Council for a decision a proposal for a Community Research and Development programme in the field of biomolecular engineering.

This programme is essentially motivated by the need to allow the optimal exploitation by man of recent discoveries in modern biology and to stimulate in the Community the developments in applied fields where nations such as the U.S.A. and Japan have gained a considerable advance.
Two main themes form the basis of the integrated research proposed for Community action. The first one deals with the development of the second generation of enzyme reactors, that is to say, with the exploitation of complex enzymatic reactions for the synthesis of elaborated products important to European industries. The second concerns the application of genetic engineering methods to organisms of importance for European agriculture and industry. Considerable attention is given here to the development of suitable host-vector systems and to the solution of the important practical problems which prevent the control of expression of foreign DNA.

The programme proposal is composed of six integrated projects and brief details are given below:

1. Development and evaluation of new reactors using immobilized multi-enzyme systems including those requiring multiphase environment and cofactor regeneration.

   including:

   - reactor kinetics and geometries for multi-enzyme reactions
   - immobilization of multi-enzymes and cofactor regeneration
   - stabilization of lipid-requiring enzymes and development of reactors for reactions at a hydro-organic interface
   - stabilization of sub-cellular organelles such as mitochondria, chloroplasts and peroxisomes and of membrane-bound enzymatic complexes in the form of microsomes, thylakoids, and inner mitochondrial and plasma membranes
   - immobilization of intact eukaryotic plant and yeast cells

2. Development of bioreactors for industrial and human detoxification

   including:

   - bio-compatible insoluble matrices
   - targeted carriers, with specific tropisms, for administering medical enzyme products
   - enzymatic detoxification of poisons

3. The transfer of genes from diverses sources to the bacterium Escherichia coli, Saccharomyces cerevisiae and other suitable micro-organisms

   including:

   - overcoming expression barriers
   - construction of "synthetic genes"
   - development of mutational tools to produce, for example, harmless vaccines or stabilized enzymes

- modification of enzymes to inhibit their degradation in a foreign environment
- development of "libraries" of expressed genetic information and rapid screening techniques for their identification

4. Development of cloning vehicles

   involving:

   - organisms which are likely to be of greater importance to industry than E. coli, such as the bacteria Actinomycetes, Pseudomonas, and Bacilli, the fungi Penicillium, and algae, using plasmids, viruses, and mitochondrial DNA; and in plants using potential vehicles such as Agrobacterium plasmids, plant DNA viruses, chloroplasts and mitochondria.

5. Novel gene transfer in species important to biological industry

   including:

   - regulation and expression of transferred genes
   - regeneration in vitro of plant cells

6. Studies of strain stability and improved methods for detecting contamination in fermented strains

These six projects represent a continuum for industry and agriculture and fall into the area of mission oriented research. As such, they are concerned with recognising and investigating a series of "bottlenecks" which at present restrict the transfer of ideas and techniques in biomolecular engineering to industrial and agricultural problems. The programme is envisaged as an indirect action involving cost sharing contracts with both private and public organizations in the member states. The proposed Community contribution is 23.5 million European Units of Accounts (about £ 16 million).

Full use would be made, in the case of commercially exploitable discoveries, of the Council regulation (EEC no. 2380/74) which adopts provision for the protection of intellectual property. Consultation would be established, with the help of an Advisory Committee for Programme Management, for the continual adaptation of the programme to the specific needs of European industry and agriculture.

PANEL DISCUSSION I

Panel:

Prof. Dr. Ir. A. Rörsch, chairman
F. van den Akker
Dr. G. M. A. van Beynum
Dr. R. E. Cape
Prof. Dr. A. L. Demain
Dr. J. de Flines
Ms. D. Haber
Dr. M. H. Nielsen
Dr. P. H. Pouwels

(Van der Kerk) We will start now with the panel discussion. Its chairman will be Prof. Dr. Ir. A. Rörsch, until recently Professor of Biochemistry at the State University at Leiden, and now a Member of the Board of Directors of TNO. There are two other members who have to be introduced. The first is Dr. P. H. Pouwels of the Medical-Biological Institute TNO, who has a great reputation in the field of biomolecular research. The second is Mr. F. van den Akker, who represents Dr. Ginjaar and his Ministry of Health and Environmental Protection.
Prof. Rörsch, now I leave the floor to you and the members of the panel.

(Rörsch) Thank you. Ladies and gentlemen, when five months ago I was asked to be in the chair this afternoon, I was not yet an employee of TNO. I think I should mention this to stress that the title of this Conference is not my responsibility. I would never have moved into the field of biotechnology twentyfive years ago if it had been hiding its past, and neither would I have done so if I had any doubt about the shining future. I guess that most attendants feel the same way.

Having got that off my chest, I turn to the first question, which is: "Could each member of the panel comment upon the reasons for the meagre successes of biotechnology in industry, especially when compared to petrochemistry. Only antibiotics and enzymes were the main industrial breakthroughs in Europe and the USA".
Professor Demain?

(Demain) Well, personally I would not like to be quoted as having said that the results of industrial biotechnology have been meagre. I am not familiar with the petrochemical area, but I could perhaps agree with a statement saying that the diversity of products from petrochemistry is greater than from industrial biotechnology. But the production of amino acids has been a major biotechnological event and although we produce mostly lysine and glutamic acid, there are at the moment processes available for the production of many others that are waiting in the wings, so to speak. In the US it appears that there may be a major development in the area of sweeteners for the food industry; we may see the development of a dipeptide sweetener, and I am sure that the aspartic acid and certainly the phenylalanine that will be needed for this large volume product, will come from biotechnology.

(Rörsch) Thank you. Would Dr. Nielsen like to comment?

(Nielsen) I agree with Prof. Demain; the results have not been as poor as the question suggests. But there may be some truth in the question, because I think that development could have been faster is more capacity had been devoted to it. Still, starch hydrolysis has been a major development, and the installations for glucose isomerase are of a rather great size. I agree, however, that in petrochemistry capacity and diversity have been much greater than in biotechnology, especially in the USA and Europe.

(Rörsch) Are there any comments from the floor? No, then I'll go on with the next question. It is from Mr. Heijn of the Ministry of Science and addressed to the

representative of the Ministry of Health and Environmental Protection. To me this always seems to be part of a game called 'hide and seek among civil servants'. The question is: "Could you describe the kind and the extent of scientific facts necessary to get the Dutch Government to propose to Parliament to lift the ban on CIII work, which is hampering innovative research and development in this country". Mr. Van den Akker.

(Van den Akker) At the moment the policy of the government foresees but one C III facility for the whole country. Accepting that as a starting point, standards and guide-lines should be worked out as fast as possible, as that is in the national interest. In November last year the 'Committee ad-hoc for Recombinant DNA-Work' was created, which has as members representatives of the government, of research institutions and others. The Committee should deal with the problem and bring out an advice. As I have said, I think this should be done as fast as possible.

(Rörsch) Dr. Pouwels wants to comment on the question, I think?

(Pouwels) What I am going to say is my personal view, and not necessarily the official point of view of TNO.
I think that there are at least three arguments on which the decision should be based. The first is political and ethical, the second is about the benefits we get, or can expect, from these types of experiments, and the third is scientific. I want only to touch upon the last one.

C III types of experiments are carried out at a well defined containment level, both physically and biologically. You are doing the experiment in a building which is especially designed for the purpose, and you are doing the experiment with organisms that are special, that do not occur in nature. The experiments that fall in the C III category are clearly outlined in special guide-lines.

Now a good - and hotly debated - experiment is the one in which you have incorporated a piece of tumourigenic virus DNA into the E. coli bacterium. It is my belief that you should be allowed to do this type of experiment, if risk-assessment studies show that the experiment poses no extra risks. I would like to remind you that these experiments have been done in the USA and that another group there showed that the bacteria used are highly crippled. They do not colonize germ-free animals and do not survive when taken by human volunteers. I think that the risk assessment studies show clearly that these experiments pose no special hazard.

I still have a last point. Yesterday we had a meeting between people from the scientific community and from industry, and there it was agreed that there is a big gap between the type of research carried out in universities and that carried out by industry. Prof. Demain has pointed out that universities and other institutes should train people in the kind of research that is done in industry. So I would urge the government to create a C III facility in the Netherlands, in order to close this gap between university and industrial research.

(Rörsch) Thank you, Dr. Pouwels. The next question runs: "Could each member of the panel give his opinion about the three most promising products or processes in industrial biotechnology that will come up in the next five years?". I would like to direct this question to someone from a non-competing industry, as a competing industry might be tempted to deceive the questioner. Dr. Pouwels from TNO, may I call on you again for a first answer to this most challenging question?

(Pouwels) I have been intrigued by the question because it mentions just three products or processes. I have thought about it and I could come up with only three areas where, to my opinion, successes can be expected in the next five years!

The first area is that of hormones, such as insulin or the human growth hormone. I expect that these hormones will be produced in a relatively short time - say a few years - by fermentation. It are relatively simple peptides and you can either pull out the messenger RNA from the organs where the hormones are made, and convert the

RNA into DNA which then can be cloned, or you can synthesize chemically the DNA which codes for the hormone.

The second area are vaccines, for example against hepatitis-B, food-and-mouth disease, influenza and, may be, also polio. These vaccines will be made within the next five years, I think, because the genetic material of these viruses is relatively easy to isolate and also relatively easy to handle.

The third area might be the diagnostics, not only antibodies, but also DNA and RNA probes for infectious viruses in animals and man. I think this is a reasonable expectation because at the moment we are already able to detect new sub-types of viruses, as we can sequence the DNA and then detect differences in sequences between various viruses we discover in animals and man.

(Rörsch) I think Mr. Van den Akker would like to comment?

(Van den Akker) Yes, I do. Although I do not feel competent to answer the question in detail, I can give an impression of what the government could do to stimulate the development of biotechnology.

Generally, every process can be regarded as a system which has raw materials and energy on the input side and gives, as output, product(s), by-products and wastes. Taking that as our point of depart, we think that the government should aim in the first place at stimulating the international competiviness of industrial activities in the country. But, secondly, we think we should try to stimulate the introduction - and we have a policy for that - of products and processes that save raw materials and energy. An important by-product of such a policy can be a lowering of pollution of the environment by industrial activities. We have a name for it: stimulation of clean technology.

In my opinion biotechnology could make an important contribution to the cleaning up of present technology, and I think that most speakers of today intimated that.

However, the government of this country has only limited financial means to stimulate the introduction of clean technologies. The Ministries of Economic Affairs, Science Policy and Health and Environmental Protection have joined forces with industry, research institutions and universities to try and promote the introduction of clean technologies. Also, the European Communities are active in this field through their research programmes, for example the Energy Programme, the Waste Programme and the Environmental Programme.

One of the most important things is, in my opinion, the exchange of information on the activities going on. The Dutch government participates in the organisation of symposia on the subject; one will be held on 'clean technology' early in November of this year. It will be organised jointly by the Polytechnical University of Delft and the Ministry of Health and Environmental Protection. At the end of 1980 the European Communities will hold an international seminar on clean technology.

These are only a few examples, of course.

(Rörsch) Of course I am interested in clean technology, but I think industry is interested in money making too. The next question is about that, I think. It is: "Do small companies or entrepreneurs have any chance of success in the field of biotechnology? May I direct this question to Dr. De Flines of a not so small company?

(De Flines) Well, I believe that it is rather difficult for a small company or an entrepreneur to start in this field. The Cetus Corporation of Dr. Cape is a good example. He said this morning that he started it as a small company, and that it grew very fast in the beginning and was quite successful. However, after a time it was necessary to attract more capital, and some bigger companies provided that. He also said, quite lightly, that he had to enlarge his group to about 250 people. But then you are no longer a small company.

So I think that small companies can have a start, but the only way to accomplish something is growing very fast and being very successful. Cetus Corporation did that, but it is one of the exceptions I know of.

There might be, however, possibilities for small companies in isolated fields. I could imagine a small company developing a specific enzyme system for diagnostic purposes. That might be a possibility. And something in small-scale waste-water treatment might be another example. But in general, it seems to me to be a very difficult field for smaller companies.

(Comment) This is a clear exposition of the business mentality and I don't have anything against that. But how do you square this with the need for producing human growth hormones, as only a small amount of children have to be treated with the hormone?

(De Flines) This is a very good question and it points to an important problem in pharmaceutics in the Western World. Few companies want to invest money to produce drugs for, so called, small diseases. I think that there are two possibilities. The government can take over and provide money from the public purse to develop the drug. The other possibility is that the drug would be very expensive and could be developed by a private company that would make some profit on it.

(Rörsch) Thank you. I think Prof. Demain has a comment.

(Demain) Yes I have, as I am not that pessimistic about entrepreneurial motivation. The specific example mentioned was addressed by two small companies last summer. In general I think that smallness of the market will not prevent private enterprise to investigate all possibilities of recombinant DNA or other genetic engineering techniques.
As a consultant of industry I know that private enterprise is investigating subjects for which there are no marketing possibilities at the moment, but might become a reality in the future. In biotechnology you have to take gambles, you will have to accept that you won't see a return on your money for five or ten years. And if you are not prepared to accept that kind of risk, you should not step into the business in the first place.

(Rörsch) Ms. Haber indicated a comment?

(Haber) Yes, a brief one. It is an interesting question, especially when taken with the one before about the most promising areas in biotechnology. Yes, there are a small number of children who will need human growth hormones. Given that situation it is important to that small number of children. But if you are deciding on social priorities, where to put resources in developing biotechnology, I would say that the more promising field is, perhaps, single cell protein, because there are more hungry people in the world than children who need human growth hormones. I know that it is a difficult decision to make. But if you are working with limited resources, then you have to decide where the greatest need and the greatest social need is.

If I had to distribute money - which I am not, as some of you will happy to hear - then I think I would put my money into the other field. That may be not profitable, but I think it fills a greater social need.

(Rörsch) I think there is a comment from the floor.

(Comment) I am rather shocked by the solution that has just been propounded. It means that you should not develop drugs for, so called, small diseases, because other needs are greater. Are those needs greater in reality? If you don't treat a child in need of human growth hormone, you may get a sub-normal adult who may live for many years. What is the cost to society? I think that should be taken in account too.

Then I would like to point out that it is very easy for Dr. Cape to say that we should go out and start our own companies. That may be true in the US, but it is not here. Here the general climate is against it.

(Rörsch) Thank you. I am afraid that I'll have to go on to the next question, which is to Dr. Cape. "Could you give further information about bioproduction of hydrogen as an energy source?"

(Cape) No, I can't, I have no answer to that one.

(Rörsch) Anyone in the audience who wants to comment?
No, then I'll go to the next question, which I think is about 'clean technology'. It runs: "How do you get rid of mycelium in a fermentation industry?". Would you have a go at it, Dr. De Flines?

(De Flines) Certainly. You have to get rid of the mycelium, otherwise you would have an enormous problem. You can do it in a number of ways. The most expensive is to burn it; that takes extra energy because of the water content. Then you can put it into a waste-water treatment system. There is no problem, as the material is biodegradable. Another way, rather widely used - and we use it too - is to filter off the mycelium, remove traces of material that are not desirable and sell it as a feed for cattle; you get some money for that. You can dry it and sell it as a feed for animals. But the drying process is expensive and the wet stuff can go bad easily, and if it does it cannot be sold as feed, so then you have a problem.
You can put it into the sea; because fish can eat it and thrive upon it as the stuff is biodegradable. The last possibility is to use it as a kind of fertilizer and to dump it on land. But that can be rather smelly, and it should not be done near to houses or cities.

(Rörsch) Thank you. Not surprisingly, there are a large number of questions to Ms. Haber. However, I would like to answer the first one myself.

(Haber) Is that up to me or up to you?

(Rörsch) It is up to me. It is about your statement that the majority has the right to be wrong. This has led to: "Can scientists accept something that is not true, even if the majority says the contrary?". This is the Galileo situation. My answer is: scientists should always be honest, and I believe they usually are. Jim Watson may call himself an ass - which by the way he is - but he is also a brilliant and honest scientist. I think there often is a communication gap between scientists and non-scientists. A scientist will never say that he knows the truth, but he is always prepared to give his honest opinion, which, I am afraid, many people do not like to hear.

I want to leave it at that, and now I have the first question to you, Ms. Haber. It runs: "How does GMAG inform the public as to avoid that GMAG and the public get a different perception of risk after some time?".

(Haber) So it is my turn now?

(Rörsch) Yes, of course.

(Haber) Well, GMAG is perhaps somewhat, and in my view unfortunately so, too secretive in its deliberations, our meetings are closed which I consider as unfortunate. We do have our published annual reports, which list the work we have done, we do publish 'guidance notes' in between annual reports, and we have quite an extensive mailing list of people who receive the guidance notes - mostly those who are doing recombinant DNA work. So we do have some communication.

Then we have organised a few public conferences where we invited people to discuss problems of recombinant DNA work with us. My union sponsored a public conference about a year and a half ago where the pro's and con's - risks or not-risks - of recombinant DNA were discussed again.

But now that I have the microphone, I would like to comment on the first question that was answered by the chairman.

(Rörsch) Sorry to interrupt you. I have nothing against it, but let me give you the next question, because it is related. It runs: "The statement of Ms. Haber was directed to the point that governments, private companies and scientists should not make decisions that are wrong. But at the same time she put forward the proposition that the people at large have the right to make decisions that are wrong. It strikes me as a non-scientific contradiction.".

(Haber) Well, I am afraid that it does not strike me that way. As I said in my talk, if you accept democratic principles, then you have to accept that the people have the right to make decisions, and some of these decisions may be seen by scientists as being wrong. Then the problem is how to stop the people from making wrong decisions. And as every other rational person I want to have decisions that are not wrong. But I think that the responsibility for seeing to it that this happens, rests to a great degree on the shoulders of the scientists. It is up to the scientific community to educate the public and to state clearly what they think is right. For the scientist it is no good to come and give a lecture in his language, if he does it he should use the language of his public. I know that is very difficult, but I know too that it is very important. If scientists communicate adequately with the public, by that very fact they will help the public to come to decisions that are not wrong from their point of view.

I still want to go back to the first question, answered by the chairman, which he compared to the Galileo situation. I can understand that situation quite well. But when a scientist or a small group of scientists believe that they know what is correct and the rest of the world disagrees with them, the view of the rest of the world will prevail. It is not a question of knowing what is right, it is about convincing the public that you are right. Galileo did so in the end because his arguments were unassailable.

Perhaps a short anecdote about Bernard Shaw will clarify what I am talking about. It was the opening night of one of his plays, and the audience was very enthousiastic, cheering and calling for the author. After a while Shaw walked on the stage, accepted the cheering and the enthousiasm, and when it died down he heard one lady in the audience shouting: 'Rubbish, horrible, awful, terrible play'. Shaw turned to her and said: 'I quite agree with you, my dear, but who are you and I against hundreds?'.

I think there is a lesson to be learned: unless you can convince people - and I think scientists can do it although it takes an effort - you will not be supported by society, and you will have a lot of conflict, like the present conflicts about nuclear energy, for example. If those in biotechnology don't convince the people they might get the same conflicts.

(Rörsch) There is another question for you, it is a rather long statement and I gave you a copy, but I think it boils down to this: "The trade unions should give their advice, but not make the decisions and try to control affairs". Could you give a short comment?

(Haber) Basically I think that decisions should be made jointly. The trade unions have a part to play, but as decisions will have to be reached after discussion, they cannot be the only partners in the debate.

If I were an autocrat, I would say: yes, I'd like to control everything and make all decisions myself; I don't mind if they are made by one person as long as that person is myself. But I believe in democratic methods for decision making, and so I think that the trade unions have a part to play as have the employers organisations, the scientists and all the other sectors of the community that are affected by the work that is being done. Trade unions may have a slightly larger part to play as, just as I said in my paper, if there are any hazards in certain types of work, it is the work-force which will be first and foremost at risk.

(Rörsch) Thank you. Here comes the last question I have for you and again it is a long statement. But I think it can be summarized into: "Biotechnology is in its infancy. Do you see at the moment any new elements about which the public should be informed?".

(Haber) I would like to tackle that from the way the public could react. The public could become quite emotional about a number of issues: scientists are now playing around with life itself, scientists can create new forms of life not occurring in nature by just playing around with genetic material of micro-organisms, could that be extended to human beings and, if so, what will be the consequences?

I think that are real fears. For the scientist the only way to allay those fears is to

explain what he is doing and what the risks of his experiments are. It is the only way I see to defuse the emotions. If I may, I would like to enlarge upon something that has stuck me: it is the fact that in discussions like these most parties are so terribly one-sided. Quite often they cannot see both sides of the question, they only see their side and argue from that, which is not the best way of starting a real discussion.

What should we do to educate the public? One place we could select is the schools. I think it could be quite worthwhile to inform children at school, the young generation, not only about the benefits of biotechnology, but also about its hazards. For if you don't inform the young generation about the hazards of biotechnology and the ways of science to contain those hazards, they will find out for themselves. Then they will start to ask questions as: Why is science not honest with me; why did the scientist not tell me about the hazards? I think the only approach is an honest approach, saying quite clearly what the hazards are and what we are doing to contain them.

(Rörsch) Is there a comment from the audience? Yes, there is.

(Comment) The discussion on this subject that has been going on in the USA, the UK and elsewhere, is admittedly imperfect. But I think it is the most extensive public discussion on any issue I have heard of. It even may be a model for other problems that may arise in the future. I think there is an enormous amount of good-will involved and that we are moving in the right direction.

(Comment) In all these comments on modern technology and biotechnology I detect some negativity. Of course, the public will have to be informed about hazards and consequences, but I think we are going too far in that respect at the moment. May I point out that in the time of Fleming hazards were not mentioned, or very softly only, and that, as a result, we developed some very important medicines very quickly. The present tendency to paint all possible hazards on a large canvas is rather one-sided, we should point to the possible benefits too, especially for the sufferers of those diseases that cannot be cured at the moment.

(Cape) May I have ten seconds? Of course I agree with the gentleman in the audience, but I would like to point out that I have never seen anything compared to the favourable press and the process of public education by the press on the subject of biotechnology and the possibilities in this field.

(Rörsch) Well, my solution is also continuous education, but I know that it is so damned difficult to get the attention of the public for something that is not dangerous. Does Ms. Haber want to comment?

(Haber) Yes I do. I agree with the gentleman in the audience too. Perhaps I have been putting a lot of emphasis on hazards and risks, and it might be that I always do so. If I do, it is to counter those who are delivering sales talks, who tell us how wonderful everything is without pointing to the hazards and risks. But I am not blind to the points that have been just raised in the audience.

(Rörsch) I have a factual question for Professor Demain: "Could you enlarge somewhat on direct fermentation of cellulose to alcohol, on the producing strain, the economics and future prospects.

(Demain) At MIT and I think at a few other places Clostridium thermocellum is being investigated for this purpose, and the organism has some attractive characteristics. It can break down cellulose to sugars and it can convert those sugars to ethanol. The sugars are cellobiose and glucose, as is usual with these types of cellulolytic organisms. Clostridium thermocellum has also the interesting characteristic of being able to break down hemicellulose, although it cannot use the zylose that is a break-down product. Dr. Wang at MIT has been conducting mixed fermentations, using Clostridium thermocellum and Clostridium thermosaccharoliticum which is an organism that can convert zylose, cellobiose and glucose to ethanol. So we have the possibility of converting hemicellulose, which is a waste material, to ethanol. I'll go rapidly through some other attractive characteristics of the organism as compared to the usual

cellulolytic fungi. The activity of the enzyme is not influenced - to be honest only slightly - by the concentration of the sugars in the solution, and in this respect it is unique among cellulolytic organisms. It is a thermophile, it grows at 60°C and this could help to reduce the costs of extraction of ethanol, and of course is helpful in retarding contamination. It is also an anaerobe, and this has the benefit of eliminating the costs of oxygen transfer, which can be very expensive. Another benefit is that little cell material is formed during the fermentation, so you don't lose half of your substrate because of cell formation.

However, there are some problems too. One is the formation of acetic and lactic acid along with the ethanol; another is ethanol tolerance as these organisms are not as tolerant to ethanol as yeast. Lignin is a serious problem, there has to be developed an economic process to remove lignin if we are going to grow trees for the conversion of cellulose and hemicellulose to ethanol. And the enzyme contents of the cells are considerably lower than the enzyme contents of some other cultures. So it is a promising method for conversion of cellulose, af agricultural energy into ethanol, but it will take at least several years to turn it into an economic process.

(Rörsch) Thank you very much. I have given a large number of questions to Dr. Cape, and he will not have time to answer all of them. Could you make a selection of two or three?

(Cape) I'll try in any case. I have one here about the difficulties of expression because of differences in transcription, and another asking me whether I think that there are real fundamental things that theoretically might be expected to be impossible. I am inclined to say 'no' to the last question, and 'yes' to the first, asking immediately 'so what?'. I am remembering, and I guess with some retrospective amusement, that years ago when we were talking about the possibilities of genetic engineering and its glorious future, there always were people who could provide us with ten or more reasons why it would not work. Up till now we have always proven them wrong. I think we should recognize clearly that in the last twenty or thirty years a tremendous amount of fundamental research has been done and that we now are in a position to reap the fruits. To coin a trite phrase: it is the fundamental understanding that comes first, and only then the practical results will start to arrive.
In one of the first questions it was suggested that the results of biotechnology have been rather meagre, and that will seem to be so if one forgets the fundamental effort that has been put into it. If one looks at the state of the art in 1945 or 1950, one sees what specific advances are behind us. At present nobody is postulating fundamental questions yet to be answered that compare with the ones that have already been answered. Of course there will be difficulties, but these are no longer problems, only challenges. They represent opportunities, and we know pretty sure how to get around them.

(Remark) What about the production of interferon and its difficulties?

(Cape) That is an extremely difficult problem, but I could postulate that perhaps a yeast would be a better organism than a bacterium. It might turn out to be a real difficult problem that will take years to solve, but I don't think it a fundamental theoretical problem, and that is all. We may have to deal with, what I would call, some antics and it might take years to remove these.

The third question on which you might be interested to hear my comment is about intervention and help by governments. I guess that I come philosophically from a society that believes that industry is the implementing body. Practical things get done in the private sector; government is to leave well alone unless there is a hypothetical, or a clear and present danger that requires regulation. Most of us in the field have the feeling that we don't want the help of anybody, and certainly not of government. What government should really do is to stimulate fundamental knowledge which will advance the field, and that is proper to universities.

To coin a rather simple simile: If a country wants to join in international hockey, it should see to it that the population gets enthousiastic about hockey. But if the population does not, that country will never be in an international hockey tournament with a team.

(Rörsch) It is a pity that there is not time for it, otherwise I would have liked to comment on the answer you just gave. But thank you all the same, Dr. Cape.

I now have a question for Dr. Van Beynum: "Are there immobilized enzymes available on the market?".

(Van Beynum) I thought that I had answered that question in my paper, but I can do it again. Glucose isomerase is an immobilized system that is available on the market and can be bought by anyone. It is produced by Novo in Denmark and by my own firm, Gist-Brocades in this country. Large amounts are sent every year to the USA by the Danish and the Dutch. Anyone who wants it, can buy it.

The Japanese have immobilized systems for malic acid, amino acids and amino acid acylase. To my knowledge they are not 'on the market' as they are not sold to other firms or other countries. Then we have penicillin acylase which is produced by several firms using immobilized systems. But again these systems are not sold to outside customers.

(Rörsch) Thank you very much for your short and clear statement. Dr. De Flines, you expressed preference for liquid submerged fermentation over solid state. The Japanese industry indicates an opposite preference. Would you like to comment?

(De Flines) I don't think I can agree with that statement, it does not seem to be true. I have been to Japan a number of times and I have visited many fermentation industries over there. One big company, the biggest soy souce producer in the world, uses the solid system for producing their product which as all of you know, is a fermentation product. It is a very old one and the industry does not want to change it because they fear it would affect the taste. Other fermentation industries I have seen produce antibiotics, glutamic acid and other products and they all use deep fermentation. But perhaps the man in the audience who asked the question in the first place, would have a comment?

(Comment) Yes, I'll do so. In your paper you were talking about fermentations in general and it is in this context that I raised my question. You said, I think, that solid fermentation was used in the past, but that we now prefer submerged fermentation, because it has less cooling problems, transfer of contamination and so on.
But I could also argue in favour of solid state systems from a theoretical point of view: volume is less, contamination might be easier to control, separation might not be such a problem and so on.
To be more specific on the Japanese situation, we know that they are active in cellulolytic enzymes, but Japanese industries usually are very secretive about the things they develop. But I have the impression that they do not neglect the solid state approach.

(De Flines) You may be right there and I did not want to say in my paper that we should neglect the solid state approach. But at the moment other processes are preferred for economic reasons, and I think that is a good reason to prefer them.
But I agree that we should never lose sight of the solid state process.

(Rörsch) Ladies and gentlemen, our time is up. We would have liked to go on, but the time-table does not permit it.
May I thank all of you who posed so many interesting questions, and I thank in particular the members of the panel who did their utmost to answer the questions.

Industrial Aspects of the Biotechnological Production of Steroids

Professor Dr. K. Kieslich
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Biotechnology covers all processes that synthesize, convert or degrade substances with the help of micro-organisms, plant or animal cell cultures. As a rule it is accompanied by the formation or modification of biomass and by the recovery of certain components of cells.

Quickly, people in a number of fields became interested in biotechnological methods, examples are: pharmaceutics, foodstuffs, animal feed, basic chemicals, agriculture and environmental protection. However, it has now become common knowledge that the exploitation of biotechnological processes on an industrial scale requires a careful consideration of a large number of industrial aspects.

In this paper a number of these factors are reviewed for a special application in the pharmaceutical industry, i. e. the biotechnological processes used for the production of steroids, which will serve as an illustration of the multiplicity of conditions that have to be considered.

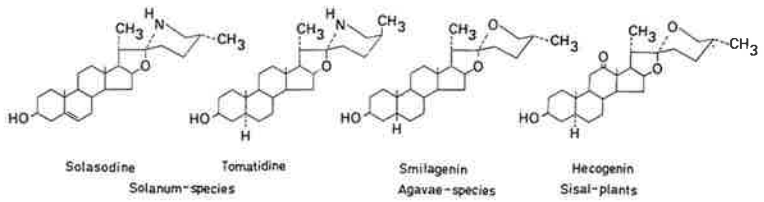
A few years ago the quantity of steroid raw materials required to satisfy world-wide needs, was already in the order of 1000 to 2000 tonnes a year. The raw materials were converted in commercial preparations, serving a world-wide market of over $ 300 million a year, a figure indicating the importance of the field.

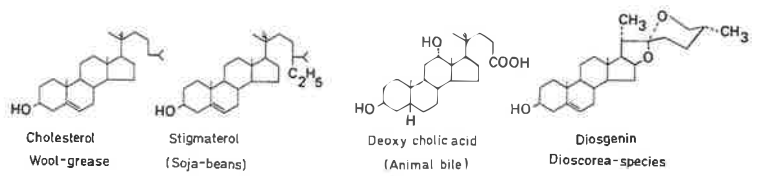
The biotechnological processes that have been in use for several years now, are mostly employed in two fields only:

- production of suitable starting materials for steroid synthesis;
- effecting specific transformations in the, otherwise, chemical synthesis of sex and adrenocortical hormones.

Production of suitable starting materials

Compounds possessing the characteristic steroid structure abound in the flora and fauna of this world, although concentrations vary widely. However, as parent materials for steroid synthesis, only the following structures offer a chance of adequate economics, and are therefore fit for consideration:





Of these natural products, the first three are hardly out of the development stage. They are transformed by purely chemical means into basic structures that can be used for further synthesis.

The sole exception is the hydrolysis of the sugar residue dirhamnoglycoside from dioscin, present in yams (Dioscorea) for the production of diosgenin. This is accomplished by an enzymatic process. Soil bacteria which cling to the open concrete basins ferment the mash of crushed yams, and so it can not be qualified as a controlled biotechnical process.

The compounds industry needs, have to be recovered from plants, fruits or animal organs, and the only things industry could do were to rationalize their gathering as far as possible, to improve recovering and purification methods and to try to cultivate the wild plants in plantations.

Biosynthesis trials by submerged cell structures

1. There has been a rather heavy research effort to obtain steroid structures from plants by biotechnological processes. It was hoped concentrations would be higher than in the natural products, and of course it was attractive to become independent of climatic conditions and of sudden changes in the foreign policy of the producing countries. It was shown that both solanaceae and dioscorea species could be grown on solid nutrient media in the shape of callous tissue with adequate steroid contents, and that these could be propagated in a fermenter by submerged culture. Unfortunately, matter-volume-time yields - in other words the amounts of recoverable steroids in relation to volume and time - as attained to date, are still at a far too low level for the process to become economically interesting. The cause is the low propagation speed of the vegetable mass, and this is illustrated in the following figure:

A) World market price of diosgenin $ 25-35/kg

B) Average total costs of fermentation with normal product recovery $ 50-100 per m^3 in 24 h.

C) i.e. 2-4 kg diosgenin should be obtained from 1 m^3 in 24 h.

D) Optimum formation of diosgenin up till now 0,05-0,07 kg diosgenin/m^3, day.

2. Plant cells have rather long generation times, but micro-organisms multiply very fast. So biosynthesis of steroid materials with the help of a micro-organism seemed much more promising at the outset. Previous results, obtained by Squipp Company, indicated that use could be made of the lower fungus Polyporus sulfureus, which forms more than 4 grammes of eburico acid per litre in just 48 to 72 hours of fermentation. The acid is an extracellular product and can be isolated from the culture broth by extraction without any difficulty. Biotechnologically the process is not very difficult, and costs are reasonable from an economic point of view. Unfortunately, its suitability as a starting material for steroid synthesis proved to be poor, as the elimination of the methyl groups in the 4 and 14 positions requires expensive sequences of chemical reactions. Other steroid structures, such as fusidic acid and helvolic acid, which are known as antibiotics, have modified ring structures and are not acceptable as starting materials either.

3. Ergosterol formed in yeasts, on the other hand, has long been a starting material for vitamin D production. In itself, yeast fermentation is one of the oldest biotechnological processes, and it is accompanied by cellular growth of remarkable rapidity. Previous efforts with the fermentation of Saccharomyces strains particularly rich in sterol had led to yields, as a mixture of ergosterol and dehydroergosterol, of up to 750 grammes from one cubic metre of culture broth in just 30 hours, using a technically feasible recovering method for this intercellular product. The medium used was simple and consisted of 10% molasses and 1% cornsteep.

Although the conversion of the product into a suitable starting material for steroid synthesis involves only relatively simple chemical processes, the biotechnological product is still too costly in this case.
In view of the rising prices of molasses the chance that it can become cheaper in the future, is low.

The same conclusion goes for the other examples given as yet, and here we have an essential factor in the application of biotechnological methods on an industrial level: the product-volume-time yield.
If one does not aim at high-quality and, therefore, high-priced products, a biotechnological manufacturing method will never be competitive unless appreciable quantities of substance are formed rapidly per volumetric unit. A good example is the inexpensive amino acid lysin, which can be produced biotechnologically because no less than 57 grammes are formed per litre.

A study by the French firm of Rhône Poulenc gives the following distribution of costs:

Raw materials:			
42 %	Molasses	}	40 %
21 %	Sucrose		
14 %	Starch		
6 %	Corn-glucose		
17 %	N-source + inorganic salts		
Energy			16 %
Operating costs			27 %
General costs			17 %
Fermentation costs		total	100 %

(Data obtained from Rhone-Poulenc, E. Gwinner, Chem. Ind. XXXII, 86, 1980)

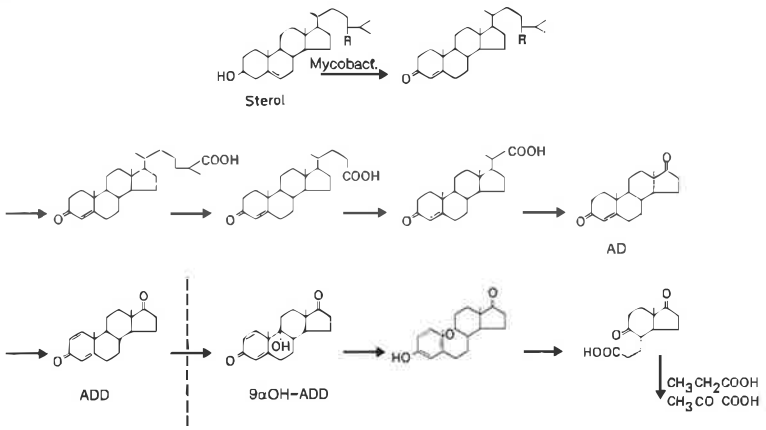
It can be seen that the nutrients are the most important cost factor in these types of biosynthesis, and this points to a way of lowering of costs: either use micro-organisms with high biosynthetic potential or utilize low-cost wastes as basic nutrients.

At the moment there is no method for biological sterol synthesis that is attractive from an economic point of view. Nature still is the most important producer of raw materials for the synthesis of steroids. The most important are diosgenin, which has been mentioned already, the bile acids from cattle gall bladders and stigmasterol, which occurs in soy-beans. They are isolated by purely chemical methods.

Sterol side-chain degradation

However, with biotechnological methods one might tap new sources of raw materials, used no longer or not yet because a purely chemical route to an intermediate or an end product is too costly. From 1935 to 1956 Schering AG of Berlin used cholesterol as a starting material for steroid synthesis. It was reduced to a steroid raw material fit for further utilization by chemical oxidation. However, multiple secondary reactions caused the yield of the chemical oxidation to remain minimal.

Ever since 1913 it has been known that micro-organisms too are able to oxidize the sterol side chain. Mycobacteria are especially effective, but they have a serious draw-back. Besides the desirable degradation of the side chain by the well-known mechanism of β-oxidation, these bacteria also cause a 9α-hydroxylation tantamount to the initial stage of fission of the steroid skeleton, which, of course, is completely undesirable.

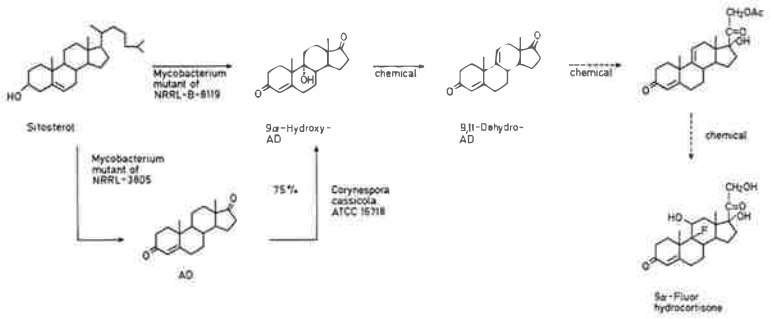


In 1965 Charles J. Sih - and independently Arima - elucidated the degradation mechanism and discovered a brilliant method which permits the elimination of the undesirable reaction. The structure of the substrate is modified to 19-hydroxy-cholesterol and then it is possible to steer the transformation directly to estrone. This indicated the way towards a practical method of sterol side-chain degradation using micro-organisms.

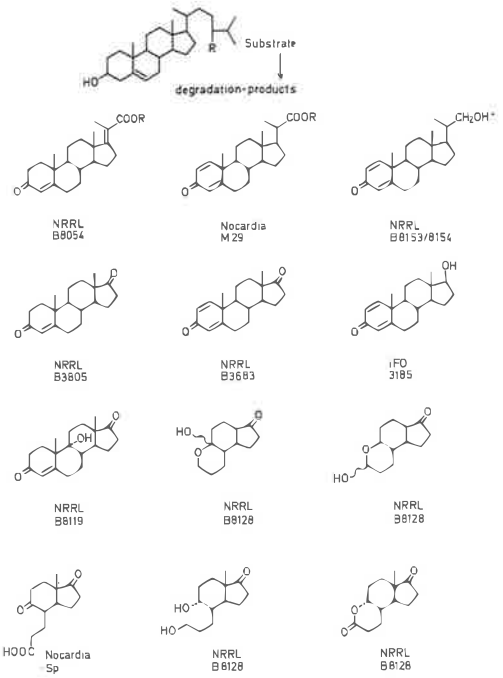
In the last years a number of interested operators in this field, such as Gist-Brocades, Searle, Upjohn, Schering AG, Mitsubishi and GBF Braunschweig, also discovered methods to transform sitosterol, which occurs together with stigmasterol in soy-beans and could not be utilized hitherto. Degradation is carried out mainly by Mycobacteria and Athrobacter strains. All these methods are based on the blockage of 9α-hydroxylase, with or without an additional inhibition of 1-dehydrogenase.

In particular, the use of suitable mutants and the development of suitable forms of administration of substrate led to technical processes which, using relatively high substrate concentrations, give 4-androstene-3,17-dione or 1,4-androstadiene-3, 17-dione at low costs.

A further utilization of sitosterol side-chain degradation was patented by Upjohn, based on the development of a mutant inactivating the 1-dehydrogenase instead of the 9α-hydroxylase. This gives 9α-hydroxy-4-androstene-3,17-dione, an excellent starting material for corticoid synthesis, as the 9α-hydroxy group is dehydrated easily into the $\Delta^{9,11}$-dehydrosystem, and so offers a starting structure for the production of 9α-halogen corticoids. The 11-hydroxilation, which would be required otherwise, is thus combined through a 9α-hydroxilation with side-chain degradation in a single fermentation step.



An alternative would be to take advantage of the highly optimized degradation to 4-androstene-3,17-dione in combination with a separate 9α-hydroxilation in another microbial step, making use of Corynespora cassicola fungus. The addition of the necessary corticoid side-chain might be carried out at a suitable point of the synthesis by various methods. Although this way of total degradation of the side-chain followed by a partial reconstruction does not look good at first sight, it should be realized that the known micro-organisms, contrary to a mammalian cells, are unable to form a progesteron structure from the sterol side chain. Nevertheless, other mutants derived from Mycobacteria of Corynebacteria can transform phytosterols into further intermediates, which accumulate by an inhibition of various degradation reactions.



Such intermediate structures may also serve as starting materials for the partial synthesis of new steroid analoga.
The industrial advantage of these processes lies in the use of very low-cost substrates for the production of high-quality starting materials for synthesis. The profits can be increased still more by using sterol mixtures, like those deriving from tall oil, a waste product of paper manufacture.

In the beginning, the use of Mycobacteria seemed a doubtful issue, because of their taxonomic kinship with pathogenic germs. But the proofs of harmlessness demanded by the public health authorities through animal experiments, exclude strains with any toxic property from industrial application. All mutants too, have to be investigated in the same way in each individual case. In addition, the danger of a non-pathogenic strain changing by mutation to pathogenicity, is far smaller than that of a lack of stability of the varied enzymatic properties within the mutants.

However, it is to be expected that the qualifications, imposed in the field of environmental protection, will grow much more severe when, in the future, we will start to use micro-organisms into which by the way of plasmids, the level of desired enzymes has been increased or has been introduced with markers of antibiotic resistance.

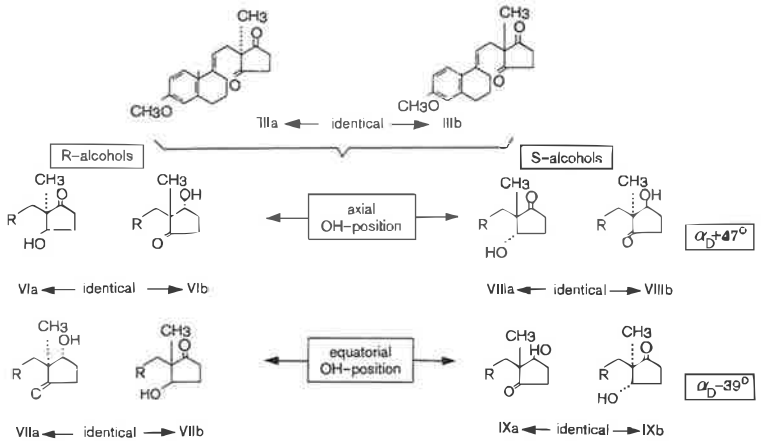
In general, these measures will add to the costs of biological fermentation methods as, besides the usual precautions against reactor contamination by foreign germs, the industry will also have to protect the environment against production organisms. Possible methods are post-combustion of outgoing fermenter air and sterilization of fermentation broth prior to product recovery.

3. Total synthesis

A third and ultimate method for the manufacture of steroid hormones is integral synthesis which, of course, offers total independence of natural raw materials which are not available in unlimited quantities. For this, an optimization of the well-known total synthesis proposed by Torgov and Wyeth, was required. Schering and Roussel-Uclaf discovered independently of each other a microbiological step that is of decisive importance for the economics of the process.

When the so-called secodione, a very cheap synthetic starting material, is cyclisized, C_{13} becomes the first asymmetrical centre, and a racemate is formed because there are two possible ways for ring closure. The formation of a racemate at this stage, however, can be prevented if, prior to cyclization, the C_{13} configuration is made asymmetric.

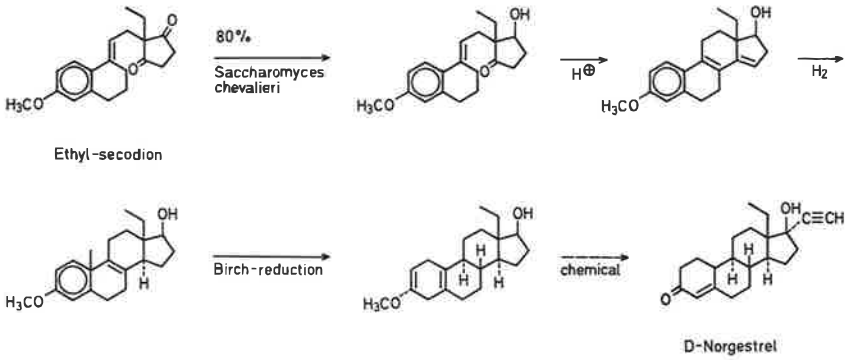
This may accomplished by microbiological reduction of one of the two ketone groups. Theory indicates that this should give four enantiomeric ketols, but dependent on the micro-organism used, three pure forms are obtained actually, the 17-keto-14β - alcohol not having been found up till now.



Possible Stuctures of the Hydroxy-Keto Compounds Formed by Microbiological Reduction of Seco-dion.

For the synthesis of estrone, the 14-keto-17-alcohol configuration is needed. This compound may be made by Saccharomyces uvarum with a yield of 70 to 75% of pure product in 42 hours of fermentation and substrate concentrations of several grammes per litre. After cyclization the compound forms a pure estrapentaene characterized by a natural ring configuration. The chemical steps that follow, are plainly controlled by the chirality, introduced by enzymatic means, and the total synthesis of the natural product estradiol is achieved in a single microbiological and five chemical steps, without racemization occurring.

For the manufacture of estrone other equivalent methods were developed, such as one based on the pyrolysis of 1,4-androstadiene-3, 17-dione, which is obtained at very low cost by the microbiological side-chain degradation of sitosterol. In synthesis of the important contraceptive D-Norgestrel with the unnatural 13-ethyl group, on the other hand, the microbiological stereospecific reduction step is still used in an otherwise total chemical synthesis.

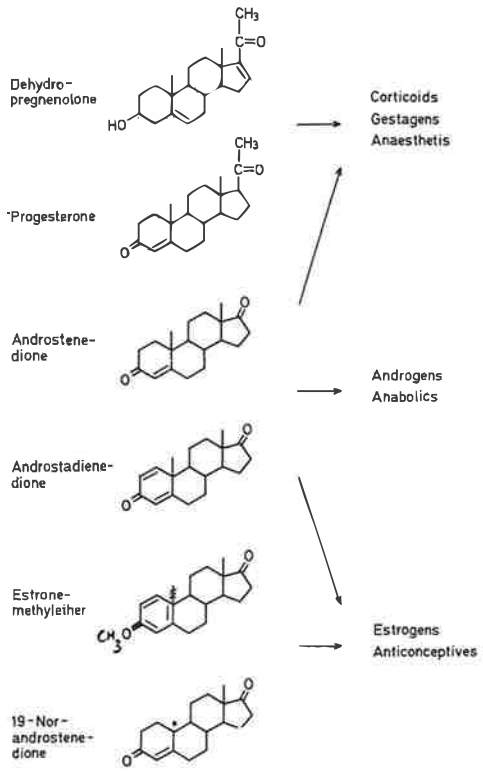


As in any other industrial microbial process, competitive success depends on the identification of the most suitable micro-organism for carrying out the reduction and on a cheap nutrient. As steroid substances are poorly soluble in water, the form of substrate administration is another decisive factor. Here, one can use a number of techniques, such as: micronization, preparation of special suspensions and emulsions, addition of surfactants and apportioning of substrate addition. In some cases these techniques or a combination of them, may lead to a significant increase in the concentration of substrate in the culture broth, a factor as decisive from the point of view of economics as is volume-time yield in the case of the manufacture of biosynthetic products.

In summary we can say that industry is always on the look-out for:

- Suitable new biosynthetic processes
- New sources of raw materials
- Total syntheses with or without a microbiological transformation step.

The chief commercial considerations in this field are the price and accessibility of raw materials, and the competitive advantages or disadvantages of the biotechnological, or chemical processes that are available.
At the moment most processes for steroid synthesis start from:

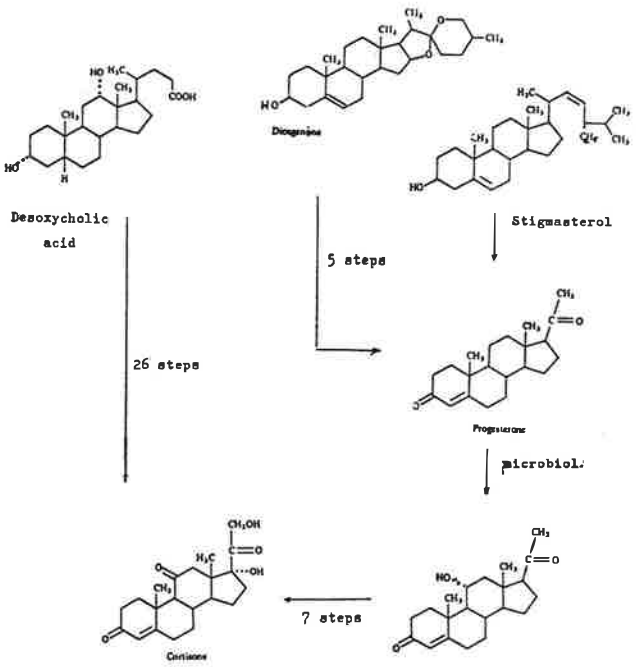


Other microbiological transformations in steroid synthesis

A number of other microbiological transformations might be used to replace chemical steps in a synthesis route. In industry just a small number are used, though.

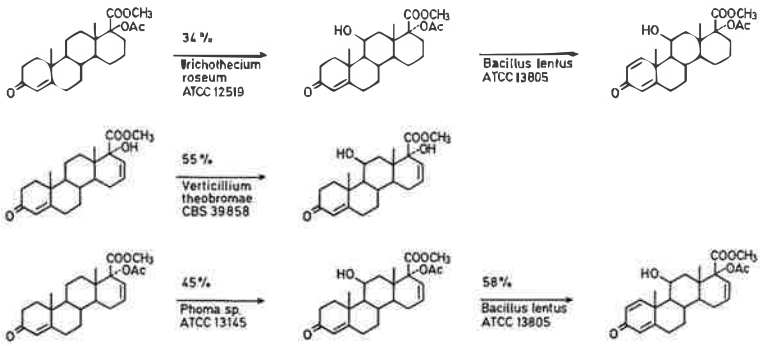
Hydroxylations

Of these, the most important microbial transformations are hydroxylations - in other words the introduction of hydroxy groups - and they are particularly important in the synthesis of corticoids. At present, the classical 11-hydroxylation, first developed by Peterson and Murray using Rhizopus or Aspergillus strains, has attained an elevated stage of development. On a substrate of progesterone, or related structures, in concentrations of up to 50 grammes per litre the hydroxylation may be carried out in 2 to 3 days of fermentation with yields of 80% or over.

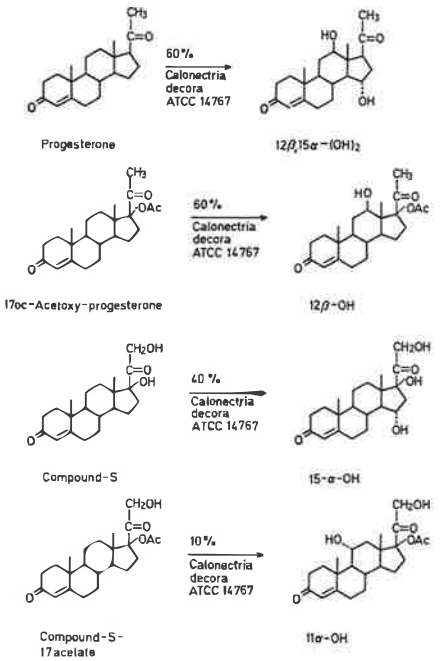


As is well known, a purely chemical synthesis of corticoids was based on bile acids which had a 12-hydroxy group in the steroid, this group being transferred to the 11-position in a route of many steps.

However, 11 α-hydroxylation is not the natural one, and consequently the compound is just an intermediate. It is clear that an introduction of the 11-hydroxy function in the native configuration leading from Reichenstein S to hydrocortisone structures, is of much greater significance. Knowledge of the inactivation mechanisms of cortico-steroids pointed to a way to increase the activity of the original hydrocortisone by the introduction of substituents near to inactivation centres, or by the introduction of new functions. In this way it was possible to minimize undesirable effects or to enhance desirable ones. The result was a large range of modified corticosteroids with strong anti-inflammatory action, which required 11-hydroxylations for their synthesis.
A number of different fungi are known which can carry out the required hydroxylation. Curvularia lunata frequently proved to be the best agent with substituted Reichenstein S compounds. To cope with structures that have been modified more strongly, other fungi have to be used now and then, as in many cases the substrate structure heavily affects enzymic activity and the orientation of the attack.

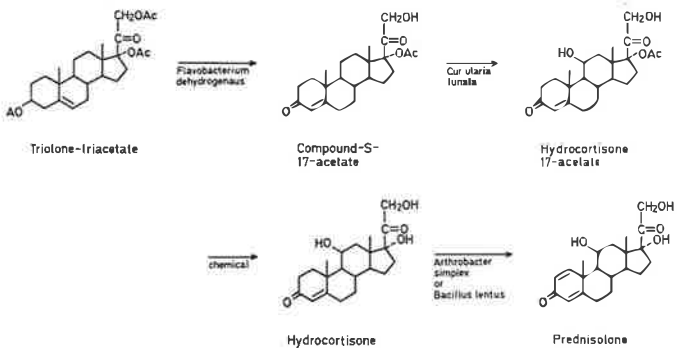


Suitable strains may be selected from known collections or may be obtained by thorough testing of freshly isolated new strains. In addition, we can modify the substrate chemically to ensure that we get the transformation we want to have. If we use Calonectria decora, the possibilities are illustrated in the following diagram:



Results obtained by Schubert show that Calonectria decora hydroxylates progesterone is the 12β- and 15α-positions. When the 15α-position is sterically blocked by a 17α-acetate, only 12β-hydroxylation occurs, as expected. If the hydrophylic character of the substrate (for instance in the case of compound S) is raised, the 12-β reaction is stopped, as the 15α-hydroxy group is introduced faster. Dihydroxylations are observed only in relatively lipophilic substrates. Let us close these remarks with the surprising fact that compound S-17-acetate is hydroxylated neither in the 12β- nor in the 15-position, but in the equally equatorial 11α-position. It will be clear that here we have a method for steering the direction of attack in some degree.

In the Gist-Brocades process this method is applied technically. It uses Reichenstein-S-17-acetate as a substrate and effects the 11β-hydroxylation with Curvularia lunata.



The secondary hydroxylations in the 7α- and 14α-positions which are usual with this fungus, are completely suppressed by the voluminous acetate residue in the 17α-position, so that the microbial process yields hydrocortisone-17-acetate which is easily saponified in a weakly alkaline medium into the hydrocortisone desired.

In addition to the 11-hydroxylation and the 9α-hydroxylation mentioned before, the microbial introduction of a hydroxygroup in the 16α-position is well known in the synthesis of the corticoid triamcinolon.

For the preparation of steroid structures on a laboratory scale other hydroxylations are used. In general we can say that the introduction of hydroxy-groups in any position of the steroid skeleton is possible now, using a number of different micro-organisms.

1-Dehydrogenation

The anti-inflammatory effect of the corticosteroids mentioned stems from the double bond in the 1, 2 position. This structural characteristic can be introduced by chemical and microbial means, but the chemical processes are markedly inferior to the microbial ones, both in purity and in yield. There are known a large number of bacteria, and some fungi which can carry out the 1, 2-dehydrogenation. Arthrobacter simplex is used often for the dehydrogenation of the 1, 2-configuration. In the fermentation of hydrocortisone to prednisolone this bacterium can convert substrate loads of up to 100 to 200 grammes per litre. Frequently, however, such high loads lead to incomplete conversion, and then an expensive step is needed for the separation of the prednisolone and the unconverted starting material. So in most cases a lower substrate concentration is actually accepted.

But conversion costs are not the only parameter when assessing the competetive potential of a microbial method. Degradation of substrate and product, or both, may be just as important.
Arthrobacter simplex has the unpleasant tendency to degrade both hydrocortisone and prednisolone, and as the prices of the substrates are often more than $ 1000,- a kilogramme, the search for another micro-organism that does not degrade the substrate or the product, can be quite worthwhile.

Immobilized biocatalysts

Undesirable reactions can be avoided by the exclusion of living cells and the use of immobilized enzymes. That looks quite attractive, but it should be realized that the industrial utilization of immobilized enzymes is still limited to enzymic reactions that do not need expensive co-enzymes. Many oxidative and reductive conversions require co-factors, and the development of effective methods for their recovery or regeneration is an enormous technical challenge.

An alternative might be the immobilization of whole cells, as their enzymic performance can be regenerated more easily.
Although the dehydrogenation of hydrocortisone to prednisolone with fixed Athrobacter simplex cells has already been developed to a fairly advanced stage, a technical application of the process is not in sight yet.

Waste treatment

Although the mycelium left over from the production of antibiotics may be used as an animal feed in some cases, the biomass generated by steroid conversions still is nothing but a waste. Sometimes it can be dumped, but otherwise it will have to be degraded and, as Dr. De Flines pointed out in his paper, that can be a major operation which adds significantly to the costs of the product. It is here that immobilization of cells could become attractive. Immobilized cells allow repeated use of the biocatalysts and this tends to keep production of waste biomass low. So immobilized cell techniques are quite attractive from an environmental point of view.

Fermentation conditions

In addition to the selection of a suitable strain, either from known collections or by the testing of freshly isolated strains, another factor of paramount importance is the optimization of the conditions of fermentation.

In general, these conditions may be affected by the following parameters:

1.) Chemical or biochemical variables
  1.1) Composition of the nutrient liquid
    1.1.1) C-source
    1.1.2) N-source
    1.1.3) organic salts (trace elements)
    1.1.4) growth factors and vitamins
  1.2) Fermentation additives
    1.2.1) precursos, substrates
    1.2.2) inhibitors, promoters
    1.2.3) substrate solvents, emulsifiers
2.) Variables with a biological effect
    2.1.) quantity of the inoculum for pre-fermentation
    2.2) quantity transfered from the pre-fermenter

3.) Physical and physicochemical variables
  3.1) form of the added substrate
    3.1.1) dissolved, micronized, partially dissolved
    3.1.2) concentration and addition time
  3.2) Nutrient medium additives
    3.2.1) additives for enhancement of the buffer capacity
    3.2.2) additives for changing of the redoxy-potential
    3.2.3) anti-foam agents, mechanical foam destruction
  3.3) pH-value
  3.4) temperature
  3.5) aeration and agitation rate

A complete investigation of all possible permutations requires a considerable analytical effort. Present measuring and control techniques allow accurate control - continuous or in small intervals - of parameters as temperature, pH value, concentrations of dissolved O_2 and CO_2, concentrations of these two gases in the outgoing air stream, redox potential and so on. Unfortunately, control of the concentrations of substrate and product is far more difficult, and, due to the time-lags occurring, continuous on-line control is an impossibility, at least with present techniques.

The number of variables that have to be investigated, and the fact that there are many steps between the first screening tests and the commercial implementation of a microbiological process, ensures that one has a long way to go. Systematical verification of 10 parameters with just 3 variations for each gives, as the number of tests that have to be carried out, $3^{10} = 59\ 049$. On top of this, the parameters themselves may have to be varied in time and volume, as the conditions at the start of fermentation may either be kept constant, or changed after different intervals. If one introduces just 3 variations in time-volume in a 10-parameter programme, the total number of tests increases to $3 \times 59\ 049 = 177\ 147$.
Although statistical confounding methods and mathematical modeling with suitable computer programmes may reduce this number drastically, and although some parameters may be investigated by pulse variations in continuous fermentation, known values based on former experience are usually introduced for basic parameters.

In general, the effort needed to develop a commercial biotechnological process in terms of engineering research and optimization, is in no way inferior to that required for chemical processes. In addition, biotechnology suffers occasionally from the additional burden of biological fluctuations.

Usually, one starts with shaking glass tests and then goes on to pilot fermenters with a volume of 10 to 50 litres. If the process still looks promising, the scaling up to commercial production may involve the development of special fermenters. However, in most cases industry has recourse to the standard agitator fermenter, the more novel types being used for special processes, such as, for instance, the production of single cell protein - which requires substantial aeration - or in waste-water treatment.

Biotechnological and chemical engineering compared

This paper has treated biotechnological processes in the steroid field only, but some conclusions may have a far wider importance.

In biotechnological processes one often has to employ high-quality apparatus, measuring and control techniques are expensive and methods for the isolation and purification of the product can be costly. The costs of implementation of biotechnological processes are frequently, but not always, higher than those of chemical processes.

But biotechnological processes have some advantages too. Yields can be high and degradation or side reactions can be far lower than in chemical processes. And in some cases, a biotechnological process uses the starting materials far more effectively.

But difficulties with yields and isolation of products, that are problems common to both chemical and biotechnological processes.

Although it is far too early to compare in an objective way the advantages and disadvantages of chemical and biotechnological processes, the last table summarizes some of the advantages and disadvantages of biotechnology.

Biotechnological procedures compared with chemical processes.

Advantages	Disadvantages
1. Stereospecificity of enzymatic reactions.	often lower volume/time efficiency,
2. Introduction of chiral centres.	
3. Several coupled reactions involved in one fementation step.	
4. High biosynthetic yields of complicated structures.	sometimes expensive purification procedures,
5. Mild reaction conditions.	sterile operation,
6. Usage of cheap raw materials as nutrient media and substrates.	yield variation with different batches of raw materials and occasionalty the formation of noxious odours,
7. Low temperatures (exept for sterilization)	energy requirement for aeration and sterilization,
8. Operation in aqueous media (except for product recovery).	sewage contamination with consumed nutrient media,
9. Reduced formation of chemical waste and recycling of extraction solvents.	formation of waste biomass,
10. Manufacture of products, which are not available by means of chemical synthesis.	increased number of control and regulation parameters,
11.	biological fluctuations due to the use of living cells,
12.	complaints by the sanitary authorities about the hazards

The ICI Single Cell Protein Process

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with a contribution by

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United Kingdom.

The industrial application of biotechnology in pursuit of profit is costly, time consuming and subject to most if not more than the usual constraints of operation within the commercial world. In most cases it is capital intensive and thus it is quite natural that the large industrial corporations, such as BP, Mitsubishi, Gas Chemical, Shell, Novo, ICI, Tate and Lyle, are represented at this meeting.

I will start my paper by being perhaps deliberately controversial in saying: 'In biotechnology all the easy things have been done and there is little likelihood, on both scientific and economic grounds that we are staring a revolution in the face'.
It is my belief that biotehcnology is in danger of overselling its potential to prospective investors, and thus industry, funding agencies and government research institutions should apply strict commercial criteria to their decision making in this field.

As my examples of 'easy' biotechnology I would cite brewing, traditional food fermentations, the addition of α-amylase to a vat of starch slurry to produce glucose, waste-water treatment and the production of soluble metabolites such as antibiotics, citric acid, glycerol and ethanol.
Conceptually, the simplest piece of biotechnology is the growth of micro-organisms, followed by harvesting and drying with the final product placed in a bag and sold to the animal feed industry. No complex processing, few if any hazardous operations, apparently no need for complex machinery in this low temperature, low pressure, neutral pH world of the microbe. In short: grow, dry and sell. Well, from my experience the Single Cell Protein processes that have been developed, have stretched the imagination and innovative skills of all those involved in their development to a degree that the conventional chemical industry has not experienced before.

I shall now describe the ICI Single Cell Protein process and use specific aspects of the development work to exemplify my earlier comments. I hope to avoid the macroeconomics of SCP processes and concentrate on those factors of a scientific and technical nature that have influenced the viability of the ICI process.

In the late 1960's we commenced research on a bacterial SCP process using methane gas a feedstock for direct feeding to a micro-organism. Methane was cheap, plentiful and readily accessible from the North Sea. Micro-organisms known to metabolise methane had been studied in detail in the academic world for several years and thus methane was a natural choice for ICI.

We soon found that methane as a feedstock had some major problems:
- Methane being gaseous and of limited water solubility, energy had to be used to supply the organism with a soluble substrate.
- The microbial yield of the organisms in pure culture from methane was poor. (I shall use the term $C\eta$ - carbon conversion - to define yield as percentage of methane or substrate carbon incorporated into cellular carbon.)

Growth in mineral salts medium using the continuous culture technique gave carbon conversions of < 55% with massive oxygen requirements and cooling loads. Cheap as

methane was, these poor yields could not be tolerated. We predicted that too little biomass was produced from too much methane and oxygen to yield an economically viable process. Shell persisted with this route using mixed cultures, but they finally shelved their SCP project several years ago. A number of other corporations looked at methane as a carbon and energy source, but all have now come to roughly the same conclusion; the costs associated with methane feedstock are too high to allow a sufficient gross margin - capital penalties associated with protection against explosion hazards are great. The poor yields of cells on methane can be explained on biochemical grounds: the enzyme methane mono-oxygenase consumes reducing equivalents that would otherwise be directed to ATP production and biosynthesis.

This energy loss is not incurred by cells growing on methanol and yields are significantly higher on a carbon conversion basis.
As the oxydation state of CH_3OH is closer to that of cells than CH_4, less oxygen is required per tonne of cells, and this means smaller compressors, smaller cooling towers and less expense. So in the early 1970's methane was abandoned at ICI and a CH_3OH process development commenced. The normal procedure of isolation of organism, product testing etcetera was followed, and a 37 m^3 continuous culture pilot plant was developed. The fermenter design is a novel pressure cycle fermenter with a distinct circulation pattern, engineered to facilitate maximum culture exposure to all nutrients and cooling elements.

Even at this early stage (1973) the project had a full complement of biochemists, mathematicians, engineers, nutritionists and microbiologists and process workers running the pilot plant. A design team was busy looking at possible designs for a large plant and the process of re-educating the chemical engineer to accept the restrictions imposed by microbial growth and a proteinacious product was well under way. About this time a new breed of re-educated biologists arose - these biologists spoke the chemical engineer's language and had a rudimentary grasp of the restrictions on biology imposed by engineering constraints. The engineers and biologists between them covered the middle ground of compromise whose domination in making decisions was, and still is, the most important part of our project's development. Indeed, I would go further than that and assert that any project in biotechnology which does not go through this period of education in compromise between professional engineers and biologists is doomed to failure. I find it difficult to envisage that the skills necessary for this occupation lie in the grasp of one person and therefore I cast some doubts on the value of education in biotechnology alone.

To return to the story. Commencing in 1973 the pilot plant was operated and produced product for testing in toxicological models and in target animal species. Throughout this period the yield on methanol was poor and many theories and hypotheses were tested to understand these low carbon conversions ($C\eta$'s). All submerged microbial cultures are subjected to perturbations in environment because of imperfections in mixing. In small laboratory fermenters, where energy input per unit volume is uneconomically large, these perturbations are usually quite small, but in a pressure cycle fermenter they are quite large. On average laboratory fermenters gave better results than the pilot protein plant. The oscillations experienced by the culture in the pilot plant, dissolved oxygen tension, partial pressure of CO_2, pH, temperature and hydrostatic pressure were tested in laboratory fermenters whose configuration was such that simulation was possible. The odd point was, however, that different lab fermenters gave different yields on the same experiment. To cut a very long story short, we found it was the way in which we added methanol to cultures that determined the yield.
We designed a fuel injection system where a solenoid valve was operated by a timer such that discrete shots of methanol could be added to the culture. For a 10 second pulse for example, the 10 second equivalent flow of methanol was added in < 1 second. At a fixed growth rate of 0.2h^{-1}, $C\eta$ collapsed as pulse frequency was dropped. $C\eta$ did not decline further at lower frequencies and, of course, the pilot plant was at a low frequency because of the single point addition of methanol. There were, therefore,

four solutions to the problem of low yields in large fermenters:

- Increase the growth rate such that periods without methanol were shortened. Oxygen transfer constraints made this impossible.
- Use another nutrient limitation so that methanol was in slight excess at all times. Low yields on all other limitations of growth prohibited this solution.
- Instead of putting methanol in through one pipe, distribute it through many small nozzles throughout the fermenter. Although complicated and expensive this was the solution chosen and we now have two methanol addition points per m^3 of our 1500 m^3 commercial fermenter.

A fourth solution was to drop the maximum methanol uptake rate from the equivalent of $D = 0.55\ h^{-1}$ to the growth rate of the plant, which is $0.2\ h^{-1}$. This would then be a μ_{max} culture. This option was suggested by the following results. As D is increased, 'off' time decreases (at constant pulse frequency) and at $D = \mu_{max}$ there is no off time and a shot of methanol no longer subjects the organism to an effective concentration perturbation. Our understanding of the biochemistry of the microbial yields has continued to produce further improvements.

So by this piece of research we achieve the high yields of cells required for the economic production of 'PRUTEEN', the brand name of our Single Cell Protein product. The molecular basis of this manipulation of bioenergetic efficiency remains unknown, although we believe that protracted periods of cyclical growth acceleration and deceleration lead to the futile use of energy in maintaining intracellular pools of metabolites.

We now had the basis for investment in a methanol/SCP system and in 1976 a capital sum of £ 40 million was sanctioned for the construction of a large plant. After a further period of fermentation research we realised that improvements to cellular efficiency were unlikely to be achieved by manipulation of the fermentation environment alone. We therefore turned to the techniques of genetic engineering. Biochemical studies showed that our organism had the low efficiency, high affinity GOGAT/CS ammonia assimilation pathway:

- GOGAT: α-oxoglutarate + NAD(P)H + glutamine $\longrightarrow$ 2 glutamate + NAD(P)
- GS: Glutamate + NH_3 + ATP $\longrightarrow$ glutamine + ADP + Pi.

Other microbes were known to have the low affinity, high efficiency assimilation path involving GDH:

α-oxoglutarate + NAD(P)H + NH_3 $\leftrightharpoons$ glutamate + NAD(P).

We decided to try and save some energy by deleting the GOGAT function and replacing it with a foreign GHD gene.
My colleague Dr. J. Windass of our Corporate Research Laboratory will tell you exactly how this was achieved and the results in terms of improved yields.

(Windass) Our strategy has been to:

A - identify and isolate a suitable donor GDH gene;
B - devise vector plasmids for transfer to ASl;
C - to develop means of appropriate gene expression in ASl;
D - to isolate GOGAT mutants of ASl.

The criteria set for (A) were simple. The source of GDH had to have a highly active enzyme and for simplicity we chose Escherichia coli. Our first attempt at gene mobilisation from a $GDH^{(+)}GOGAT^{(-)}$ E. coli used the properties of lysogenic Mu phage to mobilise the GDH gene onto the promicious plasmid RP4.
RP4 $GDH^{(+)}$ was identified, but when transferred to ASl did not give expression.
Using RP4 $GDH^{(+)}$ we did in vitro recombination reactions using the E. coli vector plasmids pACYC184. In these experiments SAL1 restriction was most successful and a 7.6Kb E. coli fragment was transferred to pACYC184. Transfer back into a

glutamate-requiring E. coli conferred glutamate independence. Furthermore, GDH levels in the latter were enhanced, but growth characteristics were quite normal. Unfortunately pACYC184 was not transferable to ASl. The hunt was on for another vector to which the GDH fragment could be attached by in vitro work. Several derivative plasmids were examined and we finally chose pTB79, a derivative of R30 OB to which Tn5 has been transferred from the plasmid JR66a, thus providing a single SAL1 site. pTB70 like R30 OB is multicopy and although not in itself mobilisable to ASl, can be transferred as a 'passenger' on RP4 when present in the donor.

The SaL1 GHD fragment was cloned into pTB70 at the Sall site and the recombinant plasmid transferred by mating to ASl.
Enzyme assays showed that:

- The E. coli GHD enzyme was expressed;
- GHD activity was gene dose related;
- There was sufficient GHD to support growth at the same rate as the previous GOGAT/GS system.

So we had now completed three steps in our strategy. It only required the mutation of the $GDH^{(+)}GS^{(+)}ASl$ to $GOGAT^{(-)}$ to achieve our goal.

Temperature sensitive mutants of ASl were isolated after NTG treatment, which grew at 30°C but not at 37°C; pTB70 GDH recombinants were mated into all these mutants from E. coli and scored for ability to grow at 37°C. Four were identified. Other studies showed that:

1. It was the E. coli DNA present on the plasmids which was complementing the mutations.
2. The strength of complementation was gene dose dependent.
3. Complementation only occurred at high NH_4^+ concentrations (>70mM).
4. These mutants lacked GOGAT but had GDH.

(Senior) Our target was achieved. Fermentation studies showed enhanced yields, thus proving that the carbon-limited growth of our organism was energy limited. We believe that this is one of the earliest examples of commercially significant genetic engineering and is most encouraging in this growing technology.

In addition to fermentation physiology, nutrition science and biochemistry, there has been the powerful influence of chemical engineering, which of course continues to be the keystone of the enterprise. Dewatering the culture to produce a concentrated product stream is essential and the dewatering process has to be cheap. Furthermore, the water removed from the culture contains soluble nutrients, thus providing a large effluent treatment problem. The solution is to recycle this fluid back to the fermenter and add more nutrients. Our engineers have solved this problem by lysing, flocculating and floating the product under aseptic conditions and recycling the clear supernatant back to the fermenter. The only effluent water from the plant is the water driven off during flash drying. This recycle system requires close control, as nutrient excess of P and S in the fermenter are possible through increased buffering capacity during continuous operation. The translation of this simple concept into an operable plant has been a major achievement of chemical engineering and biology.

At the end of 1979 construction of the plant was complete and our operating team is now commissioning the plant. The plant has a capacity of 50 - 70 kT/yr and the technology is now largely obsolete, because of long construction lead times and continued research activity. This first plant is relatively small, and our design team and research staff are working on the next plant for the 1980's, which would be as big as 300 kT/yr.

What of the future? In SCP technology we must improve the product by increasing the content of essential amino acids, increasing the yield of cells per tonne of methanol and simplifying the process. The current economic climate is somewhat chilly, and £/$ ratios plus bumper harvests of soya beans have conspired to make soya a very cheap commodity. Soya broadly sets the price structure of all protein meals for animal feedstuffs, and thus we simply have to improve our productivity and added value to reap the full commercial benefits of our venture.

We see SCP technology as the beginning of 'Big Biology" within ICI. The multidisciplinary team assembled is now working on other ventures in biotechnology. It is our belief that this mixed team of scientists and technologists, united by the SCP project, will be the key to success in future biotechnologies. I will conclude my talk by returning to an earlier point. We believe we have got over the worst of 'easy biotechnology' and are now going to tackle the 'advanced course'.

The Recovery of Metals by Microbial Leaching

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Introduction

The use of hydrometallurgical processes for the recovery of metals from low grade ores has become increasingly important over the last two decades. In these processes, an acidic oxidising liquor is allowed to react with the mineral material and thereby solubilise the metals which can then be recovered from solution. If the activities of micro-organisms are involved in this solubilisation process, the technique is know as microbial leaching. In its simplest form, the process consists of the percolation of acidified water through a bed of a mineral, wherein bacterial activity results in the oxidative reactions that lead to metal release.

Metal leaching has had a long history but the role of micro-organisms has only recently been appreciated. Thus the presence of copper in acid mine drainage waters was noted by the Phoenicians and the Romans and the latter probably recovered the metal from this source. (1,2,3) Large scale leaching of copper from sulphidic ores was carried out at Rio Tinto, in Spain, in the mid-eighteenth century (4) but it was not until 1963 that the involvement of bacteria in this particular system was confirmed. (5) Similar natural leaching has been observed in many areas of the world over long periods of time. For example, dumps of mine waste have long been known to pollute surrounding soils and waterways with inorganic material but the biological implications of this remained unsuspected.

At present, the only metals that are recovered on a commercial scale by microbial leaching are copper and uranium. The scale of this operation is indicated by the fact that 10 - 15% of all copper produced in the USA is obtained by microbial leaching processes. (2) The potential of the technique is, however, not limited to copper and uranium. It can, in principle, be extended to all sulphide and some oxide minerals since microbial leaching is, by definition, a biochemical oxidation process, catalysed by living organisms, in which an insoluble mineral is oxidised to a soluble form. In the case of sulphide minerals, sulphates are formed (equation (i), M is a divalent metal). Equation (i) is extremely general and vastly oversimplifies what is a very complex process. It is the purpose of this article to examine these complexities.

$$\underset{\text{insoluble}}{MS} + 2O_2 \longrightarrow \underset{\text{soluble}}{MSO_4} \qquad \text{(i)}$$

The Advantages of Microbial Leaching

The use of hydrometallurgical techniques gives several advantages over other processes. Present pyrometallurgical technology is, naturally, based on the higher grade ore minerals which are in increasingly short supply; lower grade ores are not economically treatable by these methods. Leaching techniques however, whether chemical or biological, are applicable to all ore grades and are particularly relevant to materials containing low amounts of metal. These include waste materials from conventional processing.

A further advantage of the leaching technology becomes evident if the environmental aspects of pyrometallurgical operations are considered. The sulphurous emissions traditionally associated with smelting operations are eliminated and any process wastes produced are in liquid or solid form that can be contained. This advantage is considerable as the environmental impact of any large scale metal recovery operation is a prime consideration in many parts of the world.

Furthermore, microbial leaching has lower energy requirements than competitive,

conventional processes, with consequent major economic advantages. This is because, in its simplest form, the process operates at ambient temperatures and hence costly heating steps are avoided. Comparable chemical leaching processes require either high temperatures and pressures or costly oxidising agents to generate the required lixiviants. The simple technology of microbial leaching also gives economic advantages in that lower capital costs are involved.
For these reasons, interest in microbial leaching is growing and considerable research effort is being expended. The successful exploitation of the method for the economic recovery of metals requires developments in physical and chemical, as well as biological, areas and much knowledge on the fundamental aspects of the process is still lacking. In the following sections, some aspects of what is known of the microbiology, chemistry and process technology of microbial leaching will be outlined and some possible future prospects for leaching will be considered. The whole topic has been the subject of several recent extensive reviews in which more detailed discussions can be found. (1, 2, 6, 7, 8)

Microbiological Aspects of Metal Leaching

There are several specialised types of bacteria that are able to derive the energy they require for growth from the oxidation of inorganic materials such as ferrous iron, sulphur and soluble and insoluble sulphides. Some members of this diverse group can exist and proliferate at temperatures as high as 80°C and all are able to withstand environmental pH values of 2 or less. The ecological niches that allow this unique mode of existence are usually waters that have been in contact with sulphur-rich minerals and coals. The low pH values traditionally associated with this areas are due to the activities of these micro-organisms.
The energy-yielding reactions employed by the microbes are expressed in equations (ii), (iii) and (iv) indicating the oxidation of iron, sulphur and soluble sulphides, respectively

$$4FeSO_4 + O_2 + 2H_2SO4 \longrightarrow 2Fe_2(SO4)_3 + 2H_2O \quad \text{(ii)}$$

$$S^o + 1.5O_2 + H_2O \longrightarrow H_2SO_4 \quad \text{(iii)}$$

$$H_2S + 2O_2 \longrightarrow H_2SO_4 \quad \text{(iv)}$$

Equation (i) can be taken to represent the generalised reaction for the bacterial oxidation of insoluble metal sulphides. The iron oxidation reaction (equation (ii)) is catalysed by bacteria at a rate 10^5-10^6 times faster than the inorganic rate at the low pH values and temperatures favouring the growth of the organisms. (9) Hence, in the presence of iron and of sulphur or a sulphide, the net result of the bacterial activities is the production of a mixture of ferric sulphate and sulphuric acid. This constitutes the lixiviant that is active in microbial leaching.
The organisms are, in general, autotrophic and hence are able to satisfy their cellular requirements for carbon by fixing carbon dioxide. Some, however, are not fully autotrophic and require complex organic supplements to growth media to allow development. Other nutritional requirements can be satisfied by inorganic supplements. The metabolism of these organisms is summarised schematically in Figure 1.
The most widely studied micro-organism of the metal leaching group is Thiobacillus ferrooxidans. (1, 6) This organism, discovered originally in drainage waters from a coal mine, (10) is a motile, non-spore forming, gram negative, rod-shaped bacterium and is commonly found in metal leaching liquors. It is characterised by its ability to oxidise iron, sulphur, soluble and insoluble sulphides and compounds such as thiosulphate and tetrathionate. It is an obligate autotrophe and an acidophile. Some strains have been shown to fix molecular nitrogen. (11)
Many studies have indicated that T. ferrooxidans is able to utilise insoluble sulphidic minerals as growth substrates. (1, 2, 6, 7, 8) These include pyrite (FeS_2) and other iron sulphides, such as pyrrhotite and marcasite, and minerals such as chalcopyrite ($CuFeS_2$) chalcocite (Cu_2S), covellite (CuS) and sphalerite (ZnS). This metabolic diversity as regards insoluble substrates accounts for the ubiquity of T. ferroxidans in natural metal leaching systems involving many minerals.

Many results indicate that various strains of T. ferrooxidans are able to develop a high degree of tolerance to metal ions in solution (see refs. 1, 2) and this undoubtedly contributes to its success in oxidising a wide range of complex ores from which these ions can be released. Thus, the organism has been shown to tolerate zinc at 120g/l, copper at 55g/l, iron at 160g/l, nickel at 72g/l, cobalt at 30g/l and uranium at 12g/l. These concentrations are considerably above the inhibitory levels normally associated with other groups of micro-organisms. Cultures of T. ferrooxidans are, however, inhibited by low concentrations of mercury, silver and molybdenum but it has been shown that silver inhibition, at least, is not significant in an artifical leaching system. (12)
In many leaching and drainage situations, T. ferrooxidans occurs along with another thiobacillus, T. thiooxidans. These two organisms are similar in many ways but T. thiooxidans is only able to grow on sulphur and some soluble sulphur compounds, and is incapable of the oxidation of iron. Its limited abilities, however, are of importance in leaching operations where free sulphur exists or is formed by other activities.
Mixed cultures of acidophilic organisms have been shown to possess activities similar to those of T. ferrooxidans. Thus, Leptospirillum ferrooxidans, an organism that, in pure culture, can oxidise and grow on ferrous iron but not sulphur or metal sulphides, can in the presence of sulphur-oxidising thiobacilli, grow on and oxidise pyrite and chalcopyrite. (8,13) The thiobacilli shown to be active in these mixtures included T. thiooxidans, T. organoparus and T. acidophilicus. Neither the thiobacilli nor Leptospirillum ferrooxidans were active against the mineral sulphides singly in pure culture. Hence, an iron-oxidising organism in the presence of a sulphur oxidiser can mimic the activities of T. ferrooxidans. In fact, in laboratory experiments, the rate of iron release from pyrite by the mixed cultures was greater than that found with a pure culture of T. ferrooxidans. Enrichment cultures from iron-oxidising acidophiles using pyrite as a substrate can lead to stable mixed populations, which probably contain organisms similar to L. ferrooxidans in addition to thiobacilli. (13) This result has led to speculation (18) that mineral dissolution in vivo may not be due entirely to T. ferrooxidans, but may involve the combined attack of several species of micro-organisms.
The upper temperature limit for the thiobacilli so far discussed and for L. ferrooxidans is 35 - 40°C but since temperatures in the interior of actively leaching heaps can, as a result of chemical and biological oxidation reactions, reach 80°C, (14) other organisms must be involved if bacterial leaching is to continue. Bacteria with the necessary thermophilic character have recently been discovered and some of their characteristics described. Thus several cultures of moderately thermophilic, thiobacilli-like organisms are now known. (15,16,17) All are capable of growth at 55°C, but unlike the mesophilic thiobacilli, they are dependent on additional organic compounds for growth. In laboratory studies, these requirements are satisfied by yeast extract or glutathione. Members of this group are able to grow on sulphur, ferrous iron and minerals such as pyrite, chalcopyrite and covellite. These organisms are unable to fix CO_2 effectively while growing on yeast extract (16) although details of their metabolic systems are still lacking. A somewhat similar microbe, termed Sulphobacillus thermosulfidooxidans has also been described, (18) but in addition to the activities described above, this thermophile is able to grow heterotrophically on glucose and sucrose. It differs also from the thiobacilli-like organisms by being a gram positive spore-forming micro-organism.
Organisms with even greater temperature optima have recently been isolated. Several strains of spherical bacteria, probably all similar to Sulpholobus acidocaldarius (2,19, 20) have been isolated from sulphurous hot springs. These organisms can grow at temperatures up to 80°C but in general have a growth range of 50° - 70°C. They are able to obtain energy by the oxidation of sulphides, sulphur and ferrous iron but need small amounts of yeast extract for growth in laboratory media. Additionally, they are able to grow heterotrophically on simple organic compounds. Their importance in metal leaching may lie not only in their thermophilic character, but also in their apparent ability to leach some minerals more effectively than mesophiles. Thus, for example, when compared with T. ferrooxidans, Sulpholobus sp. has been shown to oxidise chalcopyrite more effectively (21) and will also rapidly solubilise molybdenite

(MoS_2). (22, 23) The organism is also more tolerant to high (>lg/l) concentrations of soluble molybdenum than other chemoautotrophs. (23)
These extremely thermophilic Sulpholobus-like strains do not possess the usual cell wall structure found with other micro-organisms, They have instead an atypical cell envelope, lacking peptidoglycan, but containing a lipoprotein complex with a high proportion of charged amino acids on the surface. (24) The lipids of the organism are also unusual in that they are predominantly ether linked with almost no fatty acids present. (25) These characteristics are presumably necessary to allow Sulpholobus to thrive in extreme acidophilic and thermophilic areas, and it has been suggested that the unusual cell wall structure is a major factor that permits adaption of these organisms to these environments. (24)
Few other organisms with the capability of releasing metals from ores have been described. However, many micro-organisms, including bacteria and fungi, occur in areas where leaching occurs and persist there over long periods. Their role in the ecology of the leaching process is obscure but they could act to supply organic co-factors to the leaching organisms. Thiobacilli with pH optima in the range 5 - 7 are commonly found in mine drainage water (8) and it has been suggested that these might act to reduce the pH in the initial stages of the leach, by the oxidation of sulphur or sulphides, to a level where the leaching reactions could proceed and the leaching organisms proliferate.
A further point concerning the microbiology of leaching needs to be considered. In many leaching operations, oxygen depletion can occur owing to the high oxidative demands of the bacteria and no truly anaerobic iron-oxidising organisms are known that could continue the leaching process. However, T. ferrooxidans, T. thiooxidans and Solpholobus acidocaldarius can couple the oxidation of sulphur with the reduction of ferric ions under anaerobic conditions according to equation (v), (26) and

$$S^o + 3Fe_2(SO_4)_3 + 4H_2O \longrightarrow 6FeSO_4 + 4H_2SO_4 \qquad (v)$$

hence sulphuric acid generation and sulphur removal can continue in oxygen-free conditions. Obviously, this can only proceed as long as a supply of ferric ions is available, and these are only formed when oxygen is present as the terminal electron accepter for the bacterial oxidative reactions.

The Chemistry of Microbial Leaching

The energy-yielding reactions of the leaching micro-organisms result in the production of ferric sulphate and sulphuric acid, according to the reactions shown in equations (i) - (iv). Ferric sulphate is extremely effective in the dissolution of many minerals (27) according to the general equation (vi)

$$MS + 2Fe^{3+} \longrightarrow M^{2+} + 2Fe^{2+} + S^o \qquad (vi)$$

Specific examples are as follows:-

$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3FeSO_4 + 2S^o \qquad (vii)$$
(pyrite)

$$CuFeS_2 + 2Fe_2(SO_4)_3 \longrightarrow 5FeSO_4 + CuSO_4 + 2S^o \qquad (viii)$$
(chalcopyrite)

$$CuS + Fe_2(SO_4)_3 \longrightarrow 2FeSO_4 + CuSO_4 + S^o \qquad (ix)$$
(covellite)

$$Cu_2S + 2Fe_2(SO_4)_3 \longrightarrow 4FeSO_4 + 2CuSO_4 + S^o \qquad (x)$$
(chalcocite)

$$Cu_5FeS_4 + 6Fe_2(SO_4)_3 \longrightarrow 13FeSO_4 + 5CuSO_4 + 4S^o \qquad (xi)$$
(bornite)

$$ZnS + Fe_2(SO_4)_3 \longrightarrow 2FeSO_4 + ZnSO_4 + S^o \qquad (xii)$$
(sphalerite)

$$UO_2 + Fe_2(SO_4)_3 \longrightarrow UO_2(SO_4) + 2FeSO_4 \qquad \text{(xiii)}$$

(uranite)

Thus the biologically produced ferric ions are able to oxidise the sulphide or oxide minerals leading to the solubilisation of the metal. Ferrous ions produced in the reaction can subsequently be re-oxidised by the bacteria (equation (ii)), thus setting up an iron oxidation-reduction cycle in which ogygen is the oxidising agent. Sulphur produced in the reaction can also be oxidised to sulphuric acid (equation (iii)). This latter reaction is important in a leach system in that the sulphur produced by reaction (vi) could form a passive layer on the surface of the mineral, which might prevent further ferric ion attack unless it was removed. In aerobic regions, the oxidation can be catalysed by T. ferrooxidans or other thiobacilli such as T. thiooxidans: in anaerobic zones, reaction (v) could account for sulphur removal and expose fresh mineral surface to ferric ions. The net result in either case is the conversion of the sulphur atoms of the mineral into sulphuric acid, via free sulphur.

Other reactions almost certainly occur. For example, some sulphate will be formed by the direct oxidation of the metal sulphides by ferric ions (equation (xiv))

$$MS + 4Fe_2(SO_4)_3 + 4H_2O \longrightarrow MSO_4 + 4H_2SO_4 + 8FeSO_4 \qquad \text{(xiv)}$$

even though the reaction producing elemental sulphur (reaction (vi)) predominates.

The discussion so far has represented the role of bacteria in microbial leaching as an indirect one in which their main function is the generation of a lixiviant which acts in a purely chemical manner on the mineral materials. Many of the leaching organisms, however, are thought capable of effecting an oxidative reaction on minerals, in which the solid substrate is directly attacked. Reported examples of this reaction mode are as follows:-

$$4FeS_2 + 15O_2 + 2H_2O \longrightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4 \qquad \text{(xv)}$$

$$4CuFeS_2 + 17O_2 + 2H_2SO_4 \longrightarrow 4CuSO_4 + 2Fe_2(SO_4)_3 + 2H_2O \qquad \text{(xvi)}$$

$$2Cu_2S + 5O_2 + 2H_2SO_4 \longrightarrow 4CuSO_4 + 2H_2O \qquad \text{(xvii)}$$

$$CuS + 2O_2 \longrightarrow CuSO_4 \qquad \text{(xviii)}$$

$$2MoS_2 + 9O_2 + 6H_2O \longrightarrow 2H_2MoO_4 + 4H_2SO_4 \qquad \text{(xix)}$$

$$ZnS + 2O_2 \longrightarrow ZnSO_4 \qquad \text{(xx)}$$

It is not clear, however, to what extent these direct reactions actually take place, or if the direct metabolism of minerals is possible. Since iron is commonly found to some extent in all minerals, it is very easy to set up a cycle of bacterial iron oxidation followed by chemical reduction and to explain all leaching in terms of an indirect attack by ferric ions. Thus, apart from the oxidation of the elemental sulphur that is produced by the chemical reaction (vi), no additional direct involvement of the bacteria with an insoluble substrate needs to be postulated. However, the oxidation of some minerals under iron-free conditions has been demonstrated. Synthetic chalcocite and covellite of high purity are oxidised by T. ferrooxidans (28,29) suggesting that a direct attack must occur. With both minerals the rates of solubilisation were substantially increased by the addition of soluble iron to the systems. Some caution in interpreting results of this kind is, however, necessary as the presence of only trace amounts of iron in the mineral preparations or in the organisms could make a considerable difference and invalidate any conclusions concerning indirect or direct bacterial leaching.

Other workers have suggested that the bacterial oxidation of insoluble metal sulphides in the absence of iron might be due to a combination of an acid reaction with the mineral (equation (xxi)),

$$2MS + 2H_2SO_4 + O_2 \longrightarrow 2MSO_4 + 2H_2O + 2S^o \qquad \text{(xxi)}$$

followed by the bacterial oxidation of the sulphur produced (reaction (iii)). (30) These two reactions would constitute an acid leaching cycle. There are indications that the rate of reaction (xxi) might depend on the solubility of the sulphide involved. (31) In kinetic studies in the oxidation of pure, synthetic cadmium, cobalt, nickel and zinc sulphides by T. ferrooxidans, a relationship between solubility product and rate of metal solubilisation was noted such that the fastest rates were obtained with the mineral having the highest solubility product and hence the highest concentration of soluble sulphide when in a leaching solution. Thus, the reaction was postulated not to occur with the insoluble substrate. These reactions with minerals such as zinc sulphide (8) can be carried out by T. thiooxidans as well as T. ferrooxidans. Rates of reaction are increased by the addition of free sulphur and clearly direct oxidation of atoms in the crystal lattice in the mineral is not involved. However, the situation is less clear with some copper minerals; covellite and chalcocite are leached in iron-free conditions only by T. ferrooxidans and not with T. thiooxidans in the presence of sulphur. Thus acid leaching apparantly does not occur with these compounds and direct bacterial leaching is implicated.

At the present time it is difficult to be definitive about whether direct attack on insoluble substrates other than sulphur actually occurs, and it may be that it is dependent on the mineral involved and its state of purity. It is certain, however, that under leaching conditions, attachment of bacteria to minerals does occur. Many studies using the techniques of electron microscopy in both transmission and scanning modes (see ref. 2) have demonstrated this with various thiobacilli, including T. ferrooxidans, and with Sulpholobus. Clearly, independently of whether leaching occurs by a direct or indirect mechanism, it is advantageous if the organism and substrate occur in close proximity. Studies have shown that, in many cases, attachment occurs preferentially on the sulphide rather than the silicate phases of complex minerals (32) and that the sites of attachment are often associated with etching or corrosion of the mineral surface. From observations such as these, one might infer that a direct mode of bacterial attack on the mineral was occurring, but the precise mechanism of these substrate-microbe interactions and reactions remain unclear at the present time and further research is required. The whole question of direct or indirect attack has been considered in more detail recently by Brierley (2) and by Kelly et al. (8)

The Biotechnology of Microbial Leaching

The only metals that are presently recovered in commercial quantities by microbial leaching are copper and uranium. However, the contribution that this technique is making to the metals industry is growing rapidly and, in particular, is becoming a major method for the recovery of uranium. As the previous sections have indicated, there is no theoretical reason why other ores cannot be treated in this way and a range of other metals obtained. Present limitations are largely economic.

The technology employed to recover uranium and copper is relatively crude. Two main methods are involved, namely heap leaching and insitu leaching. Heap leaching is usually carried out with either low grade mine wastes, such as result from the primary processing of copper minerals, or with low grade sulphide ores. These materials are arranged in heaps, preferably on an impermeable base which in some instances is specially prepared. Alternatively, the material can be dumped in a valley allowing natural drainage pattern to be used. Normal practice with mined ores results in material varying in size from large boulders to fine particles. Copper content is never more than 0.4%. Water is allowed to percolate through the bed of material and the ever present populations of leaching bacteria can then begin to operate. Solubilised metal emerges in the liquor at the base of the heap and can be recovered by cementation with scrap iron, electrowinning or solvent extraction. The barren solution remaining, which contains a high content of ferrous iron, is then recycled to the top of the dump and the process continues. The ferric iron initially required in the process is supplied by the bacterial oxidation of pyrite, which is invariably associated with copper minerals.

The size of the heaps varies greatly but the largest in the USA can be up to 1200 ft.

high and can contain up to 4 billion tons of material. (2) Recycle times are measured in years in these cases.
In some systems, especially those involving the solubilisation of uranium oxides, the re-oxidation of the ferrous iron in the barren solutions is allowed to take place seperately from the heap of minerals. (33) This is usually carried out in aerated lagoons in which the bacterial activity can be controlled to some extent. The separation of the bacterial oxidation processes from the leaching process overcomes any problems of oxygen or carbon dioxide limitation, high temperature or inhibition by high concentrations of dissolved metals (34) and is particularly applicable to uranium leaching as no organisms are known that can derive energy from the oxidation of uranium oxide. Uranium leaching is, therefore, always by an indirect meachnism.
An example of the microbial recovery of copper by heap leaching is given by the process used at the Kennecot Copper Mine in Utah, USA. (33) The mined rock is a porphyry ore containing 1% copper and is treated conventionally. The waste accruing from the treatment of the high grade ore, amounting to 2.5×10^5 tons per day, contains less than 0.4% copper and is thus considered to be of low grade. This material is dumped and leached with a leaching solution that is maintained at pH2.1 by the addition of sulphuric acid. Copper recoveries amount to 200 tons per day. Other trace metals in the leach liquor such as yttrium, uranium and molybdenum are also recovered.
Heap leaching is mainly used for copper production and relatively little uranium is recovered in this way. Uranium is mainly recovered by microbial leaching using in-situ methods, especially from old, abandoned uranium mines. (2, 8, 33) The underground workings are sprayed with water to encourage microbial action and the metal is solubilised from the rock walls of the mine. The leach liquor is allowed to collect at the lowest part of the mine, from where it can be pumped to the surface and the uranium recovered.
The in-situ process has many economic and environmental advantages over processes involving mining operations. No rock is brought to the surface thereby saving considerably in manpower costs and eliminating the risks to miners' lives. Also no solid waste material is generated, an especially relevant fact when one considers that only a fraction of one percent of mined material is the desired metal. Liquid wastes can be minimised by the recycling of leaching solutions. The process is ideal for use on lower grade ores and for finely disseminated ores not normally treatable in a conventional manner. These advantages must be offset, however, against the difficulties of being able to predict solution flow patterns below ground and the consequent dangers of contamination of water zones. It is obvious that the maximum recovery of leach solution is desirable, not only on environmental grounds, but also for reasons of maximum metal recovery. Since large volumes of leaching solution are involved, operating blind in sub-surface regions poses many problems. A further disadvantage of the process is that extraction rates and recoveries are difficult to predict.
The in-situ leaching of uranium, however, is a very attractive technique and is becoming increasingly used. The process can be extended from working-out mining areas to virgin ore bodies by injecting the leach solution, generated in a surface facility, into drill holes in the ore body and allowing the liquid to percolate to a lower collecting area from which it can be brought to the surface. (33) It has been proposed that the permeability of some rock formations could be improved by fracturing them with explosives, and nuclear devices have been suggested for this purpose. (2, 3) The problems of working blind are even more relevant in these circumstances and adequate knowledge of the structure of the metal-bearing rock and of the surrounding geological formations is a prerequisite before the undertaking of such a project.

Factors Controlling Microbial Leaching

Although the technology of both dump and in-situ leaching is unsophisticated, it has proved successful for metal recovery. The underlying mechanisms of leaching are complex, however, and the practical methods used only increase the complexity. Many of the difficulties associated with leaching have already been mentioned but some consideration of the additional problems that arise because of the technology is relevant.

Many factors influence the efficiency of microbial leaching operations and these have been widely discussed. (2,6,33,35) In heap leaching, the availability of nutrients for the organisms, particularly oxygen and carbon dioxide, can be a problem especially if the requirements of gaseous diffusion have not been adequately considered during dump construction. Temperature rises due to oxidative reactions, and their likely effect on mesophilic bacteria, have already been mentioned but seasonal climatic temperature variations might also be a controlling factor in some operations, (6) particularly in smaller heap leaches. Temperature fluctuations within a dump, as the seasons change, might result in corresponding microbial population changes, with mesophiles being favoured over thermophiles and vice versa. The effect this might have on leaching rate is unpredictable. Other climatic conditions that could affect leaching efficiencies include excessive rainfall.

Effective leaching requires that the pH be maintained at values less than 2 so as to maximise the relevant bacterial activities and also to keep iron salts in solution. The pH value may have to be artificially adjusted if high rates of metal solubilisation are to be maintained, especially when dealing with sulphur-deficient minerals from which only limited amounts of sulphuric acid are formed. In large dumps, pH is not an easy parameter to control simply by acid addition. Acid may also be consumed by materials, such as carbonates, in the ore which would also result in reduced leaching efficiencies.

A major factor influencing solubilisation rates is the size of the mineral particles in the leach system (see ref. (6)). In general, the smaller the particles, the greater the leaching rate, corresponding to a greater surface area of mineral exposed to bacterial action. Some data suggest that there is a limiting particle size below which further rate enhancement does not occur. In theory, this size should represent one crystal structure: in practice the limiting size appears to be approximately 40μ. (36) Particle size is also of importance in terms of yield of metal recovered from both heaps and in-situ operations. Large boulders in heaps or areas of unfractures ore in in-situ systems could mean that much of the mineral is unavailable for leaching.

Future Prospects for Microbial Leaching

In the future, microbial leaching practice is likely to be extended to other metals and to ores of both high and low metals content. This move away from a method that is secondary to conventional recovery techniques to a primary production process has already occurred with uranium. High grade minerals will undoubtedly be treated economically by pyrometallurgical methods for the foreseeable future but, as these become scarcer, lower grade materials will have to be exploited and these ores, now considered of marginal economic importance, will likely be extracted by microbial leaching techniques.

A higher level of technology is likely to be appropriate in some instances. Several studies (33,35,37) have demonstrated that rates of metal extraction are enhanced by employing microbial leaching on crushed ores in stirred tank reactors. Thus, Bruyesteyn and Duncan, (35) using T. ferrooxidans in aerated and stirred reactors of 5 - 50 l capacity, showed rapid copper release from chalcopyrite. Final copper concentrations reached 50g/l. In a similar system, zinc sulphide was effectively leached by the thiobacillus to produce a zinc concentration of 120g/l. This concentration is sufficient to allow the use of electrowinning for zinc recovery. Later studies (37) with a continuous flow reactor system demonstrated chalcopyrite leaching that produced copper concentrations in excess of 50g/l. These studies have demonstrated the feasibility of the use of tank reactors on a laboratory scale but larger trials have yet to be reported and the economics of using such systems on an industrial scale are hard to assess. It is expected that this technology, on a commercial basis, would involve very large volumes of slurried ore. To achieve the gas transfer and mixing necessary for maximal bacterial activity, careful consideration of reactor geometry would be necessary. Operation would probably be in a continuous, not batch mode. The increased productivities of metal extraction would have to be sufficient to offset the ore mining and grinding costs if this technology was to compete with, for example, in-situ leaching.

Improvements in the technology of leaching can be achieved without employing stirred tanks. A plant system for the leaching of uranium has been described, (2,31,38) which

is really an advancement on the heap leaching principle. This has been operated successfully on a pilot scale. This system consisted of a series of 5 vertical columns, 23cm. in diameter and 4.5m high, each containing 330kg of ore as 3mm particles. These columns were maintained at 50°C and percolated, in series, with a lixiviant consisting of 12g/l of ferric sulphate at a rate of 180 litres per day. The uranium dissolved in the liquor emerging from the last column was removed by ion-exchange and the ferrous iron in the barren solution was re-oxidised by T. ferrooxidans in a separate reactor. This reactor consisted of a column which was aerated from the base and in which the active iron-oxidising bacteria formed a film on the column walls. It was operated at 30°C. The pH of the regenerated ferric sulphate solution was adjusted to 1.2 with H_2SO_4 before being introduced into the first column. The process is shown schematically in Figure 2. The essential features of this system are that the leaching and the lixiviant regeneration steps are separated and are operated at different temperatures so as to optimise both uranium solubilisation and bacterial iron oxidation. The series of 5 columns is leached over a 10 day cycle with a new column being added and an old one removed every two days. The ore and leaching fluid flow countercurrently, with the oldest ore being contacted with the freshest fluid.

This process has advantages over the conventional acid leach process for uranium extraction. It is cheaper to construct the plant, ore grinding costs are reduced (finely ground material is needed for acid leaching), less acid is required and operating temperatures, and hence heating costs, are lower. However, ore treatment takes at least twice as long with this process compared with the conventional method. Recoveries of metal, at 95% from an ore containing 0.12% U_3O_8, are similar but the process has yet to be tried on a commercial scale.

Other designs of reactor for the bacterial regeneration of ferric ions have been described. The General Mining and Finance Corp, (39) have developed a system in which T. ferrooxidans is immobilised on a plastic support of a honeycomb geometry. The acidified and oxygenated solution of ferrous sulphate is passed through this construction and the bacterial film on the plastic surfaces carries out the oxidation. This system is reportedly (39) being installed as a full-scale unit in a uranium leaching operation that is presently using a purely chemical acid leach process.

Advances in microbial leaching may allow the exploitation of ores that, although of reasonable grade, are not treatable in a conventional way. For example, zinc and lead sulphides often occur together in a finely disseminated form. Flotation methods are not able to separate the metals and the amount of lead occurring in the flotation concentrate is usually too high for the material to be used in zinc smelting operations. The application of bacterial leaching to the concentrate, however, could result in the separation of the metals by the formation of soluble zinc sulphate and insoluble lead sulphate. Zinc could be removed from the solution by a method such as electrowinning, while the insoluble residue would eventually become much enriched in lead and might allow economic recovery of that metal.

So far the discussion has concerned the application of microbial leaching to traditional substrates for metal production. The technique is, however, more widely applicable. For example, an application of great potential is the removal of pyrite from high sulphur coal. (40) For environmental reasons, high sulphur coal is not burnt because of the large quantities of SO_2 produced. Laboratory studies have shown that pure or mixed culturea of thiobacilli can rapidly and effectively remove pyrite from such coal, producing a clean product. Since coal is also often associated with trace metals, this process might allow the recovery of valuable metals. However, treatment to recover metals economically from this source might best be left until the coal has burnt. Leaching of the residual ash might lead to the economic removal of valuable metals from a more concentrated source. The same reasoning can be applied to other industrial wastes such as slags.

Finally some consideration should be given to the possibilities of producing cultures of bacteria that have been specially 'engineered' for metal leaching purposes. Normally, with any particular mineral, natural selection processes operate to produce a culture of organisms that is most suited for the conditions and further improvement is normally unnecessary. However, in leaching systems that might need to be seeded with

micro-organisms, or those employing separate iron re-oxidising reactors, a specially developed strain might be necessary for optimum operation. The most likely requirement for such a culture is resistance to inhibition by toxic materials present in the leach liquor. This could include not only the metals leached from the mineral but also compounds introduced into the liquor during metal recovery. This is especially true if a solvent extraction step is employed when the leach liquor is likely to become contaminated with organic materials. Direct selection for mutants resistant to the appropriate toxic agent is the simplest approach to adopt and also the one most likely to succeed since, at present, there is very little knowledge available of the genetic background of the leaching bacteria. Hence, the advanced techniques of genetic engineering are not yet applicable to these organisms.

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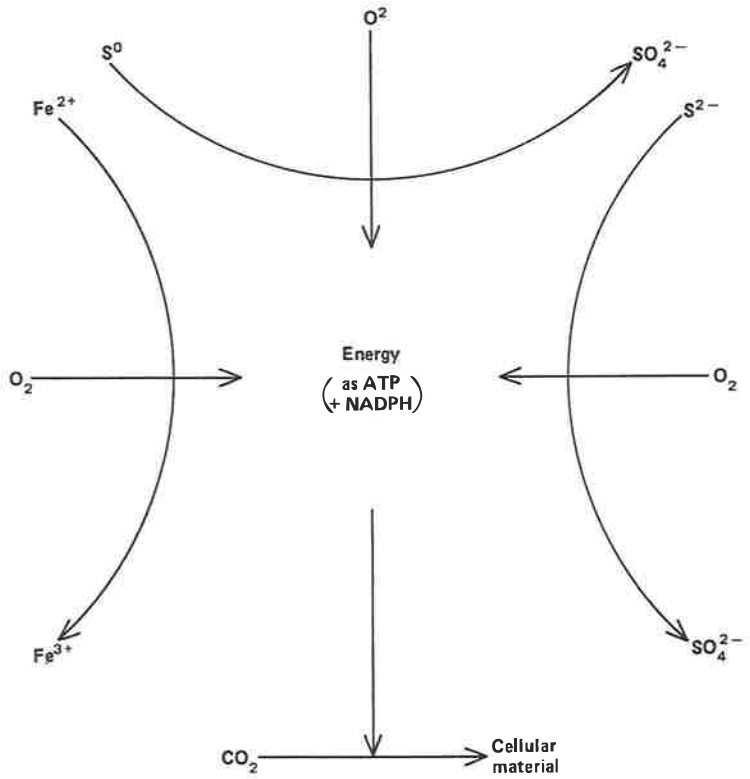

Fig 1 Schematic representation of the metabolism of the autotrophic iron oxidising bacteria

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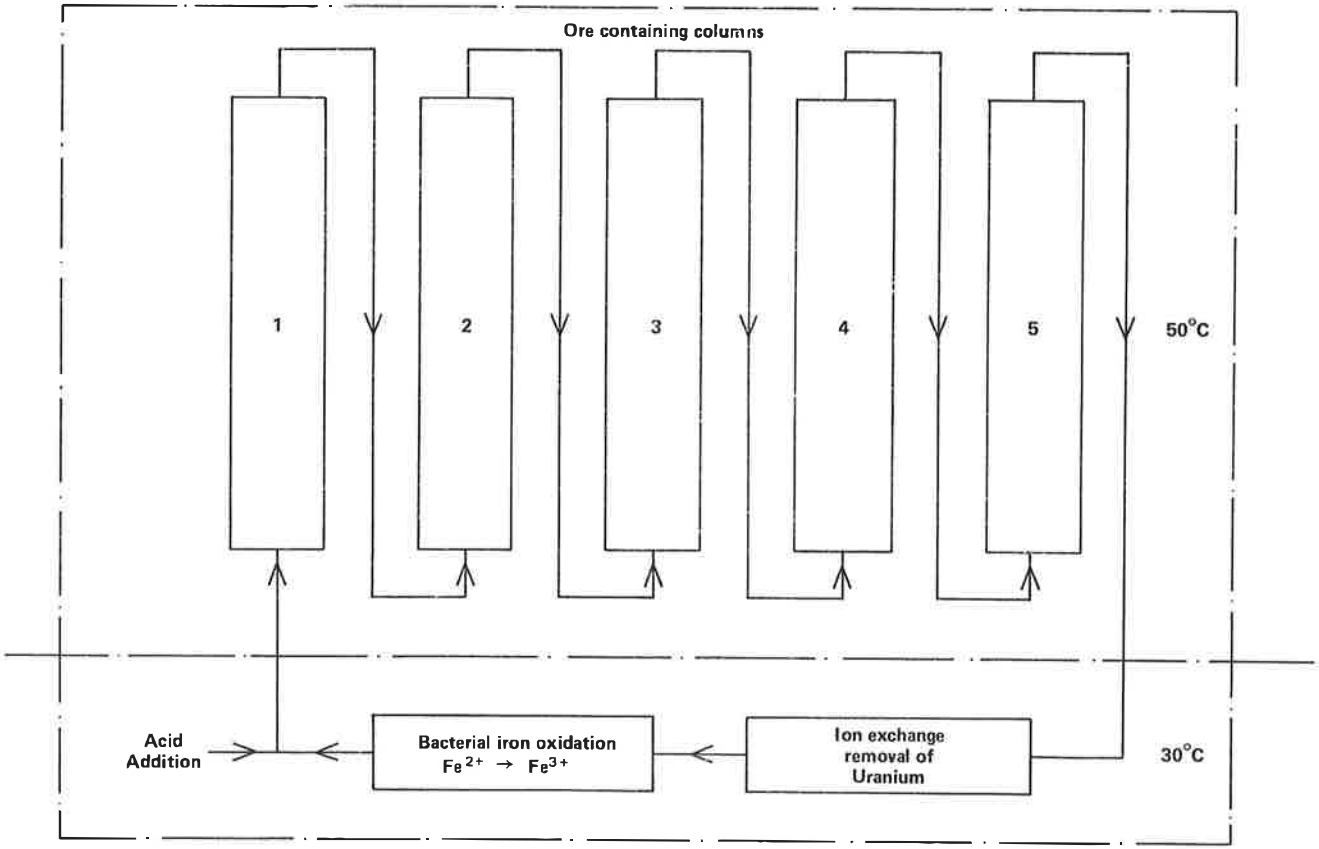

Fig 2 Schematic representation of a Uranium Leaching Plant employing bacterial regeneration of lixiviant

Probable Future of Natural Products as Chemical Raw Materials

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Introduction

The so-called energy crisis which is currently confronting the world economy has effectively focussed attention on the limited world resources of fossil carbon reserves. The urgency of the situation has probably been overstated since the reserves of oil and natural gas exceed the quantity of hydrocarbons so far extracted, most oil wells yielding only 40% of their total reserves using conventional techniques. Deposits of coal exist which are sufficient to meet the current rates of extraction for a further three hundred years, while the North American tar sand reserves are even more substantial.

The vital factor affecting recovery is economic: as the allowable cost of extraction increases so less readily accessible deposits become economic. North sea oil recovery would not have been viable without the arbitrary increase in oil prices from $2.4/ barrel in 1974 to the current contract price of $34.00 for high quality crude oil. The cost of extraction has not increased but increased prices have made low grade deposits viable and costly techniques of extraction possible.
Nevertheless, fossil carbon deposits are finite and a situation will be reached in which the cost of recovery in energy terms exceeds the energy value of the source. Before this happens other sources of energy will become competitive. Energy itself is available from the harnessing of natural sources: solar, wind, tidal, geothermal, hydroelectric and nuclear, by technologies which are being rapidly developed.
Fossil carbon resources not only provide energy in the form of heat, which is convertible into electric and mechanical power, but also a transportable, packaged form of potential energy in the form of hydrocarbons: liquid or liquifiable fuels. There is no obvious alternatives to such fuels, so that current research is being directed towards the conversion of fixed carbon resources into liquid hydrocarbon fuels, as, for example, in processes for the conversion of coal.
Because of the availability of fossil carbon resources and particularly the production of surplus hydrocarbon by-products of the conversion of oil and coal into automotive fuel and fuel oil, there has evolved a chemical industry based on hydrocarbons. With the escalating costs of crude petroleum, the industry must recoup its costs by spreading the increases over the price of main-product petrol and fuel oil and by-product chemical intermediates. In this situation alternative feedstocks to the chemical industry become competitive. Naturally regenerable resources become viable since they are unlikely to suffer rates of price increase which compare with those of a progressively depleted reserve.
The only regenerable resource is the product of photosynthesis, a process which over aeons of prehistoric time gave rise to our fossil carbon reserves.

1. Photosynthesis

The process of photosynthesis which utilizes the radiant energy of the sun to fix atmospheric carbon dioxide as carbohydrate in plants and algae, provides the only renewable source of organic chemical raw materials. Fossil carbon reserves in the form of oil, natural gas, coal, pitch, etc. are the accumulated products of prehistoric photosynthesis by marine and terrestial vegetation, in which the carbon has undergone further reduction to hydrocarbons, by a net process of disproportionation with the reformation of carbon dioxide.
The overall process can be represented by the simplified scheme:

Photosynthesis

$$6\ CO_2 + 6\ H_2O \xrightarrow{(hv)} C_6H_{12}O_6 + 6\ O_2$$

(carbohydrate)

$$C_6H_{12}O_6 \longrightarrow 3\ CH_4 + 3\ CO_2$$

(hydrocarbons)

In practice, although simple carbohydrates are the first direct products of photosynthesis, metabolic processes within the plant can lead to the formation of compounds in which carbon is in a lower oxidation state than in a carbohydrate, such as lignin, proteins, fats and even hydrocarbons. The metabolic energy required is provided ultimately by the re-oxidation of part of the photosynthesised carbohydrate back to carbon dioxide through respiration. The implication of this balance is that the utilisation of carbohydrate as a chemical raw material for traditional processes based on petroleum will require an additional input of energy equivalent to that required for the further reduction of carbon. This energy can only be provided at the expense of an equivalent amount of photosynthetic product, unless the energy is drawn from an alternative non-photosynthetic source, such as nuclear, geothermal or hydroelectric power. It is considerations of the energy balance which determine the economics of the use of carbohydrates as a renewable resource of chemical intermediates.

II. Carbohydrate Resources

Approximately half of the world's annual production of biomass, estimated at 1.2×10^{11} Tonnes (dry matter), is in the form of wood, of which 50% of the felled timber is burnt to provide energy (1). The balance of the biomass is thinly distributed as pasture, forest canopy, marine algae and agricultural crops, the latter representing the only other practical source of utilizable biomass, either as such or as processing waste.

If the regeneration and consumption of wood are in balance, the annual availability of wood is thus of the order of 3×10^{10} Tonnes. The composition of wood is approximately 40-50% cellulose, 20-30% lignin and 20-35% hemicelluloses, depending on the species and conditions of growth. The potential annual yield of cellulose is therefore of the order of 10^{10} Tonnes, with somewhat lesser amounts of hemicelluloses and lignin.

The production of woodpulp alone in 1976 amounted to 1.12×10^8 Tonnes (2), equivalent to the utilization of only 1% of the total annual available wood supply.

The world production of cereal crops in 1977 was 1.46×10^9 Tonnes (3), which, with an average starch content of 70%, would make starch the next most abundant carbohydrate after cellulose. Starch yielding root-crops, such as potatoes and cassava, provide an additional potential source of starch, a total production of 5.7×10^8 Tonnes being equivalent to a further 10^8 Tonnes of starch. The total biosynthetic yield of starch is thus around 1.1×10^9 Tonnes/annum, most of this being consumed as food, or as animal feed.

The next most abundant carbohydrate is sucrose, the total production from all sources reaching an estimated 1.16×10^8 Tonnes in 1978 (4). The major part of this production is currently consumed either directly and in manufactured foods, or in animal feeds. A small proportion, probably of the order of 10^6 Tonnes/annum is used as a chemical raw material for the production of sucrose esters, in rigid polyurethane foams and as a fermentation substrate.

The balance of the annual biomass production is distributed over the world's surface at low density, making its recovery and processing energetically uneconomic. As harvested it is a heterogeneous, low bulk density material with a high water content unsuitable

as an energy source. There is competition with food crops for available land area (only around 17.6% of the earth's land area is estimated to be suited to arable use, 11% being currently under cultivation), the requirement for which is increasing at an annual rate of 3%.

Although, in the long term there could be a net energy deficit, if photosynthesis were to be the only source of energy and food, on the basis of present agricultural yields, carbohydrates could provide an important alternative source of fixed carbon, provided that the appropriate chemical technology can be developed.

III. Carbohydrates as Chemical Feedstocks

(1) Cellulose

Despite the availability of relatively enourmous renewable carbohydrate resources in concentrated form, little use has been made of these materials as raw materials for the chemical industry.

Cellulose (Fig. 1), the most abundant carbohydrate in nature, is a polymer of the simple sugar D-glucose, into which it is converted on hydrolysis. It is separated from the other main components of wood, namely lignin and the heterogeneous group of polysaccharides known as hemicelluloses, by digestion of the wood with aqueous alkaline sulphide or calcium bisulphite, which dissolves the constituents other than cellulose. Equally well, sugar cane bagasse may be substituted for wood, though the pulp does differ in that the cellulose fibres are shorter than those from soft woods, which is important in paper making.

Sulphite extract is used to recover lignin sulphonate in the U. S. A. approximately 20% being used directly in various applications. Lignin is used as a binder or filler, as an extender in phenol-formaldehyde resins, as an emulsifier, and dispersing agent and as a sequestering agent, in a variety of industrial processes and products.

Synthetic vanillin and its methyl ether, veratraldehyde, are the only chemicals produced commercially from lignin, though compounds, such as catechol and dimethyl sulphide can be obtained in low yield (2-3%) by the high temperature distillation of lignin either directly, or with the addition of sulphur, respectively.

Cellulose itself is not used as a chemical feedstock, except for the production of regenerated cellulose fibres (e.g. viscose rayon) and film (cellophane) and chemically modified cellulose derivatives. The solubility of cellulose in water and organic solvents is increased by the derivatisation of part of the available (three) hydroxyl groups, as esters or ethers. The nitrate is well-known as a component of lacquers and of nitrocellulose explosives. The acetate and acetate-butyrate are widely used in plastics, fibres, plastic film and in lacquers. Of the ethers, ethyl cellulose is thermoplastic, soluble in organic solvents, the hydroxyethyl ether is used as a thickening agent, while carboxymethylcellulose is widely used as a substitute for natural gums, as a thickener and in adhesives. Numerous other derivatives have been prepared and evaluated.

No use is made of the hemicellulose fraction of wood. Birch xylan is the raw material for the production of xylitol, the wide use of which as a sweetener has yet to be accepted as safe. The production of furfural makes use of agricultural by-products as a source of hemicelluloses, in the form of oat hulls, corn cobs, rice hulls or sugar cane bagasse. High temperature digestion with dilute sulphuric acid leads to the formation of furfural in yields of upto 22% on raw material solids.

(2) Starch

Starch, like cellulose is a polymer of D-glucose, in which the repeating unit is in the

alternative α-configuration (Fig. 2). It is more readily hydrolysed to the component sugars, either by acid or enzymically, than cellulose. The production of glucose from starch is, in fact, a major industry in North America. Nevertheless, the total production only amounted to some 4.65 million tonnes in 1975 (5), though with the demand increasing for high fructose glucose syrups, this production could double over the next ten years (6). An important source of starch is cassava which can complement sugar cane by providing fermentable sugar outside the usual cane campaign.

Starch itself finds important industrial uses as an adhesive, as a sizing agent in textile and paper manufacture, in laundering and as a lubricant in weaving and in foundry casting. However, starch is not a pure chemical substance, comprising a mixture of the linear amylose and the highly branched amylopectin polymers in proportions which vary with the origin of the starch.

Chemically modified starches are becoming increasingly important as new applications are developed. Such derivatives are essentially produced by the partial reaction of the hydroxyl groups of starch to give esters or ethers. Prior to 1950 only the acetate, used as a substitute for gelatin and natural gums, and nitrate were produced in commercial quantities, but since then several other esters and ethers have become commercially available.

Other derivatives, including carbamates, hydroxyalkyl, cyanoethyl and carboxyalkyl ethers, acrylic ester co-polymers and oxidized starches, are used as cross-linking agents in resins and paper manufacture, as chelating agents in detergents, and as hydrophilic plastics. Generally, these products have specialised applications, in which the particular properties of starch as a carbohydrate polymer impart special attributes, such as high viscosity, ability to form coherent films or increased water absorption. Such products have unique properties rather than offering alternatives to those derived from hydrocarbon raw materials.

Hydrolysis of starch gives glucose which is the starting material for fermentation and for the production of carbohydrate derivatives such as sorbitol, mannitol, gluconic acid, ascorbic acid, glucoheptonic acid and methyl glucoside. These must be regarded, however, as sugar derivatives, rather than as raw materials for chemical industry. The total production of sorbitol, for example, in Europe is only of the order of 150,000 Tonnes and of mannitol less than 1500 Tonnes/annum.

(3) Sucrose

As a feedstock for chemical industry, sucrose has several unique advantages. It is obtained as a pure defined organic compound of relatively low molecular weight (342) and with multiple chemical functionality, giving the molecule a high potential for versatile application. Secondly, it is produced as a food in ever increasing quantity by an established world-wide industry at relatively low cost (Fig. 3). Thirdly, its extraction from sugar cane is energetically self-sufficient, the energy required to extract, concentrate and purify the sugar being provided by burning the by-product cane fibre or bagasse.

Sugar cane itself is amongst the most efficient natural converters of solar energy and atmospheric carbon dioxide into fixed carbon known, achieving a photosynthetic efficiency as high as 2% compared with average of 0.1% for the world's biomass production (1). In this respect, it makes optimal use of available arable land, which will ultimately be the limiting resource, in terms of the proportion of recoverable solar energy and carbon immobilised/unit of land area.

Despite the abundant availability of sucrose, and the consistently low price, its use in non-food applications has so far been negligible. With the recent steeply increasing cost of recovering and distributing fossil carbon resources, the potential of regener-

able energy/carbon resources is becoming increasingly important. The constraints on the development of such resources, though, are mainly technical and economic.

IV. Economic considerations

Biomass, in general, is characterised by low density of distribution and high degree of dilution with water. In thermodynamic terms it has a high entropy; the energy input required to utilise the fixed energy/carbon content may be higher than its intrinsic energy. In economic terms the cost of recovery is higher than the value of the product.

Agriculture, sylviculture and fermentation are examples of methods designed, albeit intuitively, to channel solar energy into a more concentrated energy product, such as a starch or sugar crop. This in itself requires an energy input in the form of cultivation, fertilizer application, harvesting and processing. For use as a chemical feedstock, such material will need additonal processing, the cost of which will reduce its competitive advantage relative to an alternative. For example, cellulose can be obtained from straw by conventional processes, hydrolysed to glucose and fermented to ethanol. The energy content of the ethanol and hence its value will be less than the energy input into its production. Even if the energy were derived by burning straw, the energy required to collect and transport the straw would render the process inherently uneconomic.

Thus although agricultural waste, or process waste, such as from the production of wood pulp, may be available in virtually unlimited quantity at a notional zero cost (actual costs having been absorbed into the primary product cost), further processing to obtain a suitable feedstock effectively increases raw material costs above that of alternatives.

Carbohydrates considered as a feedstock to traditional chemical industrial processes are also at a disadvantage since these processes are based on a hydrocarbon feedstock. Carbohydrates must either be converted into conventional raw materials, or new technology and products must be developed which utilise the particular properties of carbohydrates.
These two approaches will be considered with reference to sucrose, and in particular, to cane sugar.

V. Degradation of carbohydrates

Controlled degradation of carbohydrates will in general lead to products of simpler chemical structure. This may be achieved by chemical, thermal or microbiological means.

(1) Chemical degradation

The degradative reactions of sucrose which have been studied (7) are summarised in Figure 4. Despite extensive research, the yields of the isolated product tends to be low making the processes, at present, non-competitive with alternative routes from petroleum-derived starting materials. Apart from the inherently low weight yield consequent upon the loss of elements of water, degradative reactions tend not to be readily controllable, giving rise to a mixture of products. For example, the production of glycerol by the high tempertaure hydrogenation of sucrose, is accompanied by the formation of ethylene glycol and propylene glycol, which cannot be recycled.

The production of laevulinic acid in the high temperature acid degradation of sucrose (8), depends on the intermediate formation of 5-hydroxymethylfurfural which is only formed from the fructose moiety of sucrose under the conditions of the reaction. The yield is further lowered by the alternative polymerisation of hydroxymethylfurfural in competition with the ring opening reaction yielding laevulinic acid.

The yield of lactic acid from the reaction of sucrose with an excess of lime at elevated temperature is relatively high (9), but is, nevertheless, unfavourable compared with that obtained by fermentation, a process which is currently commercially viable.

The formation of nitrogen-containing heterocyclic derivatives in the reductive ammonolysis of sucrose, is also a consequence of the formation of two-, three- and four-carbon fragments and resynthesis. Consequently, the yields of desired product from the complex sequence of reactions tends to be low.

None of these reactions has, so far, achieved any economic significance. The commercial manufacture of glycerol was discontinued in 1969, in preference to the synthetic route from propylene (7), though with the increasing cost of propylene, the manufacture of glycerol from sugar by hydrogenation or fermentation could be economically attractive.

(2) Thermal Degradation

The direct pyrolysis of organic materials, such as wood, agricultural waste products, paper, or domestic refuse to produce energy by generating steam is an example of thermal degradation of biomass in the presence of an excess of oxygen. Usually the water content of the biomass should not exceed 50% if useful energy is to be obtained, since the vaporisation of internal water absorbs otherwise productive energy.

In the absence of air, pyrolysis of organic matter usually gives rise to carbon (as charcoal) and liquid and gaseous products such as methanol, acetic acid, carbon dioxide, carbon monoxide, ethylene, hydrogen and methane. Such products can be separated for use as conventional organic syntheses, though such a source is regarded as uneconomic at present.

In the presence of restricted amounts of oxygen or steam or with the addition of hydrogen, the organic matter can be completely gasified to give a mixture of carbon monoxide and hydrogen (synthesis gas) or methane, depending on the pressure and temperature of operation. Synthesis gas can be further converted into methanol, methane or liquid hydrocarbons by conventional processes which have been developed for coal gasification.

Although several processes for the gasification of coal are in commercial operation, the potential for the utilization of biomass is only as yet being explored, and the conditions for economic feasibility have not been established. It seems unlikely that such a process would be suited to a small or intermediate scale of operation owing to the technical complexity of the process involved.

(3) Fermentation

Although the fermentation of sugars usually results in the formation of chemically simpler compounds, from which standpoint it is a degradative process, it is more readily controlled and generally gives higher yields than thermal degradation. In the presence of oxygen, heterotrophic microbes generally gain energy for the processes of growth and maintenance by the oxidation of organic molecules to carbon dioxide and water; deprived of oxygen many organisms are capable of using partially oxidised organic molecules, such as carbohydrates, as sources of energy. Biochemically the anaerobic fermentation of carbohydrates is characterised by the oxidation of the sugar to pyruvic acid, by one of several routes. The glycolytic pathway has the overall stoichiometry:

$$\text{glucose} + 2\ \text{Pi} + 2\ \text{ATP} + 2\ \text{ADP} + 2\ \text{NAD} \longrightarrow 2\ \text{pyruvic acid} + 4\ \text{ATP} + 2\ \text{NADH}_2$$

There is a net gain of 2 ATP which represents energy available to the cell; of its hydrolysis in the reactions in which it partakes recycles ADP and inorganic phosphate. For

the glycolytic pathway to continue operating it is also necessary to re-oxidise the reduced pyridin nucleotide $NADH_2$, and it is in the diverse mechanisms of re-oxidation that the wide range of fermentation products are formed (Table 1).

One of the simplest solutions is the single step reduction of pyruvate to lactic acid:

$$CH_3CO\cdot COOH + NADH_2 \longrightarrow CH_3\cdot CHOH\cdot COOH + NAD$$

Lactic acid is a common fermentation end-product which can be produced in almost 100% yield from glucose, conserving 95% of the calorific value of glucose. Lactic acid has some utility as a chemical intermediate and in numerous food applications, but is not suitable for fuel use. Its range of applications is restricted by the present production technology which is costly in terms of energy and chemicals.

In the ethanol fermentation each molecule of pyruvate is reductively decarboxylated giving rise to one molecule each of ethanol and carbon dioxide:

$$CH_3\cdot CO\cdot COOH + NADH_2 \longrightarrow CH_3CH_2OH + CO_2 + NAD$$

Yields from glucose approaching the theoretical 51% w/w can be obtained conserving 93% of the calorific value of the glucose as ethanol. The energy density, 29.6 GJ/tonne is nearly twice that of carbohydrates. It can readily be used as a liquid fuel in existing internal combustion engines with minor modifications. It can also be catalytically dehydrated to yield the key chemical intermediate, ethylene.

Many bacteria have pathways which extract further useful energy for the cell in the form of ATP and produce end-products which may have more industrial significance in the future. One such group of bacteria is the Clostridia which produces carbon dioxide and the acids formic, acetic and butyric, together with hydrogen from the cleavage of formic acid, and acetone. Further reduction of butyric acid and acetone by these bacteria yields butanol and propan-2-ol. The ratios of the end-products vary according to the bacterial species and the fermentation conditions. The Propionibacteria produce propionic acid, acetic acid and carbon dioxide from glucose in the approximate molar ratio 4 : 1 : 1, with an overall yield of near 100%.

Some strictly anaerobic bacteria oxidise hydrogen, using carbon dioxide as the electron acceptor and producing methane (10). These bacteria are found in mixed cultures and carry out the final step in the production of biogas from carbohydrates. The overall stoichiometry from glucose is:

$$C_6H_{12}O_6 \longrightarrow 3CH_4 + 3CO_2$$

with fatty acids, alcohols, hydrogen and carbon dioxide as intermediates. The methane may be converted to methanol by conventional routes through synthesis gas. Hydrogen, the other potentially valuable gaseous metabolite, is generally found in low yields from carbohydrates, but may be obtained in much higher yields using photosynthetic microbes (11).

In addition to the extracellular products summarised above, there are reduced products of some microbes which accumulate intracellularly to form the greater part of the cell mass. Among them are triglycerides and other lipid products (12) and the polyester poly-3-hydroxy-butyric acid (13).

For those metabolites that are primary products of the pathways of energy generation for the cell, it is often possible to achieve yields approaching the theoretical maxima calculated from the biochemical pathways. This is done by growing the microbes at rates approaching zero.

The production of fermentation alcolhol from surplus sugar, sugar cane juice or glucose from cassava starch, for use as a motor fuel, is practicable in some countries where sugar is available cheaply, and there is a premium on imported oil (14). In Brazil

for example, the addition of 10% fermentation ethanol to motor fuel is currently practised. Substitution at such a level would be impractical in the United States where the consumption of motor fuel is so great, that the land area needed to produce sufficient fermentable sugars to provide the 7 billion gallons of ethanol needed would be 15 times the mainland acreage of the United States. Or from another standpoint, if all the corn produced in the U. S. were converted to ethanol, it would provide only 6% of the fuel consumed by passenger cars.

Since thanol can readily and cheaply be converted into ethylene in high yield (96%) by well-established technology using a process of catalytic dehydration, by alumina at 350°, carbohydrates can provide a direct route to ethylene and thus to chemicals derived from it. In fact, the view has been expressed that fermentation ethanol is preferable used as a chemical feedstock, as an alternative to naphtha, rather than as a fuel. A wide range of chemical intermediates can be derived from ethanol using conventional reactions and process technology (Figure 5).

An alternative fermentation product, lactic acid, as ethyl lactate, can be dehydrated to give ethylacrylate, a primary intermediate in the production of acrylate polymers. The controlling factor in either of these processes is relative cost. Currently, anhydrous ethanol is more economically produced by the hydration of ethylene, but the converse could apply, in situations in which the requirement for ethylene is below the minimum economic scale for a naphtha cracking plant (300,000 te/annum).

VI. Sucrose as a Chemical Feedstock

If sucrose is regarded as a primary raw material, its price becomes comparable with other bulk chemicals (Table 2). It is, however, necessary to exploit the synthetic opportunities presented by sucrose in order to produce higher valued products. This requires sucrose in the anhydrous pure state, the form in which sucrose is normally marketed.

The future availability of sucrose at an increasingly competitive price can be anticipated from a survey of the development and technology of the sugar industry. The potential uses of sucrose can be judged by a survey of its physical and chemical properties, a specialised branch of carbohydrate chemistry sometimes referred to as sucrochemistry.

In addition to the 91.8m tonnes of sugar produced in 1977, an estimated 12m tonnes was produced as non-centrifugal or amorphous sugar and a further 11.9m tonnes was disposed of in molasses. Thus, the world annual capacity for sugar production in 1977 amounted to a total of 115.7m tonnes (centrifugal raw sugar equivalent) by the year 1985, assuming an equilibrium price of 15.6¢/lb (4).

The utility of sucrose as an industrial raw material will obviously be conditioned by its cost. For high-value low volume markets the price of the sugar used will be a negligible factor, whereas the converse would be true for the competitively priced high volume product. The world price of sugar (15) has tended to be depressed below the average cost of production, though it is subject to unpredictable fluctuations, such as occurred in 1974, reaching a peak of 56.4¢/lb in November of that year (Figure 6). This extreme sensitivity to supply and demand, in fact, relates only to the 10% of total sugar production which is not consumed at source, nor subject to special quota or contractual agreements. This is, however, the price at which sugar would be available on the world market.

Although the demand for sugar is increasing steeply as the world population and the average per caput consumption increase, production capacity can be expected to expand and it would be reasonable to expect the projected equilibrium sugar price to apply.

VII. Sucrose as a Chemical

Sucrose is a non-reducing disaccharide comprising a molecule each of the hexose sugars α-D-glucose and β-D-fructose linked through their hemi-acetal hydroxyl groups by a glycosidic bond. Glucose is in the pyranose ring-form and fructose in the five-membered furanose ring-form (Figure 7).

Unlike reducing sugars, such as glucose, which are unstable in alkaline solution and readily oxidised by copper, silver or mercuric ions, sucrose is relatively stable. The glycosidic bond is subject to acid-catalysed hydrolysis. The rate of hydrolysis is determined only by hydrogen-ion activity (pH) and temperature. Being a bimolecular reaction involving water, at high sucrose concentrations, however, the molar proportion of water present will be small enough to affect the rate of hydrolysis, the reaction being:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow[+\,\Delta H]{H_3O^+} C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose — Glucose — Fructose

As an octahydric alcohol, sucrose will undergo the typical reactions of an alcohol, though being polyfunctional, selective or partial derivatisation presents problems. The relative reactivity of the hydroxyl groups will depend not only on inherent steric factors, but also on the nature of the reaction, the conditions (temperature, solvent, time, molar proportions of reactants) reagent reactivity, the stability of the activation complex, hydrogen bonding and hydroxyl group acidity.

The partial and selective derivatisation of sucrose is necessary where the properties of a particular derivative may depend crucially on the position and conformation of the substituent group. This is frequently true of biological or physiological activity where molecular conformation is fundamental to stereospecific enzyme interaction.

For many applications the degree of substitution rather than position may be crucial. Sucrose monostearate is an effective surfactant in aqueous systems, sucrose distearate is more effective as an emulsifier, while higher esters are not surface-active.

VIII. Sucrose Derivatives

Sucrose will readily undergo acylation of all eight hydroxyl groups on treatment with acetic anhydride in pyridine to give the octa-acetate. The octabenzoate is prepared by the reaction of sucrose with benzoylchloride in pyridine. Sucrose will also form ethers, urethanes and acetals, while the hydroxyl groups may be replaced by halogen, azide, amino, thiol, carbonyl and carboxylic acid groups.

Esterification of sucrose with an acyl halide requires the presence of a base to neutralise the hydrogen chloride formed, which could otherwise promote decomposition of the sucrose. Pyridine, which also acts as a solvent for sucrose, is the preferred base. An important alternative route to esters is through transesterification, in which an acyl group will transfer from an ester to sucrose in the presence of a basic catalyst. The reaction is normally reversible, though the position of equilibrium can be shifted in either direction, by continuously removing one component of the reaction. For example, sucrose heated in solution in dimethylformamide with the methyl ester of a fatty acid, such as stearic acid, in the presence of a basic catalyst such as potassium carbonate, will form the monostearate with the liberation of the methanol. If the methanol formed is continuously removed from the reaction mixture by distillation sucrose monostearate (mainly 6- and 6'-) will be formed. In the presence of an excess of methyl stearate, additional sucrose hydroxyl groups will react to give the di- and higher esters.

$$(C_{12}H_{21}O_{10})\ OH + C_{16}H_{33}CO.\ OMe \xrightleftharpoons{K_2CO_3} (C_{12}H_{21}O_{10})\ O\ CO.\ C_{16}H_{33} + MeOH$$

Sucrose Methyl stearate Sucrose monostearate Methanol

If the alcohol is glycerol, then equilibrium is reached between sucrose, the mono- and di-esters of sucrose and glycerol, and its mono- and di-esters. In this situation, it is not practicable to remove the glycerol by distillation and the reaction reaches its equilibrium composition.

Sucrose mono-esters of long-chain fatty acids have surface-active properties and are, in many respects, similar to conventional non-ionic surfactants, which find important commercial applications in detergents and as emulsifiers. Sucrose stearate, for example, is also totally non-toxic, and biodegradable, being readily hydrolysed into its components under physiological conditions. It has been commercially available for several years, being manufactured by the transesterification process from sucrose and methyl stearate using dimethylformamide as a solvent, or, in the Nebraska-Snell process, propyleneglycol, which avoids the use of a toxic solvent (16).

Since, for many practical purposes, glycerol mono- and di-esters of fatty acids are effective and preferred emulsifying agents, particularly in food applications, the mixture of sucrose and glycerol esters resulting from the reaction of sucrose with triglycerides of natural fats and oils (such as tallow, palm oil, coconut oil) is particularly effective. The mixture, known as a sucroglyceride, is marketed and permitted for use in foods as an emulsifier in several countries.

The solvent, dimethylformamide, customarily used in this reaction is a costly component of the process, both for recovery and elimination from the food-grade product. A solvent-free process has been developed in which sucrose and a fat will react in the presence of a catalyst (potassium carbonate) to give a sucroglyceride directly (17). The product may, if required, be separated using permitted food-grade solvents into its component sucrose esters, and mono- and di-glycerides, and potassium soaps, each of which have specific uses and markets.

IX. Applications of Sucrose Derivatives

Although numerous potential industrial applications of sucrose appear in the literature, many of which form the subject of patent specifications (Figure 8), they have not been widely adopted as industrial chemical intermediates. A few esters are commercially available, and these have found numerous applications as plasticisers, surfactants and emulsifiers.

(1) Plasticisers

Sucrose octa-acetate, an intensely bitter substance, finds use as a denaturant, and as a plasticiser. It is a crystalline material (mp 86°) which is not entirely satisfactory as a plasticiser for cellulose acetate films. For this application, the mixed ester, sucrose-diacetate hexaisobutyrate was found to have optimal properties (18). It has a high viscosity, low volatility and high stability to hydrolysis and discoloration. It is also non-toxic and is used as a clouding agent, to disperse essential oils, in soft drinks.

Sucrose octabenzoate (19) is also very stable to light and to hydrolysis and is completely soluble in non-hydroxylic organic solvents. It is used as a plasticising agent in nitrocellulose, acrylic and polyvinylchloride-acetate films and lacquers. It may be blended with the acetate-isobutyrate to which it is closely similar.

It is also used, like the acetate-isobutyrate, in the manufacture of transparent papers,

having a refractive index close to that of cellulose.

(2) Surfactants and emulsifiers

Sucrose mono-esters of long-chain fatty acids have surface-active properties and are completely non-toxic. Consequently, they have found numerous applications both in the food and the animal feed industries, as dispersing and emulsifying agents (20). Sucrose mono-stearate, mono-palmitate, mono-oleate and di-stearate are commercially available, having been manufactured in Japan for several years. In several countries sucrose esters are permitted food additives.

Sucrose esters are readily biodegradable, and can be used in detergents, where environmental problems can be created by persistent detergent residues. For this purpose, however, purified sucrose esters are too costly. The low cost sucroglyceride mixtures produced by a solventless process, can be economically included in detergent formulations, the detergents being competitive in cost and efficacy with those based on conventional linear alkylsulphonates. Markets include those for domestic and industrial detergents, hand cleansers, hard-surface cleaners, machine cutting lubricants amongst others.

Long-chain alkyl esters, urethanes, sulphonylurethanes and alkoxymethyl ethers of sucrose, and fatty acid esters of polyalkoxylated ethers of sucrose have also been evaluated as surface-active agents. So far, their preparation on a production scale has not been undertaken. Apart from the improved stability to strongly alkaline conditions of the mono-alkylsucrose ethers compared to the esters, these derivatives would not seem to offer any special advantages over the esters, and would not be expected to compete on a cost basis with conventional surfactants.

Sucrose esters of stearic, palmitic, lauric, and oleic acid, being completely non-toxic, bland, non-allergenic and non-irritant are suitable for use in foods and are permitted food additives in some countries; for example, in Japan, France, Belgium and Switzerland.

Long-chain fatty acid esters of sucrose have been manufactured in Japan since 1960, by a process originally developed under the auspices of the Sugar Research Foundation. Production is now undertaken by two companies, the Dai-ichi Kogyo Seiyaku Co. Ltd., and Ryoto Co. Ltd., of Tokyo. The latter have operated a process with a production capacity of 3000T/year, since 1975, using a new process in which ester exchange between sucrose and the methyl ester is effected without the use of the toxic dimethylformamide solvent (21).

Sucroglycerides are currently produced by Rhône-Poulenc S. A. in France, operating a process with a capacity of 2000T/annum, which is based on that developed by Ledoga in Italy in collaboration with the Sugar Research Foundation, and originally licensed to the Société de Melle-Bezons who commenced production in 1963. The ester exchange between sucrose and a natural triglyceride, such as tallow, is conducted in dimethylformamide, which is subsequently recovered by distillation.

The process operated by Tate & Lyle, Ltd., in England (17), avoids the use of solvents, except for the subsequent separation and purification of food-grade sucrose esters, where this is required. In this process, sucrose is heated with a natural triglyceride, such as tallow, palm oil or coconut oil, in the presence of a potassium carbonate catalyst. The product is suitable for direct use in many applications, without further processing.

Sucroglycerides are mainly used in compounding animal feeds to give improved digestion of fats. In the formulation of calf milk feeds, from skimmed milk and added vegetable fats, the sucroglyceride disperses the fat and assists subsequent spray-drying of the

product.

Sucroglycerides are also used as dispersing and emulsifying agents in the production of margarine, mayonnaise, ice-cream, artificial cream and dessert toppings, for example. Sucrose surfactant esters, are also used in chocolate manufacture to improve surface appearance, in spray-dried and freeze-dried beverages to improve dispersion, and in reconstitutable dried foods and mixes to aid wetting. In baking, sucrose monoesters are particularly effective as a dough strength improver, and to increase loaf volume and improve crumb texture and antistaling properties of bread (22). The incorporation of non-wheat protein into bread is possible without loss of loaf quality, with the addition of sucrose esters. Upto 0.4% of sucrose monopalmitate, stearate or laurate would normally be used, or appoximately 1.4g/lb. Sucrose esters also improve ingredient mixing and product texture in cake, pastry and biscuit manufacture.

Sucrose esters are used in the preparation of cosmetics, having been found to be highly compatible with the skin and totally non-irritant. They appear to have the particular advantage, when used as a surface-active component of cleansing agents or shampoos, of maintaining the natural oily protective mantle of the skin, which is removed by ionic detergents (23).

(3) Biological Applications

The extraordinary versatility of sucrose derivatives in the physiological arena, is seen in the range of basic flavour properties: several chlorosucroses are intensely sweet, the anticariogenic 1',4,6'-trichlorogalactosucrose being 650 times sweeter than sucrose itself (24). It has a pure sweet taste, indistinguishable from that of sucrose, with no side-flavours or after-taste. It is completely non-toxic, non-metabolised and virtually non-adsorbed. It is stable to enzymic hydrolysis and 60 times more stable than sucrose to acid hydrolysis.

Galacto-sucrose itself is unexpectedly tasteless, otherwise closely resembling sucrose in its physical properties (25). It would thus have valuable applications as a bland bulking agent, preservative, texturising and osmotic agent in foods. The present limitation in such use is imposed solely by cost.

At the other extreme 1',2,6,6'-tetrachlorotetradeoxymannosucrose is intensely bitter, having twice the bitterness of the octaacetate. This is being evaluated as a bittering agent as an alternative to quinine.

6,6'-Dichloro-6,6'-dideoxysucrose is effective as a male antifertility agent in rats and primates, allowing complete and reversible control of male reproductive fertility (26). It is non-toxic, but depends specifically for its activity on hydrolysis under physiological conditions and absorption as 6-chlorodeoxyglucose and 6-chlorodeoxyfructose, which are interconvertible in the normal carbohydrate metabolic cycle.

(4) Synthetic resins

An important potentially large market for sucrose and sucrose derivatives is in synthetic resins. Sucrose itself has been evaluated as a filler or substitute for phenol in melamine and novalak-type resins (27), though there is no evidence that unmodified sucrose will participate in the cross-linking reaction with formaldehyde. The expected hemiacetal link is presumably insufficiently stable at normal curing temperatures to provide any significant cross-linking to sucrose in the final polymer.

Octa-allylsucrose ether was investigated in some depth as a component of air-drying resin films, but they proved to be too brittle for practical use (26). Sucrose polyesters of drying oil acids, derived from linseed oil, tung oil or soya oil, such as sucrose heptalinoleate, were found to be superior to linseed oil as an air-drying paint vehicle,

in terms of film adhesion and alkali resistance. An ester with a lower degree of esterification could more readily be prepared, those with an average 4-5 ester groups being available by ester interchange between sucrose and methyl linoleate in dimethylsulphoxide solvent (29).

The sucrosepentaester typically would be esterified with phthalic anhydride and further reacted with a suitable diepoxide, such as bisphenol A diglycidyl ether, to give a linear polymer. The varnish is prepared by dissolving the resin in the appropriate solvent (e.g. white spirit/diacetone alcohol) to which is added cobalt naphthenate drying catalyst and pigment to give an air-drying paint. Alternatively, the resin monomer can be polymerised with a diisocyanate to give a polyurethane type resin. This product is less costly than the epoxy resin, and gives high performance surface coatings. Generally the inclusion of sucrose did not give sufficiently marked advantages over conventional alkyd-type surface coatings to offset the higher cost, and further development of sucrose-based paint resins has not taken place.

Sucrose and its derivatives have been widely explored as components of polyurethane resins, in particular rigid polyurethane foams (30). Sucrose itself can be used, but tends to lead to brittle products. The polyhydroxypropyl ether of sucrose is the preferred polyol in practice, having better miscibility with the diisocyanate and fluorocarbon blowing agent used in the preparation of rigid foams. In order to introduce flame retardant properties into the resin, halogen- or phosphorusderivatives of sucrose are frequently included in the reaction.

A rigid polyurethane foam which is essentially non-flammable can be produced from a sucrose long-chain fatty acid ester, with starch as the cross-linking polyol. On exposure to heat the foamed resin tends to char without melting, and the flame does not propagate.

X. Conclusions

Despite the very large range of sucrose derivatives, both known and potential very few have so far found industrial application. The largest non-food outlet for sucrose derivatives is in polyurethane foam resins, particularly rigid insulating foams, a market which is increasing at the rate of 10%/annum. The annual production of rigid urethane foams in the United States is projected to be around 0.65 billion lbs in 1980, which could consume up to 50,000T. sucrose, depending on relative price and availability, compared, for example, with sorbitol.

The world-wide production capacity for sucrose mono-esters of fatty acids, will only be of the order of 12,000T/year by 1980, though the demand could increase rapidly as its use in foods becomes more widely accepted.

The potential of sucrose as an industrial raw material, obviously has yet to be exploited. Major factors in determining how quickly applications of the new derivatives of sucrose will be developed are raw material cost relative to oil-based chemical feedstocks, and the cost of the derivatisation process as a commercial operation.

High value sucrose derivatives with specialised or unique properties will develop a market in their own right, the cost of the sucrose raw material being a negligible factor. For example, in the production of a non-caloric high-intensity sweetener, such as trichlorogalactosucrose, the cost of sucrose would be insignificant, compared with the cost of the synthetic process, and would not directly influence the competitive position of the product in the artificial sweetener market.

The sucrochemical industry may thus be expected to develop along these two lines:
(1) the exploitation of the unique properties of entirely new derivatives of sucrose, and
(2) the utilisation of sucrose as a fixed carbon feedstock for the chemical industry, via ethanol and ethylene.

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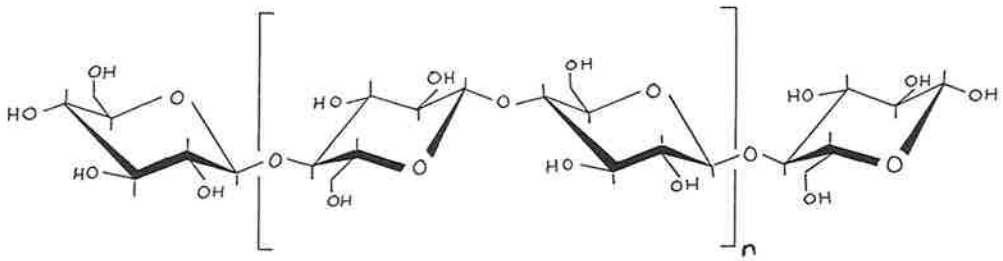


Figure 1 : Cellulose

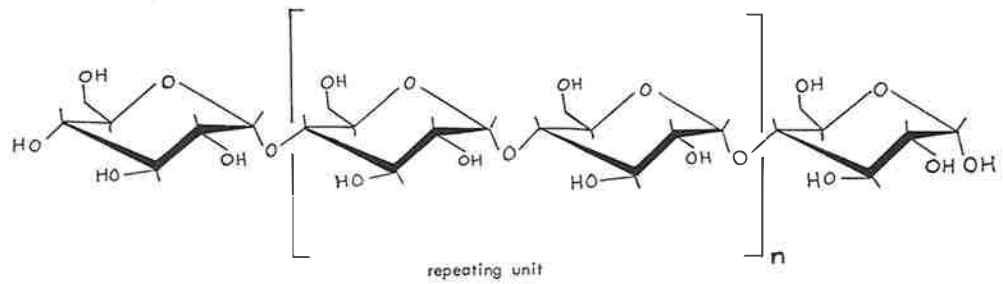
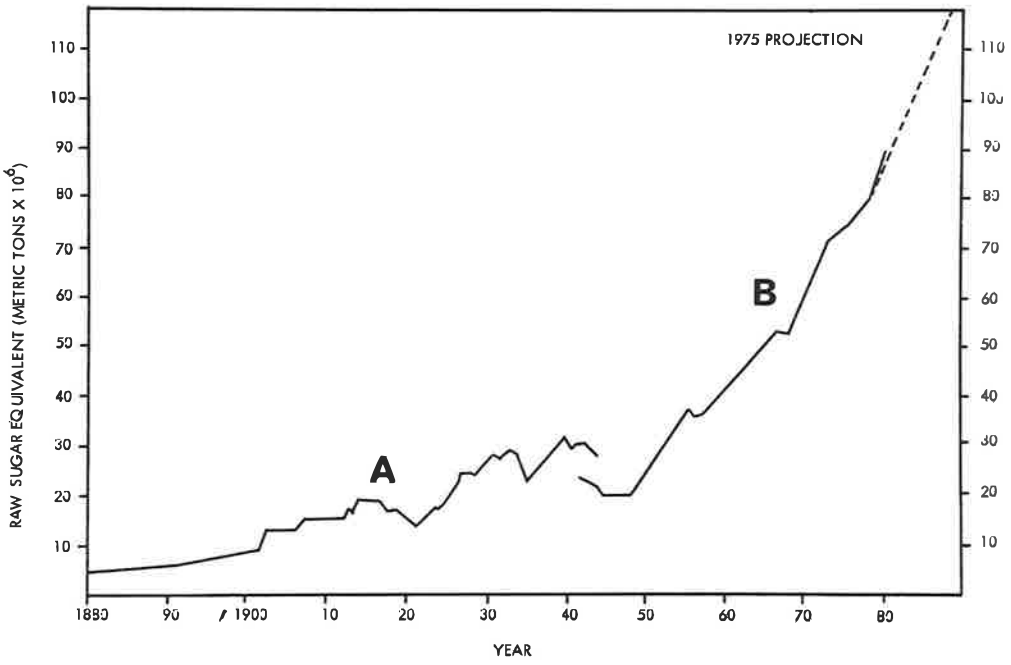


Figure 2 : Starch (amylose)

Figure 3. RECORDED WORLD SUGAR PRODUCTION
(A) FROM ALL SOURCES (B) CENTRIFUGAL SUGAR



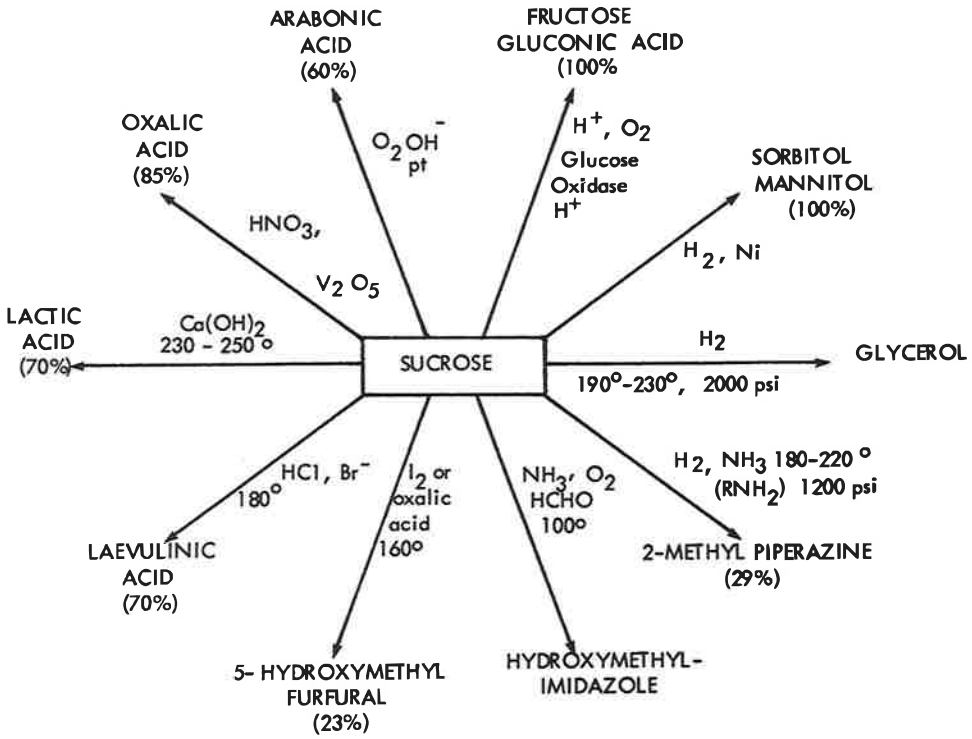
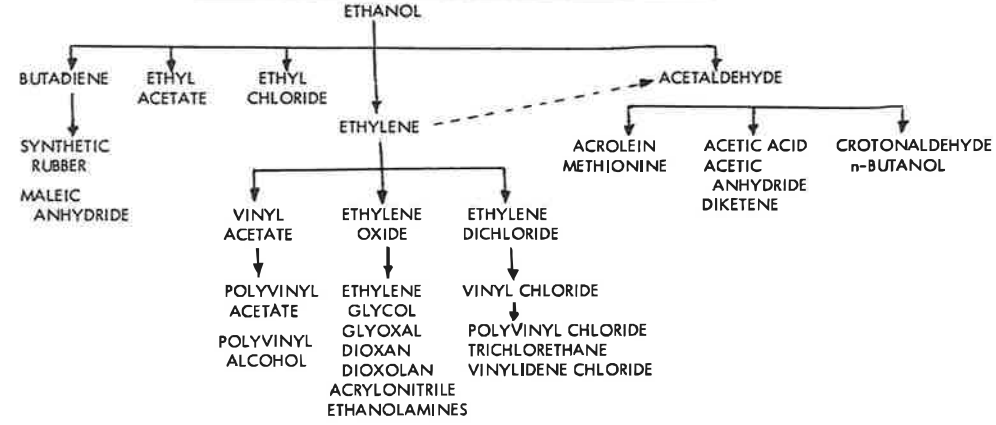


FIGURE 4: DEGRADATION REACTIONS OF SUCROSE

Fig.5 : SOME CHEMICAL INTERMEDIATES OBTAINABLE FROM ETHANOL



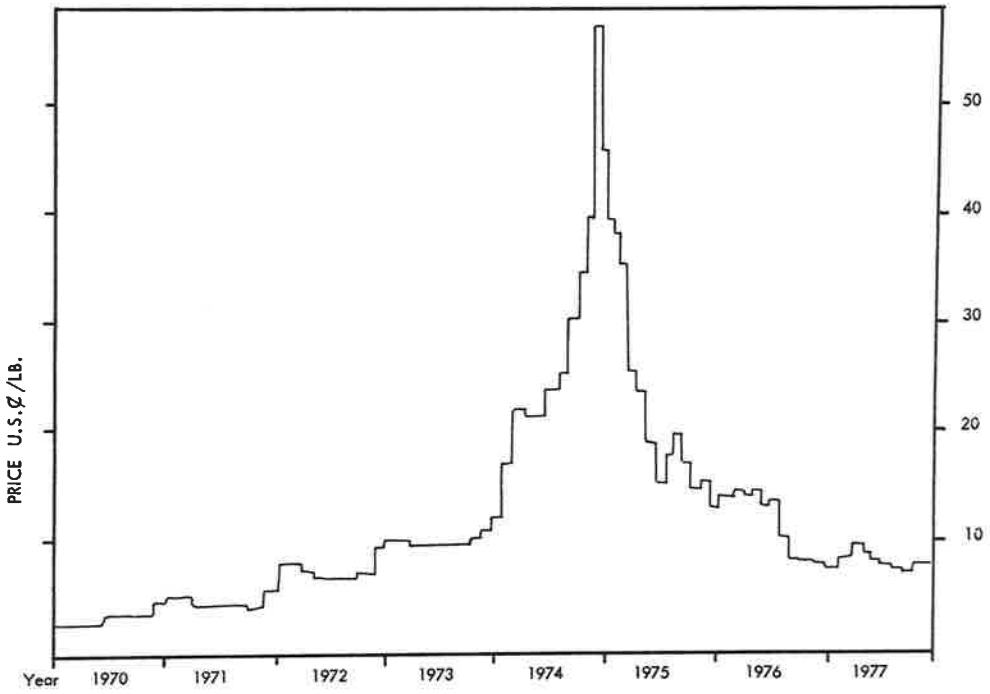


Figure 6. RAW SUGAR PRICES - MONTHLY AVERAGE LONDON DAILY PRICE (U.S.¢/LB.)

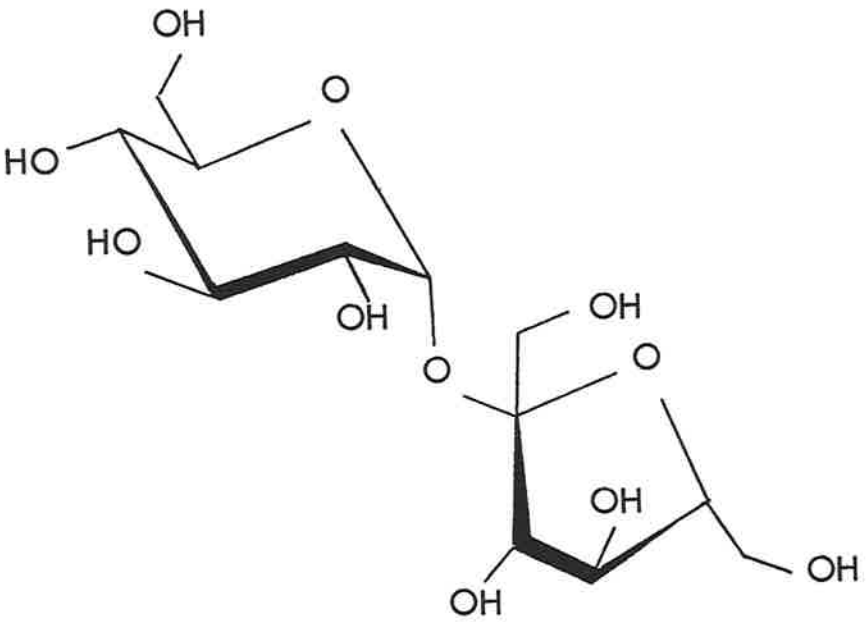


Figure 7. Sucrose

Figure 8: Derivatives of sucrose

Functional type	Examples	Applications
1. Esters	Monostearate	Surfactant Emulsifier
	Monoacetate	Humectant
	Distearate	Emulsifier
	Hexalinoleate Octa Acetate	Surface coatings Denaturant Plasticiser
	Octa Benzoate	Plasticiser
	Di-Isobutyrate Hexa-Acetate	Viscosity Modifier
	Monomethacrylate	Resin Monomer
	Polycarbonate	Resin Intermediate
2. Ethers	Mono Octadecylether	Surfactant
	Hepta-Allyl	Drying Oil
	Octacyanoethyl	Dielectric
	Octa Hydroxypropyl	Cross Linking Agent in Polyurethane Resins
	Tetra-Carboxyethyl	Chelating Agent
3. Urethanes	N-Alkyl Sulphonyl	Surfactants
4. Xanthates	S -Alkyl Monoxanthates	Surfactants Chelating Agent
5. Acetals	Cetyloxyethyl Sucrose	Surfactant

SOLVENTS	ACIDS	POLYSACCHARIDES	AMINO ACIDS
ETHANOL	CITRIC ACID	ALGINIC ACID	GLUTAMIC ACID
BUTANOL	LACTIC ACID	XANTHAN GUM	LYSINE
ACETONE	ITACONIC ACID	DEXTRAN	METHIONINE
2 : 3-BUTANDIOL	GLUCONIC ACID	PULLULAN	
ISOPROPANOL	PROPIONIC ACID	POLY-γ-HYDROXY-BUTYRIC ACID	
	BUTYRIC ACID		

ANTIBIOTICS	GASES
PENICILLIN	METHANE
STREPTOMYCIN	HYDROGEN
CEPHALOSPORIN	CARBON DIOXIDE
TETRACYCLINE	

Table 1: Some products of fermentation of sugars

Chemical	Price: US $/Tonne
Sucrose	216
Propylene	220
Ethylene	290
Benzene	295
Phenol	456
Butadiene	459
Styrene	583
Penta erythritol	1078
Glycerol	1155
Sorbitol	1340

Table 2: Comparative prices of bulk chemicals (March, 1979)

The elimination of organic wasts from surface water

Th. M. van Bellegem
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The Dutch potato-starch industry is situated in the North-Eastern part of the country, where in previous centuries peat was removed as a fuel and the underlying soil was turned into farmland, used mostly for growing potatoes.
Economically, the potato-starch industry is very important for this district: 3000 employees and 6000 farmers depend on it. It is responsible for about 30% of the world potato-starch production.
In the production process the juice of the potato is separated form the insoluble materials, such as starch granules and cell walls. In the past the juice was discharged untreated on surface water, where it caused serious environmental pollution, something which is unacceptable today. A number of years ago a research programme was started to develop a production process that would not lead to the discharge of untreated waste water.
As a first step the composition of potato juice was determined (figure 1) and it could be shown that the juice contains many useful compounds. So the obvious solution was to isolate these components from the juice. One of the first problems we encountered, was the degree of dilution of the juice, and so we started with the development of a production process in which the juice is diluted only slightly or not at all. Several of our plants use the new process and at the newest production unit the juice is even concentrated by reverse osmosis.
The next step was the isolation of the protein fraction from the juice. This is done by ultrafiltration and a heat coagulation process. The amino acid composition of the potato protein so obtained is excellent and can be compared with that of milk protein.
The third and last step was the concentration of the deproteinized juice by evaporation, which gives a material that consist of amino acids mainly and can be used as an animal feed.
Then the energy crisis intervened,energy prices started to rise, and we had to search for alternative processes. So we turned to biotechnology, because that seemed to offer some solutions, e.g. single cell protein production, aerobic treatment and anaerobic treatment.
The single cell protein (SCP) route was tested up to pilot plant scale (1), but the results made clear that SCP was not a viable alternative, mainly for reasons of costs.
Aerobic treatment is expensive because the process consumes large amounts of energy. So the anaerobic method was investigated in some depth, and the results of our experiments will be presented in this paper.

Microbiology and biochemistry of the anaerobic process

The microbiology and biochemistry of anaerobic degradation of organic materials is quite complex (2,3). In many cases the biochemical pathways consist of several phases, as shown in figure 2. First polymers, such as starch, and proteins are hydrolysed into their monomers sugars and amino acids. In the fermentation phase, which comes next, these compounds are fermented to organic acids, alcohols, carbon dioxyde, hydrogen and ammonia.
In the acetogenic phase the bacteria that take over obtain the energy they need by turning the fermentation products into acetate and hydrogen.
The methanogenic phase is the last one, and the bacteria involved in it utilize the acetate, carbon dioxyde and hydrogen to produce methane.
Thus in the anaerobic process the microbiological population consists of three main groups. In the first group are the fermentative bacteria which are involved in the hydrolysis and fermentation phase; they belong mainly to the genera Clostridium, Bacteriodes and Campylobacter. These micro-organisms can grow rapidly. The second group consists of the hydrogen producing or acetogenic bacteria, which transform the fermentation products into acetate, hydrogen and carbondioxyde. This group, which is mainly hypothetical, can only grow under an extremely low partial pressure of hydrogen.

The methanogenic bacteria form the last group (4), and they belong to the genera Methanobacter, Methanococus, Methanosarcina and Methanospirillum. Oxygen is very toxic to these micro-organisms.
Amino acids and sugars are fermented rapidly. The acetogenic and methanogenic bacteria, however, grow slowly, and the reactions are possible only when the retention time is 4 to 5 days. With a shorter retention time or when an unbalance between the different kinds of bacteria occurs, fermentation products will accumulate. As high concentrations of volatile organic acids are toxic to the methanogenic bacteria, this will lead to an inhibition of the methane synthesis. If it lasts too long, the methanogenic bacteria will be damaged irreversibly, and the reactor will have to be started up with new sludge. So to prevent failures, adequate control of an anaerobic reactor is a necessity.

Comparison of aerobic and anaerobic treatment

There are some differences between both systems that are of practical importance. Both have in common that they are composed of oxidation and reduction reactions.
But in the aerobic system carbon is transformed into totally oxidized compounds and oxygen has to be supplied as an electron acceptor. In the anaerobic system, however, the end products are methane and carbon dioxide, which are not totally oxidized compounds. These simple facts lead to a number of important differences between the two processes.
The first is the need of oxygen supply in aerobic treatment. This is a quite expensive process. To remove 1 kilogramme of COD aerobically, the energy need for aeration is between $\frac{1}{2}$ and 1 kW. In an anaerobic system the energy need does not depend on the oxygen. In this process the bacteria have an optimal growth between 27 and 37° C; with lower temperatures the load of the reactor has to be lowered. In an anaerobic system the energy input depends on the difference between the temperature of the waste water and the temperature of optimum bacterial growth. In many industries the waste water can be heated up in a heat exchanger fed by cooling water, and when necessary, the biogas produced can be used for this purpose too.
A second difference lies in production of biomass. In the aerobic process glucose is degraded into carbon dioxyde and water and a large part of the energy obtained is used by the bacteria for growth. The anaerobic process stores a considerable amount of energy in the methane obtained and as a result the amount of biomass synthesized is low. In aerobic processes the yield of biomass is between 0.3 and 0.5 kg biomass/kg COD; anaerobic processes score far lower: 0.04 tot 0.14 kg biomass/kg COD. In the world of waste water treatment, biomass is called sludge, and as sludge disposal is expensive, the low sludge production of the anaerobic process is a distinct advantage.
Another difference between the two processes is nitrogen removal. In the aerobic system nitrification - the synthesis of nitrate from ammonia - and denitrification - decomposition of nitrate into nitrogen and oxygen - both can occur. These microbial reactions make it possible to remove both ammonia and nitrates.
In the anaerobic process the nitrogen in the amino acids ends up in ammonia, and there is no biological method known that will reduce the ammonia concentration.
The last difference between the two methods is the sulphur pathway. Some amino acids contain sulphur, and aerobically this element is oxidized to sulphate. In the anaerobic process hydrogen sulphide is produced.

Anaerobic reactors, the development of their technology

The most simple anaerobic reactor is the continuous flow stirred reactor shown in figure 3. It is used mostly to treat the biomass of activated sludge systems, but if it is used for treatment of waste water, results depend on the retention time (figure 4). In about one day hydrolization and fermentation are almost complete, but degradation of the organic acids will only occur with retention times of 4 to 5 days. With short retention times the effluent will contain high concentrations of organic compounds, and the gain in reduction of the pollution is nil. With the longer retention times methane is formed and the polluting organic compounds are removed from the waste water to a high degree. Typical retention times for treatment of biomass of activated sludge are between 10 and 20 days. It will be clear that this system can not be used to treat industrial waste water,

as the retention times are far too long.
A way to solve this is to increase the biomass retention time but to keep the hydraulic retention time as short as possible; in other words, the cell retention time and the hydraulic retention time are not equal any more.
The first successful reactor incorporating this idea, was the contact process, shown in figure 5. It is a continuous flow stirred reactor coupled to a settling tank. This set-up ensures that the biomass can be recycled and re-used in the methane reactor. This type of reactor is used widely for the treatment of effluents of foods industries. Typical results of this process show loads of 0.5 - 2 kg COD/m^3/day and hydraulic retention times of 2 to 5 days.
Better results can be obtained with the hydraulic filter, developed by Coulter (5) and McCarty (6). It consists of a reactor filled with a coarse material, for example stones, coke or Rashig rings, and it is shown in figure 6. The micro organisms are supposed to attach themselves to the coarse material. We did build one on a pilot plant scale and covered the filter material with different coatings. However, we did not observe any growth of the micro-organisms on the filter material. We found that, using waste water from the potato starch industry, loads of 5 to 7 kg COD/m^3/day were possible at hydraulic retention times of 18 hours.
Then Dr. Lettinga (8) at Wageningen (the Netherlands) developed the upflow reactor. The technical details will be discussed below, but some preliminary figures may show its potential.
In this type of reactor high loads of 7 to 15 kg COD/m^3/day at hydraulic retention times as short as 4 hours can be treated. We prefer this system because it has a better performance and is cheaper to run than any other.
In figure 7 an aerobic and the different anaerobic systems are compared for an influent COD of 10,000 mg/litre.

The upflow reactor

A schematic design of the upflow reactor, or the upflow anaerobic sludge bed sludge blanket system is shown in figure 8. It consists of two compartements: the methanogenic and the settler compartment. The influent enters the reactor at the bottom, the methane produced bubbles up and is separated from the fluid. The effluent leaves the reactor at the top via the settler, where the biomass is separated from the effluent.
The biomass stopped by the settler settles into the methanogenic compartment under its own weight.
In this reactor high purification efficiencies at high loads may be attained if a number of conditions are met:
- distribution of the influent should be uniform over the underside of the reactor
- the mixing effect of the biogas produced should be optimal, i.e. the waste water at the bottom is pushed up evenly over the cross section of the reactor by methane bubbles
- the micro-organisms in the reactor should be healthy
- the settler should be effective in separating gas, biomas and effluent.

The influent should be distributed evenly over the underside of the reactor, because the micro-organisms should start to attack the organic content of the waste water immediately, and methane production should occur at the lowest level to ensure good circulation in the reactor. When the influent is not distributed evenly, the sludge bed will become criss-crossed with short-circuiting channels and the conversion of the organic content will not prodeed at the optimal rate. Uniform distribution of the influent can only be attained by installing a number of influent points (9).
The circulation in the reactor depends on the mixing effect of the biogas produced, and thus on the load. In our experiments the sludge load was 0.7 - 1.1 kg COD/kg sludge/day.
In an upflow reactor a high concentration of biomass can be obtained, because after a rather long period the sludge acquires a grainy structure and then has excellent settling properties.
The distribution of the sludge in the reactor is given in figure 9, showing the distribution at a load of 9.5 kg COD/m^3/day. It can be seen that the highest concentration of the sludge is at the bottom - where it should be - and this part is called the sludge bed. Here

concentration can be as high as 100 kg/m^3, depending on the load. Higher up the concentration falls to about 17 kg/m^3, and this part of the reactor is called the sludge blanket. Here concentration depends on the load, the mixing effect of the biogas, the settling properties of the sludge and a number of other parameters.
The settler on the top of the reactor is an important device, as it should give effective separation between gas, biomass and effluent. For these reasons it should be designed with the utmost care. In one of our pilot plants we tested different designs of settlers, shown in figure 10. The first one is the original settler developed by Dr. Lettinga, the second one was developed by CSM, a Dutch sugar refining company (10).
In the first design the sludge return and the settler influent are combined, in the second sludge return is an independent function. Our experiments indicated that, provided the design of the settlers has been done with care, there is no significant difference in efficiency between both types when priming the upflow reactor with waste water from the potato-starch industry.

A two-phase anaerobic reactor

As anaerobic degradation of organic compounds goes on in a number of phases, it might be worthwhile to separate some of them of optimize the process (11). Cohen (12), working with glucose containing influent, made a comparison between a single-phase system and a system in which the fermentation phase and the methanogenic phase were done in separate compartments. His results indicate that two-phase systems could carry higher loads. We repeated his experiments in a pilot plant, using waste water from the potato-starch industry, and we could not find a difference between the maximal loads that could be achieved. In both systems the sludge properties were equal. We don't have an explanation for our results, but it could be that the very complex composition of the waste water of our industry has some influence.
However, a two-phase system has clear advantages when strongly fluctuating amounts of influent are to be treated.
We also did some experiments with waste water from a chemical plant, and in this case it turned out that a two-phase system was a necessity, as the waste water contained toxic compounds. The toxic compounds could be removed in the fermentation phase by a special process that was developed for the purpose, and so we now can treat this type of waste water anaerobically without poisoning the micro-organisms in the reactor.
The question whether an one-phase or a two-phase system is the most suitable for treatment of a certain type of waste water, cannot be answered in general at the moment; each case should be studied on its own mertis.

Starting up an anaerobic reactor

When an new anaerobic reactor is started up for the first time, sludge is needed to inoculate it. But, at the moment, no large amounts of sludge from upflow reactors are available, although the situation may change in the future. So for starting up we have to make do with a digestion sludge, and that has serious drawbacks. The biological activity is low and the digestion sludge has bad settling properties, as it is in the form of flocks and not grainy. Bacteriological activity and settling properties improve very slowly, because anaerobic bacteria have long generation times. Our experiments showed that, using digestion sludge as a staring material, it will take two to three months before the reactor can be charged with its maximum load. During this long starting-up period intensive inspection of the reactions going on is necessary, as the process can go off the rails very easily. It is only at the end of the starting-up period that the sludge will acquire the grainy structure that is sought.
Another important point is that the potato-starch industry has a campaign of about four months and that, in consequence, the reactor will be idle for two-thirds of the year. But when the potato campaign starts, the reactor has to attain its maximum efficiency very fast. Luckily, the results of the experiments of Dr. Lettinga (7) showed that the anaerobic biomass can be stored for long periods without losing its biological activity. At the end of a campaign the influent is stopped and the sludge is left in the reactor. A few days before the campaign of next year starts, the reactor is started up again. Figure 11 gives some data on the starting up of our pilot plant after a resting period of two months. Gas

production started almost immediately and after three days the reactor was again at its maximum efficiency. During the starting-up period the COD of the effluent was always low. We analysed the fermentation products too, and concluded that an even faster start up might have been possible.

The effectiveness of upflow reactors

Figure 12 gives some results of COD removal by anaerobic treatment in an upflow system. The load was 8 kg COD/m^3/day and the difference of COD of influent and effluent is rather impressing. Figure 13 summarizes the results. With a COD influent of about 18,00 mg O_2/litre, the effluent has no more than about 800-1200 mg O_2/litre, which indicates that at a load of 7.5 kg COD/m^3/day a COD reduction of between 90 and 95 percent was obtained at a temperature of 35°C. The BOD in the influent was 10,000 mg O_2/litre and was reduced to 200 - 300 mg O_2/litre in the effluent - a reduction of more than 95%.
The amount of biogas produced depends on the amount of COD removed. In our experiments 0.32 to 0.38 m^3 of methane was obtained for every kilogramme of COD removed; figure 14 gives some data on the composition and production of the biogas.
Per cubic metre of water about 6.5 to 7.5 m^3 of biogas are produced, and its composition is rather constant: the methane content is about 75%, carbon dioxyde is between 20 and 25%, and other volatile compounds are absent or in low concentrations only.

Applications of upflow reactors

The upflow anaerobic reactor was developed in the first instance by CSM, a Dutch sugar refining company, for the treatment of the waste water form their beet sugar plants. The first reactor CSM has installed, has a volume of 800 m^3. Some other reactors, working on the same principle, are now under construction.
This year AVEBE, the Dutch potato-starch industry, will start up one with a volume of 1700 m^3. It is expected that AVEBE will start up the largest anaerobic upflow reactor in the world in August 1980 at their production plant at DE KRIM, in the north of the Netherlands. It will clean up waste water from a large potato-starch plant, and it will have a volume of 5000 m^3. It has been designed as a two-phase system.
Other possible applications of upflow reactors might be found in the fermentation industry, and in the food and chemical industries.

The economics of anaerobic treatment

A general comparative discussion of aerobic and anaerobic plants for waste-water treatment is rather difficult.
First, an anaerobic reactor has to be adapted to the industrial effluent it has to clean. Often it is not known at the start whether a one-phase or a two-phase system will give the best results, and the best system may have to be found by experiment.
Second, costs of the gas storage system can be an important item.
Third, the costs of aerobic treatment depend on the local situation and on the system chosen, e.g. whether a biological filter method or an activated sludge method is preferred.
So the dates given in figure 15 have only a limited value and should be interpreted with care. In the figure, costs are given in Dutch guilders per inhabitant equivalent for a campaign industry.

Still there are some general conclusions to be drawn.
The investment costs for anaerobic installations tend to be lower than those for aerobic processes. Operation costs of anaerobic plants are lower too. In estimating the operating costs of an anaerobic plant, we neglected some items that might be important. We did not calculate the economic value of the biogas produced, and we estimated that there would be no need for extra energy to reach the temperature of 35° C, necessary in anaerobic treatment of waste water.
The main difference between aerobic and anaerobic systems is to be found in the energy input, which is high for aerobic and rather low for anaerobic systems. In both systems the waste water has to be pumped to the reactor, but in an anaerobic system there is no need for aeration.

Methane production will be between 5 and 7 cubic metres per inhabitant equivalent, and depending on local gas prices, the sale of the methane will result in a certain lowering of operating costs. If one has full year production instead of a campaign industry, 17 to 25 m^3 of methane will be obtained for every inhabitant equivalent. In that case, the value of the methane produced may be as high as, or even higher than the operating costs of the reactor - this again depends on local gas prices. So it might be possible to make some money out of the purification of waste water, and this is one of the reasons why in the Netherlands waste from piggeries is treated in anaerobic upflow reactors more and more.

To sum up, I hope it will be clear that I consider the anaerobic treatment a process with a hidden past, which, however, will find increasing application and for that reason has a shining future.

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DRY MATTER	5%
PROTEINS	1.36
AMINOACIDS	1.44
CARBOHYDRATES	0.75
BOD	33000 mgO_2/L
COD	54000

Figure 1. The composition of undiluted potato juice

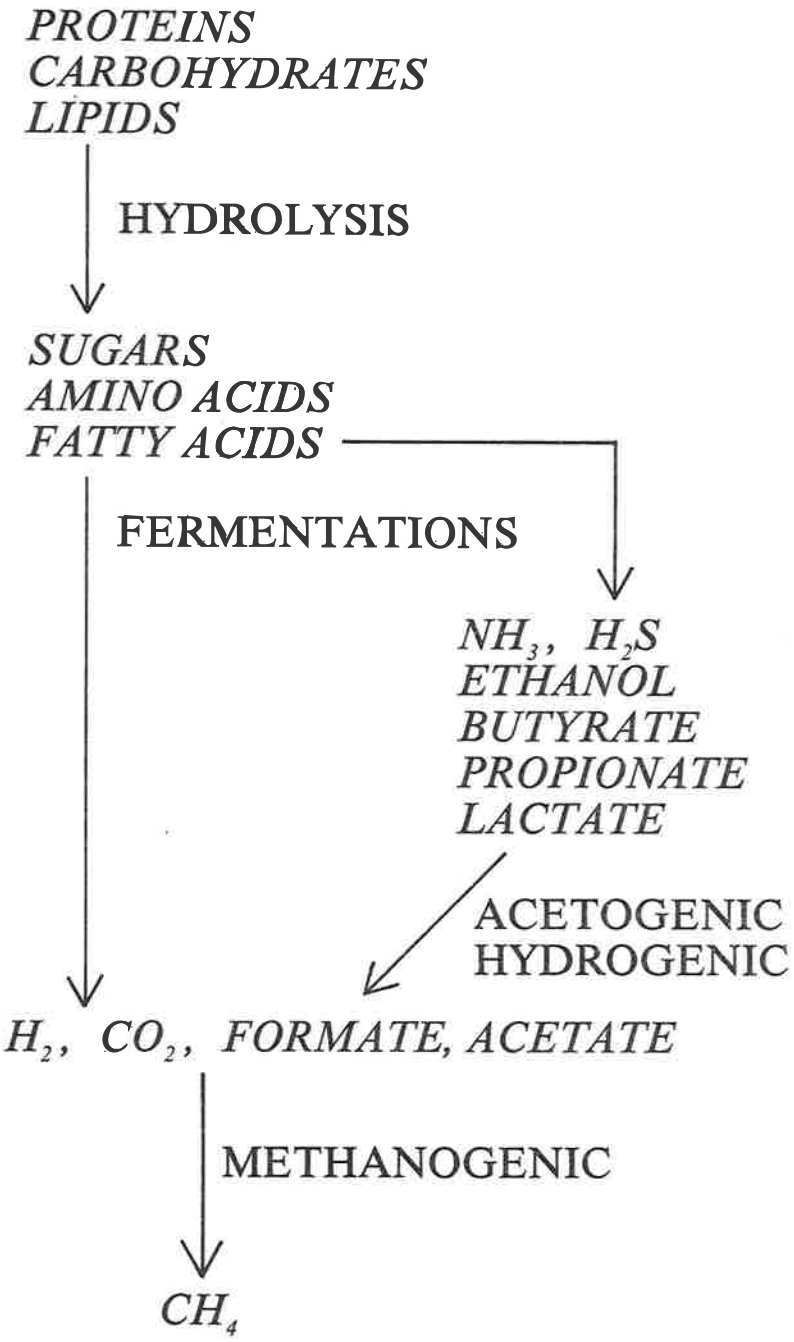

Figure 2. Biochemical pathways of the methane bio-synthesis

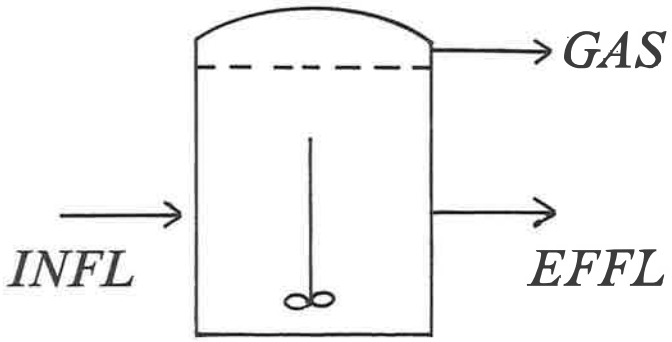

Figure 3. Flow sheet of continuous flow stirred anaerobic reactor

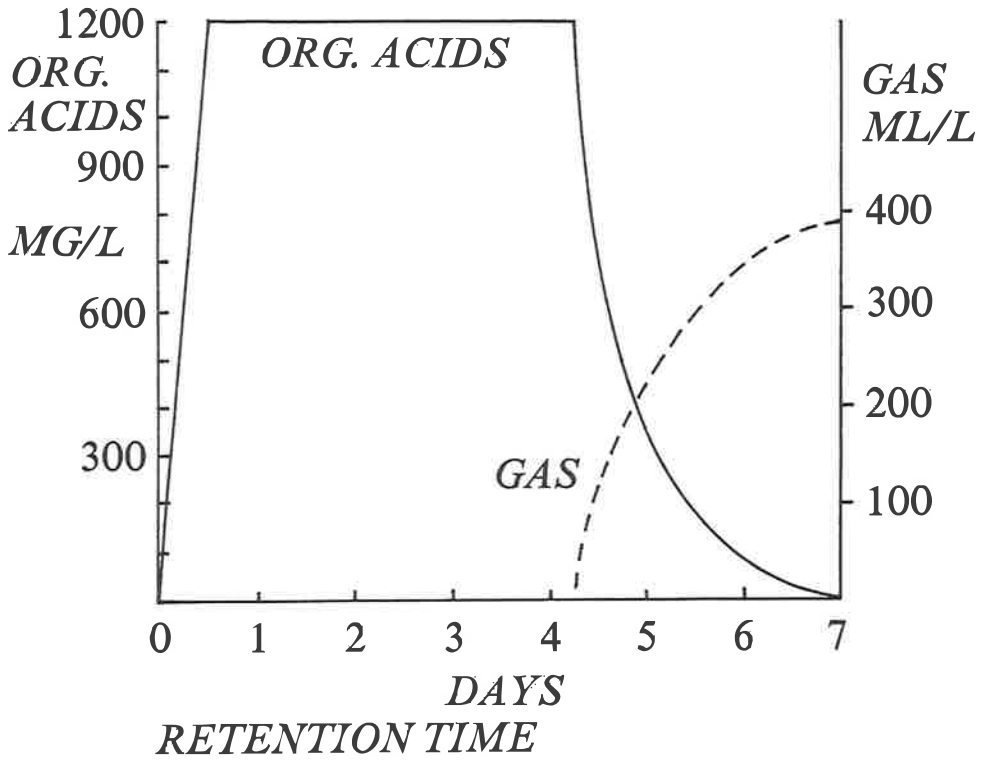

Figure 4. Production of organic acids in a continuous stirred reactor at various cell retention times (COD influent 1400 $mgO_2/1$)

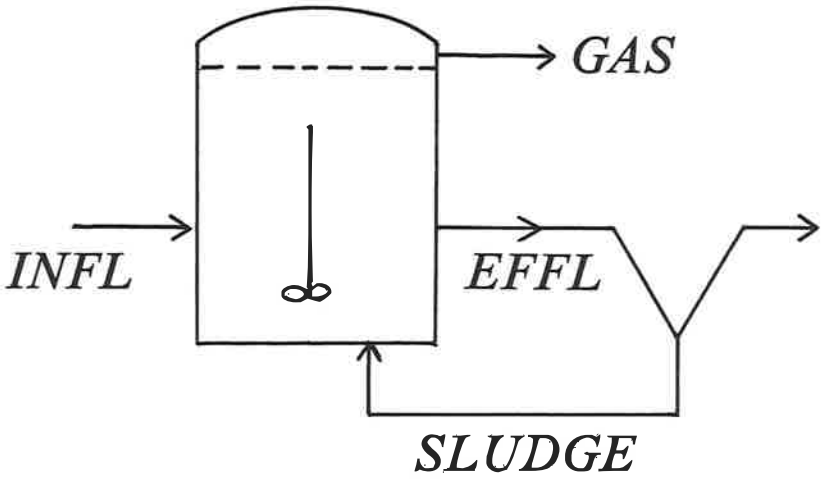

Figure 5. Flow sheet of the anaerobic contact process

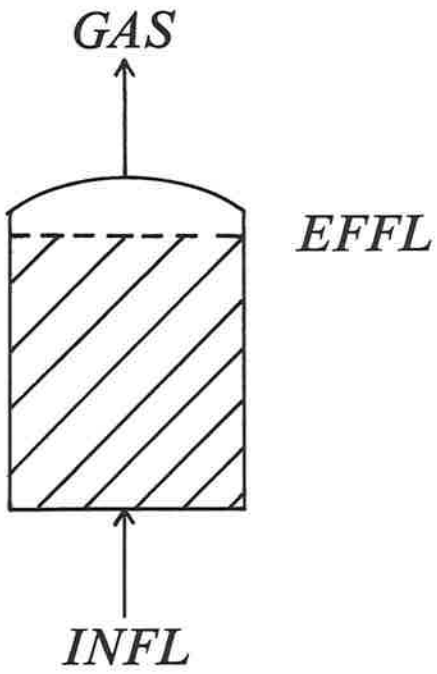

Figure 6. Flow sheet of the anaerobic filter

	RETENTION TIME (DAYS)	*LOAD KG COD/M³/DAY*	*EFFICIENCY COD %*
ACTIVATED SLUDGE	14	0.7	>90
CFSR	10	1	90-95
CONTACT PROCESS	5	2	90-95
FILTER	2	5	90-95
UP FLOW	1	10	90-95

Figure 7. Parameters of an aerobic and the various anaerobic treatment systems. (CFSR is continuous flow stirred reactor) (Influent COD 10.000 $mgO_2/1$)

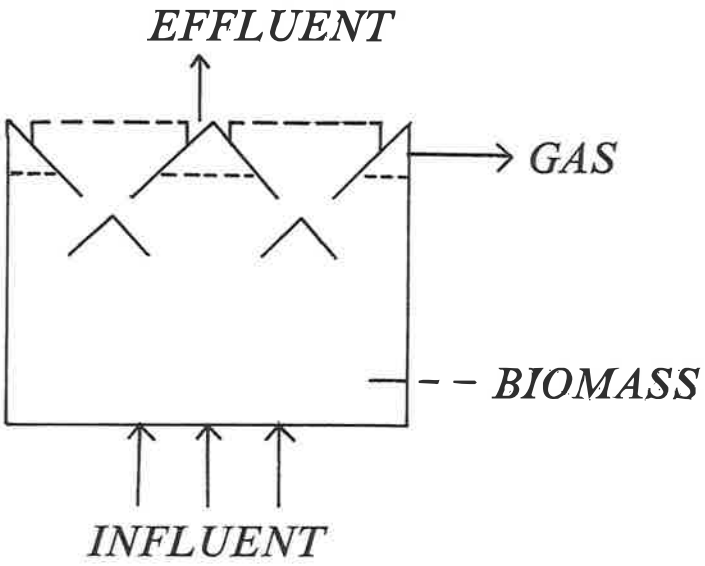

Figure 8. Flow sheet of the anaerobic up flow reactor

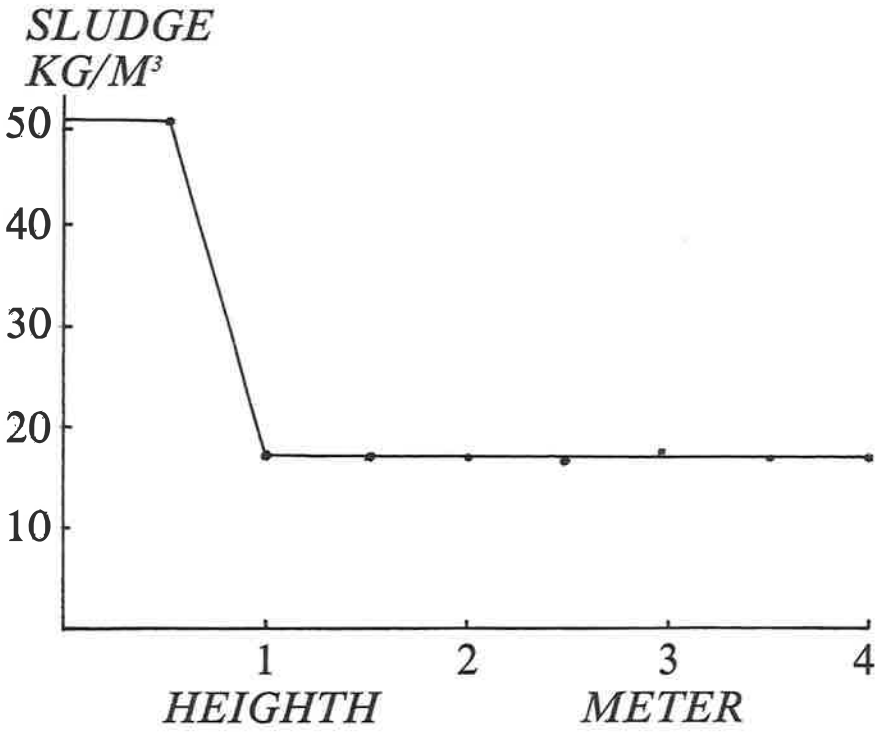

Figure 9. Sludge concentrations in an anaerobic reactor of four meter high

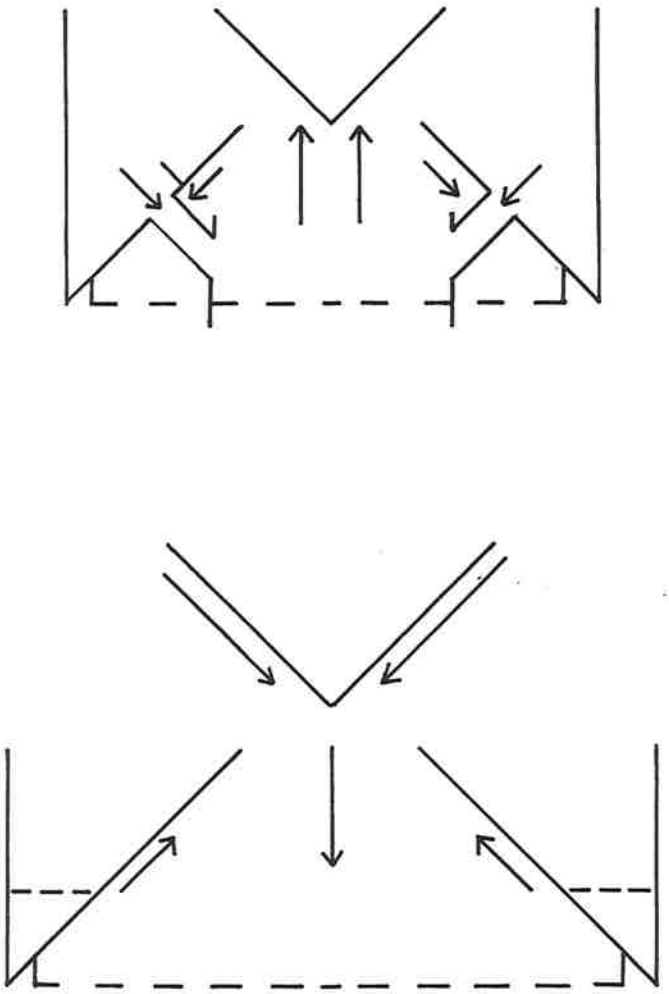

Figure 10. Settlers in the anaerobic upflow reactor

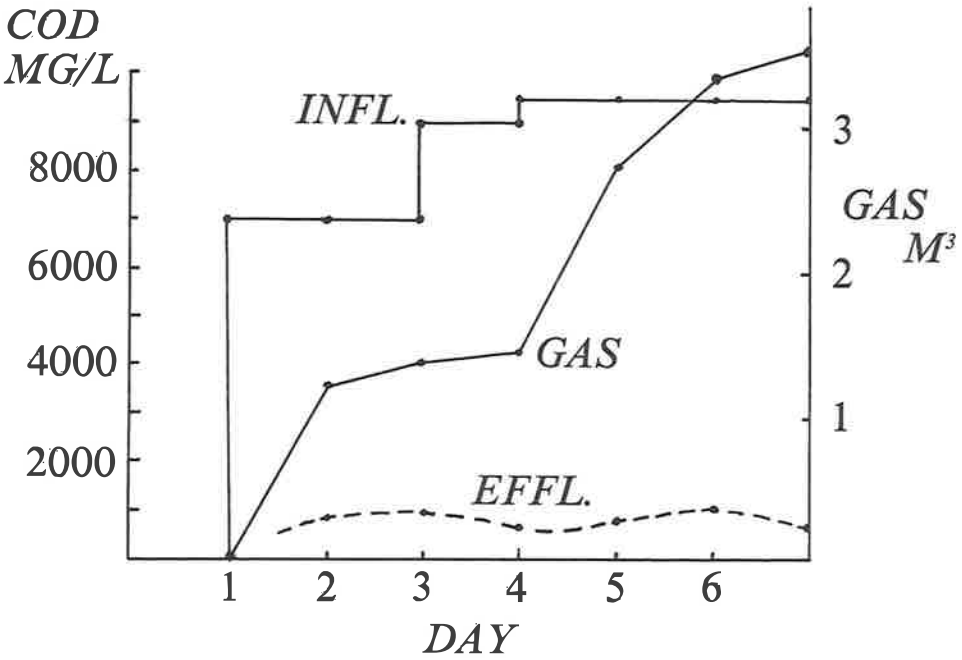

Figure 11. The start up of an upflow reactor after the reactor was stopped during two months

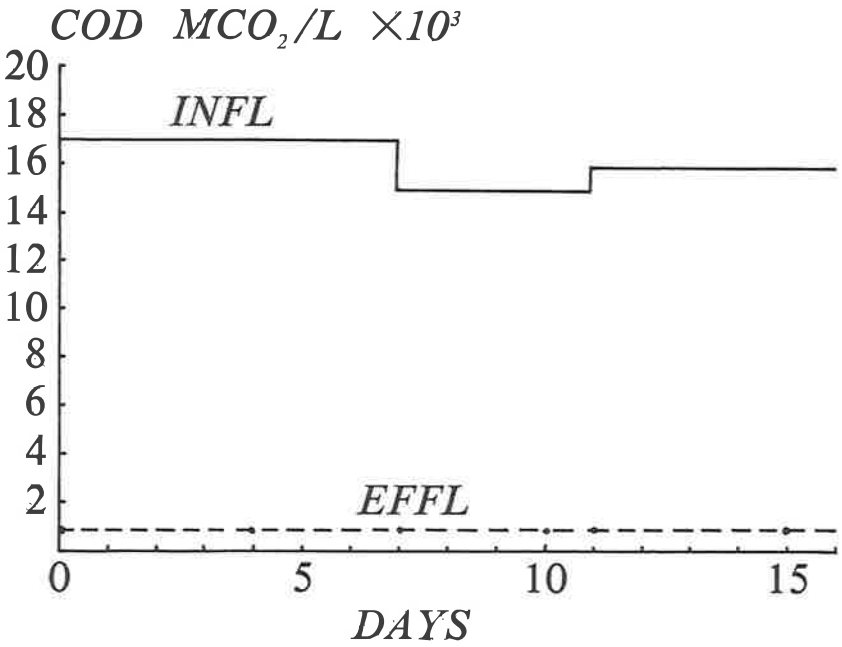

Figure 12. Influent and effluent COD of an upflow system at a load of 8 kg/m3/day

COD INFLUENT	18000 *MG/L*
COD EFFLUENT	800-1200 *MG/L*
COD EFFICIENCY	90-95%
BOD INFLUENT	10000 *MG/L*
BOD EFFLUENT	200-300 *MG/L*
BOD EFFICIENCY	95-98%
N-REDUCTION	5-10%
BIOGAS	6.5-7.5 M^3M^3 WATER

Figure 13. Purification results obtained in an upflow reactor

BIOGAS

CH_4	71- 77%
CO_2	19- 26%
N_2	0.7-1.5%
H_2, H_2S	<0.1%

Figure 14. Composition of the biogas

	ANAEROBIC	AEROBIC
INVESTMENT COSTS	10-40	40-100
OPERATING COSTS	1- 8	5- 18
ENERGY	0,5- 1 *KWh*	8- 20 *KWh*
METHANE	5- 7 M^3	

Figure 15. Economic aspects of the anaerobic treatment process.
Costs expressed in Dutch guilders for a campaign industry.

Biomass as a Source of Energy

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1. Resources and Requirements

Energy resources from fossil fuels are estimated to total about 3.1×10^{11} TJ, with an additional 2×10^{11} TJ available as nuclear fission fuels, provided that fast breeder reactors are used. If fusion would become feasible 1.5×10^{18} TJ would be available, but this source is a far and very uncertain option. When we express these recourses in the number of years these fuels would last on the 1975 total world energy consumption ($\sim$7 TW) we obtain the picture of Table I (1). Recent scenario's of the Workshop on Alternative Energy Strategies, show a levelling off of the oil production in the years to come. Around 1985-1990 the demand and supply of oil will no longer match.

The present world energy consumption is about 9 TW (1 TW $\equiv 10^{12}$ Watt) on a population of 4 billion people. There is a highly skewed distribution of the use of energy as illustrated by Table II. We see an enormous difference between the less developed countries and the developed countries. If we would say that a reasonable average of energy consumption is 4000 W per capita in a "developed" society the world energy consumption for the present population would have to be 16 TW, about double the present situation. When we realise that the UN projections for the world pupulation in 2050 vary from 9.4 to 12.2 billion people the energy consumption at the 4000 W per capita level will require an energy supply of 38 - 50 TW (2).

In addition to the primary energy resources as oil, gas, coal and nuclear fuels nature provides other energy resources: solar radiation ("solar energy"), tidal movements ("moon energy"?) and geothermal energy ("earth energy"). In this paper we will concentrate on solar energy and more particular on photosynthesis of biomass. An overview of the ways in which solar energy can be applied is given in Fig. 1. Sunlight can be applied directly by catching it on sun collectors to heat a liquid to achieve photovoltaic or photochemical conversion into electricity or an energy carrier like hydrogen. More indirect applications of solar energy are those methods in which a natural phenomenon induced by solar radiation, such as wind energy, waterfalls, temperature gradients in the oceans, growth of plants (biomass) by photosynthesis are tapped.

The order of magnitude of these "non-conventional" energy resources are indicated in Table III in TW-units. Also given in this table is the order of magnitude of recoverable fractions if one assumes that economically feasible technologies could be developed. This column thus indicates theoretical, physical limitations and not economical limitations, which may be more severe. The actual use of energy resources thus will be lower than the values mentioned in this table.

We will in this paper only consider the use of solar energy via the conversion into biomass by photosynthesis.

2. Energy relations in the agricultural/food system

2.1. Photosynthesis and crop productivity data

For our discussion of today especially the photosynthesis process in the chloroplasts of plants is of interest. Photosynthesis consists of two main reactions: an overall "light"-reaction in which chlorophyll plays the important role, and an overall "dark"-reaction.

$$2H_2O \longrightarrow O_2 + 4H^+ + 4e \quad (1)$$

$$4H^+ + CO_2 + 4e \longrightarrow CH_2O + H_2O \quad (2)$$

$$CO_2 + H_2O \longrightarrow CH_2O + H_2O \quad (3)$$

$$\Delta G = 478 \text{ kJ/mol} \quad (4)$$

The redox pair $\frac{1}{2}O_2/H_2O$ has a potential of $\sim$ 0.82 V, the redox pair CO_2/CH_2O has a potential of $\sim$ -0.42 V. Four electrons are required per CO_2 molecule transformed. Each is "pumped" over a potential difference of 0.82 - (-0.42) = 1.24 eV, taking 1 eV $\sim$ 96.37 kJ/mol we calculate the total energy input required to supply the free energy stored in the reaction as 4 x 1.24 x 96.37 = 478 kJ/mol.

The Hill-Bendall scheme, which appears to be generally accepted (7-10), gives a somewhat more detailed picture that we will need later on. The light reaction can be subdivided in two parts, photosystem II (PS II), photosystem I (PS I), as given in Fig. 2. In PS II 4 electrons are pumped up with aid of 4 photons to plastoquinone with the aid of chlorophyll P 690. A carrier chain connects this system with PS II where another boost is given to our 4 electrons by another 4 photons, and with the aid of chlorophyll P 700, which brings about the reduction of a soluble, low molecular weight protein called ferrodoxin. The reduced ferrodoxin (and 3 ATP which are also supplied by the "light"reactions) are needed to reduce CO_2 to sugar (CH_2O) in the "dark" reactions that follow. We see that 8 photons per molecule carbondioxide are required. In C_3-plants the carbondioxide is employed in the reductive pentose phosphate (RPP) cycle which begins with the carboxylation of ribulose (1,5)-phosphate which splits immediately into two C_3 molecules: 3-phosphoglycerate (PGA). Plants that have only the RPP-cycle for CO_2 fixation and reduction are termed C-3 plants, since the primary carboxylation product is a three-carbon acid. There are plants, supposed of tropical origin, which have in addition to the RPP-cycle another CO_2 fixation cycle. In this cycle CO_2 is first fixed by carboxylation of phospho-enol-pyruvate (PEP) to give a four-carbon acid, oxaloacetate in the mesophyll cells. This carbondioxide is then transported to the vascular bundle cells where it is released to join the RPC-cycle. The result is that the RPC-cycle can now operate at higher carbondioxide concentration and lower oxygen concentration than the normal atmospheric concentrations of 0.033% CO_2 and 20% O_2, which in turn leads to a reduced reoxidation to CO_2 of sugar phosphates formed by photosynthetic fixation; lower respiration losses or C_4-plants have "pep". The theoretical efficiency of the photosynthesis process in white light can be readily calculated. One einstein white light has an energy content ($E = h\nu$) of 208.41 kJ and eight einsteins are needed to fix 478 kJ, so the efficiency is:

$$\eta_t = \frac{478}{8 \text{ x } 208.41} = 0.29 \qquad (5)$$

Because only part of the solar radiation is photosynthetically active ($\eta_1 \simeq 0.43$), quantum efficiency differences ($\eta_2 = 0.80$), transmission and reflection losses ($\eta_3 = 0.85$), absorption by nonphotosynthesis pigments ($\eta_4 = 0.95$), respiration losses ($\eta_5 = 0.80$) and partial soil coverage ($\eta_6 = 0.90$), the theoretical efficiency of a C_4-plant in the tropics with a closed crop surface amounts to 5.5%. Measured maximum efficiencies are given in Table IV. We see a maximum of $\eta_4 = 0.032$ for a C_4-plant in the tropical terrestrial zone and of $\eta_3 = 0.019$ for a C_3-plant in the temperate terrestrial zone, but in practice much lower efficiencies should be taken in calculations. Table V and Table VI give some figures for natural ecosystem productivity and for agricultural systems (3-4). The data are in tons of dry matter per ha per year. When we realise that harvestable biomass, calculated as carbohydrates, has an energy content equivalent to a yield of 0.65-1.5 (W/m^2) and the solar radiation at the earth surface is between 100-500 (W/m^2), the practical efficiency, η_p, of photosynthesis is roughly 0.5%:

$$\eta_p \simeq 0.005 \qquad (6)$$

We can consider photosynthesis of a crop as an overall surface reaction in which carbondioxide and water are transformed into biomass, as indicated in Fig. 3a/3b. The net rate of formation of biomass, r_B, expressed in mass per unit area per unit time can be expressed as:

$$r_B = r_{B,max} \cdot \frac{(S)^2}{K_m + (S)^2} \qquad (7)$$

where $r_{B,max}$ is the maximum production rate, which depends on the water availability of the soil (E), expressed in mm:

$$r_B = r^o_{B,max} ((E) - 50) \qquad (E) > 50 \qquad (8)$$

In equation (7) and (8) (S) is the intake of nitrogen expressed in mass per unit area, and K_m and $r^o_{B,max}$ are constants typical for a crop under given agricultural practices. For the Dutch agriculture and soils with a moisture capacity (E) of 200 mm we can calculate these parameters for the more important crops using data given by Van Heemst et al (4). Table VII gives some typical reaction kinetic data. One should realise that (S) is the actual uptake of nitrogen by the crop, and that the data strictly pertain to Dutch agricultural practices.

Although the efficiency of photosynthesis is very low, the total result of photosynthesis on the earth is large (3). A flow of about 95 TW is fixed in biomass (annual average) of which about 30 TW in the form of poorly accessible phytoplankton and seaweeds in the oceans, about 45 TW in the forests and 20 TW in other plants. The latter figure includes about 5 TW in agriculture (3). These flows are impressive indeed when compared to the present world energy consumption of 9 TW.

2. 2. Balances and flow structures

The global energy flows in the agricultural/food systems that are influenced by man can be calculated approximately. The most important inputs are solar radiation and fossil fuels. Solar radiation is used in agriculture and forestry to produce biomass.

Part of the solar energy that has been stored in this fashion is used as human food, as animal feed, as fuel, as building material etc. In addition to this fishing activities of mankind also catches a small part of the solar energy that has been transformed into phytoplankton which in turn has been transformed via food webs into fishbiomass. These actions of mankind lead, of course, to flows of biomass which represent an energy flow each. Fig. 4 gives a scheme in which these energy flows are indicated in terawatts (1 TW = 10^{12} W). The thick arrows indicate flows that are of direct importance for human food supply and other human activities. Over the whole world these flows are 1.8 TW of plant biomass augmented with about 1 TW wood and 9 TW fossil fuels. A significant part of the plant production serves as feed for cattle and other domestic animals. Together with 0.02 TW as fish this gives 0.52 TW human food, which means 130 W per capita. To produce the wood and the food about 70 TW of solar energy has been consumed via gross-photosynthesis. Corrected for respiration and maintenance of terrestrial plants and forests ($\sim$ 30%) and phytoplankton ($\sim$ 50%) a nett consumption of about 37 TW photosynthesized biomass would be possible.

An important, but difficult, question is how this picture of the present situation might change in the future and what limits on agricultural production of biomass should be expected. There are departing views on this issue. Meadows (5) estimates the area available for cultivation at 3.2 x 10^9 ha. Mesarović and Pestel (6) assumed in their computations that the maximum area of land that can be cultivated is 2.4 x 10^9 ha. More recent and more detailed studies by a group of Dutch specialists (7-9) calculate a potentially available agricultural land area of 3.4 x 10^9 ha. This figure is the result of a study of the new soil map of the world (FAO/UNESCO (10)). The soil conditions were grouped into 222 broad soil regions and for each region the details of soils, vegetation, topography, climate and possibilities for irrigation were studied (7). Also the "absolute maximum" of food production for a "standard" cereal crop were

calculated by the group. This standard crop is healthy, green, closed, well-supplied with nutrients, oxygen, water and foodhold and therefore the production is only limited by the daily photosynthesic rate dependent on condition of the sky, the latitude and the date. The dry matter production consists of 25% roots and stubble, 37.5% grain (of which 2% is lost during harvest) and 37.5% straw. The grain is assumed to have a moisture content of 15%. Table VIII gives the main results of this study. We see that a total of about 86 x 10^9 tons per year of harvestable dry matter is the theoretical maximum. Taking an energy content of 12 GJ/ton we arrive at 33 TW of which 50% is straw and 50% is grain. Buringh (8) warns for thoughtless use of these figures because they cannot be obtained in actual practice as many economic and social reduction factors have to be taken into account.

Similar calculations can be made for the Netherlands. The rate of energy consumption in the Netherlands is about 90 GW. Fig. 5 gives a scheme of the energy relations in the Dutch agriculture/food industry for 1975. In this scheme we distinguish between three sub-systems: the agriculture/horticulture system, the animal husbandry system and the food industry. The agricultural area in the Netherlands is about 2.1 x 10^6 ha. The amount of fossil fuels required in the agriculture/food industry system is about 12 GW, including the fossil fuels required to produce imported raw materials for the animal feed industry and the requirements for transportation of the animal feed industry in the Netherlands. Fuel requirements for the food industry include transportation and packaging materials. A significant portion of the agricultural production of biomass is used for animal feed in the animal husbandry subsystem. For every ha used inside the Netherlands for animal feed production another two ha are in use somewhere else in the world to produce animal feed. This activity delivers milk, meat and eggs for human consumption. In addition to this the food industry imports 2.7 GW of energy content in the form of raw materials and intermediate food products. The food industry produces about 4 GW as food for human consumption of which about 50% is exported. The animal husbandry subsystem uses about 1.3 GW as a waste-stream from the food industry and produces itself about 6 GW of wastes as manure, methane and urine.

Also for the Netherlands it is possible to approximate the absolute maximum agricultural production of dry matter without special irrigation measures. In this calculation we used the classification of soil regions given by Van Heemst et al (4) and their productivity data for wheat, potatoes and sugar beets. Table IX gives the results of such calculations for sugar beets, winter wheat and industry-potatoes. We see that the maximum production of dry matter would be obtained with industry-potatoes that might deliver 24.5 x 10^6 tons or 11.7 GW or 13% of our energy consumption. In Fig. 6 a map of the Netherlands is given with the areas in which all the existing farmland in that region should be used for potato culture indicated. One should of course realize: that these productions cannot be obtained in practice because of diseases etc.; that this kind of an agricultural system is completely different from our present system, and that the production of fuels from the starch consumes a large amount of energy in itself so that it is questionable whether a net energy gain can in fact be obtained. We will come back to this question of energy balances later. When we think about using recent photosynthesis derived energy products in the Netherlands apparently only the waste streams indicated in Fig. 5 have potentials. But when we make a rough calculation less than 2 GW could be derived from all the municipal wastes, animal wastes and straw in this country. The potentials thus are limited indeed.

On a global basis the picture is somewhat different. I invite you to join a crystal ball gazing trip to the year 2080 and we assume that the earth has reached the enormous population of 12 x 10^9 people at that time (2, 11). We will assume that this population is supplied with 130 W/capita food and 4000 W of other energy, which is transformed from forestry, agricultural production, animal husbandry and fishing in about the same proportion as used before in Fig. 4. In addition to the food farming system we assume a developed energy farming system to exist. This system supplies biomass to a con-

version system that produces usuable energy (electricity, substitute natural gas, liquid motor fuels). This system also takes place in byproducts from food farming, cattle wastes and urban wastes. The system is illustrated in Fig. 7 and will now be discussed in some more detail.

Of course we give priority to food production over energy farming. A diet of 0.75 kg milk, 0.08 kg meat and 0.48 kg grains per capita corresponds to 120 W per capita and the agricultural system will have to supply it. We estimate the maximum potential agricultural land at 2.8×10^9 ha instead of 3.4×10^9 ha because an additional population of 8×10^9 have to live and estimates are a requirement of 0.1 ha/capita for living, roads, schools etc., Buringh (12). We assume that 60% of this required area has to come from good agricultural land because that is where cities etc. are present. Following Buringh et al (7) we base our estimates on the "standard" grain crop defined above. The 1.12 TW grain equivalents (2100×10^6 ton/year) could be produced on 191×10^6 ha assuming a harvested yield of 22 t/ha/year dry matter production of which 50% is grain and 50% is straw. This is a very high yield, but it is the figure obtained when 73% of the total dry matter production of Table VIII is taken as harvestable material.

The milk and meat require a production of forage and roughage that was estimated according to data from Alberda (13). An area of 595×10^6 ha is required for this production. Also production of cash crops such as coffee, tea, cocoa, cotton, rubber, oilpalm is taken to increase almost proportional to population. The area required in 2080 for these cultures is estimated at only 10^9 ha (12) as this area is already cultivated by advanced methods now so only modest yield increases by better farm management are expected. When we assume that agriculture of grains and forage is operating at 75% of the maximum productivity, 2×10^9 ha is required for the food farming system ("Agriculture I" in Fig. 7). This subsystem also provides 1.02 TW straw and 0.5 TW manure to the biomass-to-energy conversion subsystem.

The energy farming system ("Agriculture II" in Fig. 7) thus has available 0.8×10^9 ha on which it can grow fuelwood (eucalyptus, poplar etc.), sugarcane, cassava or other promising crops. Allowing for a generous 50 t/ha/year average productivity, 17,7 TW could be produced as a biomass feed stream to the conversion system.

Finally the forestry system has to be considered. At present 3.8×10^9 ha is forest (14) of which 30% have the function to shield other areas from erosion and cannot be harvested (12). Also 0.6×10^9 of waste land might be put back into forests (15). About 2×10^9 ha has however gone to agriculture in our attempt to maximize the high yield energy-farming potential. So we have available for forestry: 1.13×10^9 ha. A reasonable harvest, without destroying the forest, would be about 4 m^3 wood/ha/year (15, 16). An estimated industrial use of wood of 0.145 TW (15) leaves us with 1.36 TW for wood fuel use in addition to the energy farming energy flow.

Human society itself provides wastes, we estimate these at 2.4 TW (three times the present value). In summary, the energy from biomass/waste subsystem of Fig. 7 would receive 23.1 TW. A gigantic flow which infortunately can only be converted with modest efficiencies into crude oil-, or gas-like energy products. Moreover it is reasonable to require that the energy production is debited for the fuel consumption of the two agricultural and forestry system (total 1.72 TW). Taking an average conversion efficiency of 20% we arrive at 4.62 TW energy products of which 1.72 have to be paid back to the biological production system. The net result, which is in our opinion an upper bound, thus is only 3 TW. This value is of course strongly dependent on the magnitude of the world population. Of course the analysis given is a very crude one and could be refined considerably. Clearly other ways to supply solar energy such as listed in Fig. 1 in addition to biomass conversion are required.

The probability that a bioenergy system as given in Fig. 7 will be developed is rather low. Only on a limited scale, in some specific geographical locations (e.g. Brazil,

New Zealand, Australia, Canada?) could we expect a significant contribution. Reasons for this low probability are:

1. The maximum agricultural production is an upper limit that can not be obtained. Even to reach 75% of this maximum is an enormous effort which would require world-wide coordination and is unlikely to occur.
2. At present an increase in food production close to 3% per year is necessary to keep pace with the growth of the world population. In developing countries this rate has been reached with great difficulty in the past because of rate limiting factors as scarcety of human expertise, water supply, fertilizers. Energy farming on scale significant for the world energy supply, would require an impressive extra effort to remove these limiting factors and to avoid interference with food production.
3. Socio-economic factors also work in other directions. It is e.g. hard to see how a farmer could derive an attractive income out of energy farming at current and near-future energy prices. Also the interests of oil producing countries (who will not welcome competition with oil) and the poor developing countries (whose priority is to improve food production and use only a few percent of the world energy consumption anyway) are not in favor of large scale biomass-to-energy systems. Energy development programs, however, should get a larger emphasis, because availability of energy is an important stimulus for rural development. Small scale generation of energy from biomass in efficient but relatively simple equipment is important.
4. There exists no infrastructure for bioenergy systems of other than local importance. The development of such an infrastructure and energy industry on a larger scale (EEC, worldwide) will have to cope with the drawback of biomass that it is bulky, it has an unfavorable C/H/O ratio fur use as fuel, and that it often contains moisture. Also the conversion processes often have unfavorable energy input/output ratios. We will come back to this point in the next section.
5. Development of biomass energy systems will have to compete for investment capital with other choices in the coming years like coal and nuclear energy.

3. Options to use biomass as an energy resource

As we said already in the previous section agricultural wastes like animal droppings and straw are possible recent-photosynthesis based raw materials for industrial purposes. In addition to these agricultural wastes municipal solid wastes, municipal and food industrial waste waters are of course potential sources of raw materials formed by recent-photosynthesis. In addition to the waste products one could also deliberately produce biomass by agriculture forestry or mariculture and use this biomass as a raw material.

Of course there are many potential technologies to produce energy products, petrochemical substitutes and special chemicals on the basis of plant production with directly coupled chemical, biochemical or biological conversion processes. We can distinguish between the following groups of options:

1. Production of energy products (synthetic gas, synthetic crude oil, methanol, ethanol);
2. Production of base chemicals to be used as building blocks in chemical process industry (ethylene, methanol, ethanol, butanol, citric-acid);
3. Direct use of plant products as raw materials in the chemical process industries (saccharose, cellulose, starch);
4. Extraction of special products synthesised by the plants, for special industrial, pharmaceutical etc. uses (vegetable oils, fats, gums, alkaloids, pyrethrins, resins, latexes etc. etc.).

In scanning this list we see some very old familiar technologies.
All the methods just mentioned have the advantage that the energy sources are renewable and that atmospheric carbondioxide is recycled in a short loop. They do however require, except for the wastes and mariculture, an increased agricultural area, and in some way they interact with the biologically bound carbon cycle and therefore with food production.

An other approcah is to design photochemical systems for energy conversion or catalysts that are based on the mechanisms of photosynthesis and e. g. biological nitrogen fixation.

The advantages, and disadvantages, of biomass and agricultural wastes as feedstocks for energy production are listed in Fig. 8 (16). In many cases the raw material has a negative value because it gives rise to a pollution problem. Because the wastes usually are distributed sources and are bulky materials, storage-, collection- and transportation costs have considerable impact on economic viability of technologies in which they are used as raw materials. Solutions for e. g. feed lot wastes tend to be small units, for processing wastes "on the farm" (17, 18) or for regions with a radius smaller than 10 km (19). Because the wastes have poor consumer acceptance as such (e. g. straw as a fuel) the wastes have to be converted into acceptable energy products with inherent cost and losses in energy content. The raw materials often have a low energy content compared with fossil fuels because the molecules contain oxygen, and because of the high water content. In many cases the availability of the wastes for conversion into energy products is also dependent on alternative uses, as exemplified for straw in the U.K. in Fig. 9 (16). Conversion technologies for wastes can be classified in: direct combustion in incinerator systems, anaerobic digestion processes, pyrolysis (destructive distillation) processes, gasification processes, chemical hydrolysis or biochemical hydrolysis processes. In Fig. 10 a schematic of the alternatives is given, see also (20, 21).

Direct combustion heat recuperation for utilities is not particularly new and on the short term is the most promising approach to recovery of value from solid municipal/agricultural wastes. When using municipal wastes a preparation of the waste in a "frond-end system" is required to separate metals, glass etc. (22). Systems are envisaged in which the wastes are used as a supplemental fuel in regular or modified boilers along with fossil fuel to produce steam for heating purpose, or even burned as supplementary fuel in utility grade boilers for the production of steam to drive turbine generators (23). These processes are at present only feasible in high disposal cost communities.

Anaerobic digestion of agricultural/urban wastes (17-19, 24-28) aims at producing methane gas (CH_4) as an energy product. The anaerobic digestion may be described as a three step process involving (i) hydrolysis of the complex material, (ii) breakdown to simple compounds as the volatile fatty acids, alcohols, carbondioxide, hydrogen, H_2S and NH_3, (iii) methane fermentation to CH_4 and CO_2. The methane fermentation step is rate limiting. Methanogenic bacteria are strict anaerobes and mesophylic (27-30°C). The net or overall chemistry involved may be represented by the generalized reaction given in Fig. 11. We see that when e. g. cellulose is used as raw material to be digested the gas contains 0.50 mole fraction CO_2 and 0.50 mole fraction CH_4. The heating value of this gas is about 23 MJ/m^3. A CO_2-absorption process would be required to reach pipeline natural gas quality of 35 MJ/m^3. A production of 50-60 m^3 gas per ton of fresh manure can be produced by this process. When straw is used as raw material, about 200-500 nm^3 per ton of straw is obtained. From potatostarch industry a production of 1.5-6 m^3 per ton of waste water can be obtained. Nitrogen and sulphur compounds present in the original material are reduced to NH_3 and H_2S in the process and it could be necessary to remove these. The process has also potentials in industrial waste water treatment of the Dutch beet-sugar industry (29, 30), the potato-starch industry (31, 32), and wheat-starch industry (33).

Pyrolysis processes are based on the thermal decomposition of carbonaceous materials at 350-500°C by indirect heat transfer with no injection of oxygen, steam or carbonmonoxide into the reactor, Fig. 12. The decomposition products are tars and oils, some aromatics, char, low molecular weight organic liquids, methane, hydrogen, water, carbonmonoxide, carbondioxide, NH_3, H_2S, COS and HCN, dependent on the composition of the feedstock. Modifications of the basis concept are numerous. The Garret/Occidental Research Corporation process (23, p. 4-13) produces a highly oxygenated fuel oil with low heating value by low-temperature flash pyrolysis. The heat required for pyrolysis is derived from the combustion of the char and off-gas from the pyrolysis reactor in a specially designed heat exchanger part of the reactor. A variation on the pyrolysis process that has received some attention uses Na_2CO_3 as catalyst.

Gasification processes produce fuel gases by reaction of a carbonaceous material with oxygen, air, or oxygen and steam. The heat required for the chemical reactions is generated within the reactor by partial combustion of carbon. The main reactions in gasification are given in Fig. 13. In gasification of forestry and agricultural products the water content of the products is much higher than strictly required for the gasification reactions and often predrying will be necessary in order to avoid a waste water problem from the gasification reactors. The heating value of the gases produced is low, 4-6 MJ/m^3, when air is used and can be increased to 9-15 MJ/m^3 if oxygen would be used. Further increase in heating value to substitute natural gas (SNF) levels of 30-35 MJ/m^3 requires shift conversion to adjust H_2/CO ratio to about 1/3, followed by methanation. Methanation might also be realised in a microbiological fermentation of gasification products (34). Sulphur and nitrogen compounds in the feed lead to NH_3, H_2S and HCN which should be removed. In the U. S. A. there are numerous reactor systems under development, and several of these are specificly designed for forestry- and agricultural wastes (20).

In the Netherlands Van Swaaij and coworkers (35) have developed co-current moving bed gasifiers suitable for small scale production of tar-free gas from agricultural wastes. The material moves through a drying zone, a pyrolysis zone, an oxydation zone (1200°C) and finally a reduction zone where the CO_2 and H_2O formed in the oxydation zone are reduced. This producer gas has been tested out in spark ignition- and diesel engines (36).

Methanol or ethanol production.

The processes discussed so far yield either a low BTU-gas, a substitute natural gas, a biogas or synthetic fuel oils. Other candidate energy products are the lower alcohols methanol and ethanol, which can be belended with gasoline or used as a fuel as such (23, p. 2.120-2.128; 4.44-4.49). Manufacture of methanol from forestry products or agricultural products, if at all, would most likely go via a synthesis gas ($H_2/CO/CO_2$) produced by gasification (37), see Fig. 14. The major part of methanol produced today comes from manufacturing plants where synthesis gas is produced by steam reforming of hydrocarbons (natural gas, light naphta's). The synthesis gas is converted into methanol via a high pressure or medium pressure catalytic hydrogenation of the carbonmonoxide in the synthesis gas (38, p. 705). Microbial production of methanol seems hardly feasible (39). Ethanol is synthesised in the chemical industry by direct hydration of ethylene. The industrial market of ethanol is mainly covered by this process. Another process route is by fermentation of biomass sugar, grains, corn, cellulose) to an alcohol solution and carbondioxide, distilling off a near azeotropic mixture of alcohol-water, followed by an azeotropic distillation with benzene to produce pure alcohol, Fig. 15. First the biomass has to be converted into saccharose or maltose that can be fermented by the yeast. This requires enzymatic or chemical hydrolysis which is feasible for starchy crops but not particularly for cellulose as a raw material (38, p. 530; 551).

There is a lively discussion in the U. S. A. about the pro's and cons of using methanol or ethanol as gasoline extenders ("gasohol"). Facts are that the production of ethanol from grains, straw or hydrolysed timber actually uses more fossil fuel energy than it

produces as a fuel. Some figures are given in Table X which was compiled from the recent literature (40-54). Sugar cane, with bagasse fuel, and cassave seem able to give a positive energy balance especially when grown in Brasil. The energy requirements of the fermentation process are expended for the major part in the distillation steps. Energy cost of ethanol from ethylene are estimated at 34.5 GJ/t (55, p. 53).

A comparison of energy analyses from different sources is usually rather difficult because of the different base cases to which is referred. The figures in the tabel refer to a situation where all the energy inputs except solar energy into agriculture, transport and processing required for the production of 1 ton ethanol are added. All net energies are negative.

The energy balances shift significantly from the base values of Table X dependent on assumptions about (46, 59):

1. Specification of the system boundary: are crop residues and byproducts of the conversion valid energy outputs?
2. Comparison of different energy types: should the balance be calculated for total non renewable energy or only for one type e. g. fuel oil.
3. Consideration of the end use of ethanol: does ethanol mixed with gasoline give better mileage than one would expect on the basis of its heating value.
4. How are byproducts credited to the process: how is the energy content of by-products calculated and is the result credited to ethanol or not.
5. Should feed byproduct energy be added to the output or subtracted from the input.

Fig. 16 is a schematic biomass-to-fuel conversion system to produce an energy product with energy content D (GJ/ton energy product). The agricultural system produces biomass with energy content B (GJ/ton energy product), which is harvested with a yield η_h. The harvested product is processed to the energy product (G) with a yield η_p. The agricultural system requires energy inputs A (GJ/ton energy product) which are proportional to biomass production, the yield is η_a. In the processing energy inputs P_1, P_2 and P_3 are required. P_3 is an energy input that can be directly derived from the biomass input to the process, e. g. bagasse in sugar cane processing. The first column of Table X gives $(A + P_1 + P_2 + P_3)$ expressed per ton of energy product G.
The second column is $(G - A - P_1 - P_2 - P_3)$. Table XI gives a breakdown into the various energy inputs and the byproducts from process and from agriculture. For corn and wheat the byproduct is mainly distillers dry grains. For the other crops stillage effluent is the main byproduct. We assume that, in order to prevent heavy pollution, the stillage is concentrated and burned for steam generation (57). This process delivers steam so P_2 is negative and varies from -1 to -6 GJ/ton ethanol according to Kujala (57).

Depending on how the energy requirements of the biomass-to-ethanol system are realised the net energy requirements of the various crops change. Sugar cane is the only crop that generates a stream P3 (bagasse or ampas), which is always used for steam/power generation in the process itself. Therefore sugarcane has really a positive energy balance: the net energy produced is $\sim$ 12 GJ/ton ethanol produced. When cobs, stalks and straw are used as energy sources in the processing of corn, wheat and cassava the net energy values improve and cassava also gets a positive energy balance with a net energy production of $\sim$ 5 GJ/ton ethanol. The absolute limit of optimism is obtained when it is assumed that P_1 and P_2 are supplied by some "free" fuel (wood is often cited to be such a fuel), in this case all the balances are positive. In the Brazilian studies on cassava wood is assumed to be the source of fuel and also agricultural inputs are low and therefore favourable net energy values result.

Another important point is that in most studies the ethanol end use is to mix it with gasoline to produce automobile fuel. When we take a 10% ethanol/gasoline mixture ("gasohol") the decision on whether net energy is gained depends on the mileage of this mixture relative to straight gasoline. The energy cost, C, of gasoline is 52.7 GJ/ton (49). The net energy per ton ethanol produced then is (49):

$$E_{net} = 10.C.(m) - (9C + P_1 + P_2 + A - (1-\eta_h) B) \quad (9)$$

This relation is plotted in Fig. 17 in the way proposed by Chambers et al (49). The mileage multiplier is equal to 0.96 when the heating values of ethanol and gasoline are used as a measure of the possible. Scheller (45) has used a value of m = 1.06 which was based on a road test in Nebraska. This test is however not considered to have been representative. An ongoing study at University of North Dakota in both winter and summer service indicated a 3% loss in mileage (58), so m = 0.97 in that case.

Also it is possible to consider the biomass-to-ethanol system of Fig. 16 as a converter of low quality fossil fuel (e.g. high sulphur coal) into a high grade motor fuel. In such a philosophy the energy requirements P_1 and P_2 could be supplied by this source of low quality fuel. On this basis it was estimated (54) that 38 GJ coal energy is required per ton ethanol. Direct production of methanol from such coal is of course also possible and would require about 32 GJ coal energy per ton of methanol (heating value 20 GJ/ton) in a more simple process which does not need the link between "agriculture" and "energy industry". This route seems more logical for Western Europe.

Finally, it is interesting to compare the prices of the energy products from biomass we have discussed on a basis of $/GJ with the fossil fuel based energy products including coal. This is done in the Table XII.

The data for fossil fuel products are based on references (59-62); and calculations for straw, corn and wood are based on references (16, 23, 22, 62-64). Conclusions are that none of the energy products can compete with fossil fuel derived energy products at present. It also seems that fuel products derived from coal will be cheaper for the time being than products derived from biomass, perhaps mainly because of the high costs of collection and transport of biomass. Also the requirements of agricultural land for meaningful contributions to our energy supply are high.

4. Photosynthesis at sea or in vitro

We have seen above that the use of agricultural wastes for energy production is technologically feasible, but if we make a rough calculation gives about 2 GW to be derived from all the municipal wastes, animal wastes and straw in this country. Our consumption of 86 GW thus can not be met. On the global scale we saw a similar picture. We generate a waste stream of 1.3 TW and we use 9.5 TW. If we calculate on the basis of solar energy flux of 200 W/m^2 and a photosynthetic efficiency of 0.7% the area required to generate 11.4 TW biomass by photosynthesis (20% for production, fertilizing, harvesting, transport etc.), we find 8.2×10^6 km^2. At the end of the century, when world consumption is estimated at 25 TW, this area will be 21×10^6 km^2. The agricultural areas on earth add up to 12×10^6 km^2, with an additional 22×10^6 km^2 of pastures. Energy cropping apparently interferes significantly with present land-use.

This and similar reasonings lead to attempts to capture solar radiation at higher efficiencies than photosynthesis does at present and preferably in a storable form. Mariculture of kelp (Macrocystis) and study of algal-bacterial systems are examples of projects (66-70) aiming at a relief of land usage. A different approach is to produce hydrogen by in vitro photosynthesis. In such schemes the photosynthesis "light" reactions would not be followed by the "dark" reaction but by a hydrogenase induced regeneration ferredoxin (FD). The net reaction is the splitting of water into hydrogen gas and oxygen. Sodiumdithionite or pyruvate have been applied as the reducing agents in aqueous enviornments (71). Hydrogenase can be prepared from

specific anaerobic bacteria and be added to a suspension of chloroplasts. The production of hydrogen is limited because reduced ferredoxin (FD) is oxydized and the enzyme hydrogenase inactivated by the oxygen formed. If formidable barriers like: increasing stability of the photosynthetic system; avoiding reoxydation of ferredoxins; development of sufficiently active, oxygen insentitive hydrogenase; large scale preparation of the enzymes etc. would have been tackled, Vind (65) states that photosynthetic efficiencies of about 10% would be possible.

5. Conclusion

Conclusions from the survey are that technology offers many options for the use of agricultural products as energy resource. Waste materials are the most likely raw materials for energy production but will only give a modest contribution. In the Netherlands less than 2%, on a global scale less than 6%.

Ultimate potentials of energy from biomass on a global scale are strongly dependent on the magnitude of the world population. For a world population of 12 billion people at most 3 TW can be derived, for a population of 6 billion 7.7 TW is an absolute upper bound.

Bioenergy can play an important role in the rural communities, especially in developing countries where a shortage of fuel (wood) often exists.

Energy farming for motor fuels in a significant scale for the world energy supply is not likely in the future, but local conditions e.g. in countries with a low population density and no fossil fuel resources are different. Only a few crops can give positive energy balances for ethanol production, even when no drop in mileage by using ethanol/gasoline mixtures are used. Sugar cane and cassava are potential candidates.

Biological solar energy conversion not competitive with food production would require high efficiency "photosynthesis" in vitro or mariculture. These are long term options with low probability of success.

Acknowledgements

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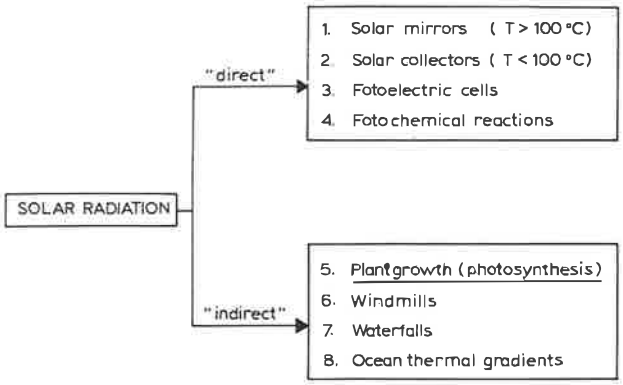


Fig. 1. Overview of ways in which solar energy can be applied.

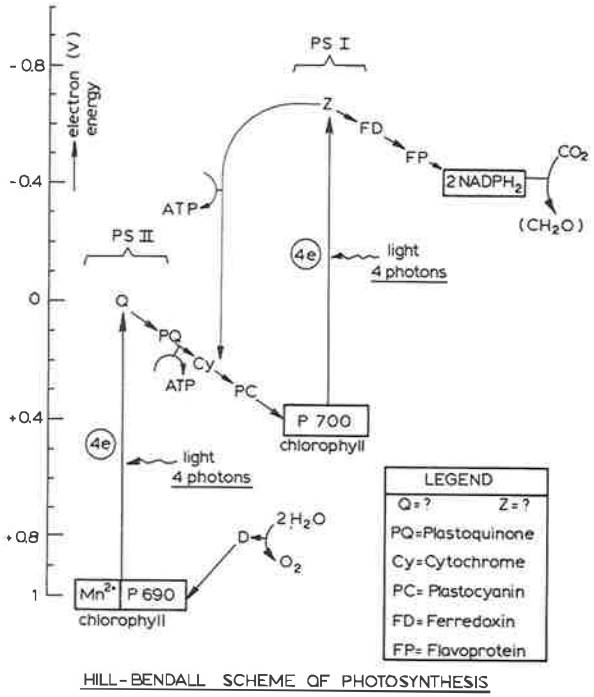


Fig. 2. Hill-Bendall scheme of photosynthesis.

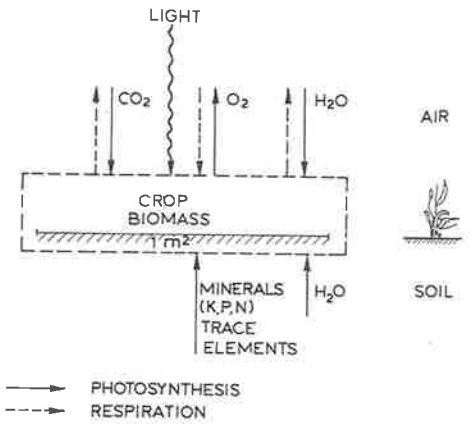


Fig. 3a. Schematic of local surface reaction kinetics for photosynthesis process.

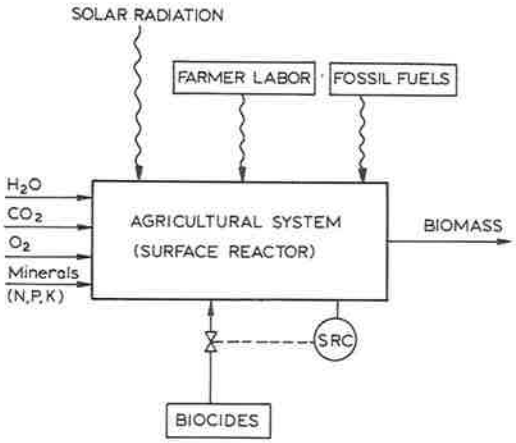


Fig. 3b. Agriculture as a chemical surface reactor system.

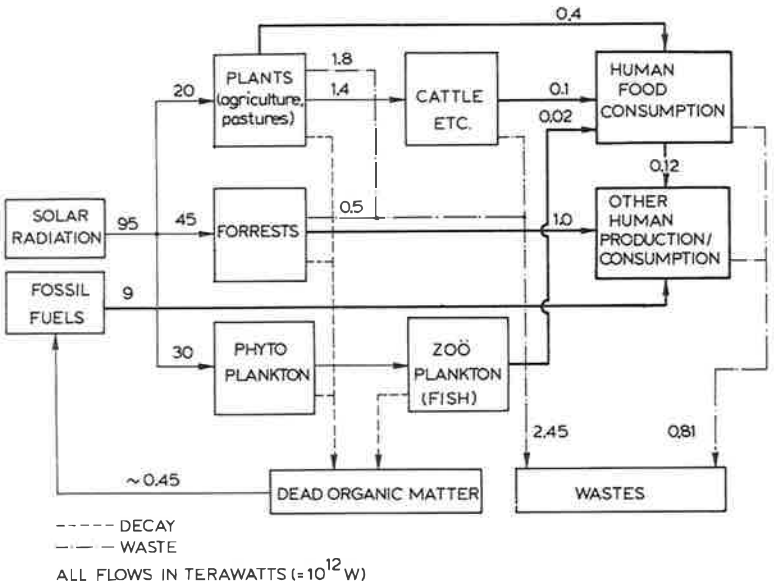


Fig. 4. Overview of present global energy relations.

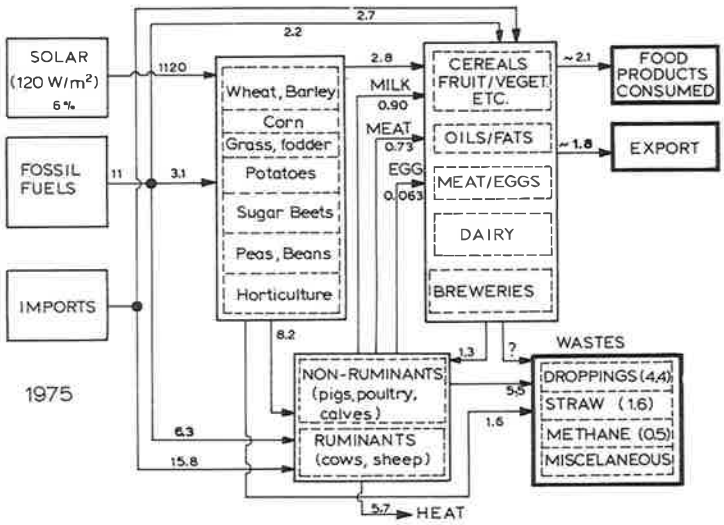


Fig. 5. Energy relations of the agriculture/food industry system in the Netherlands.

Fig. 6. Maximum hypothetical agricultural regions suitable for industry-potato culture (not feasible).

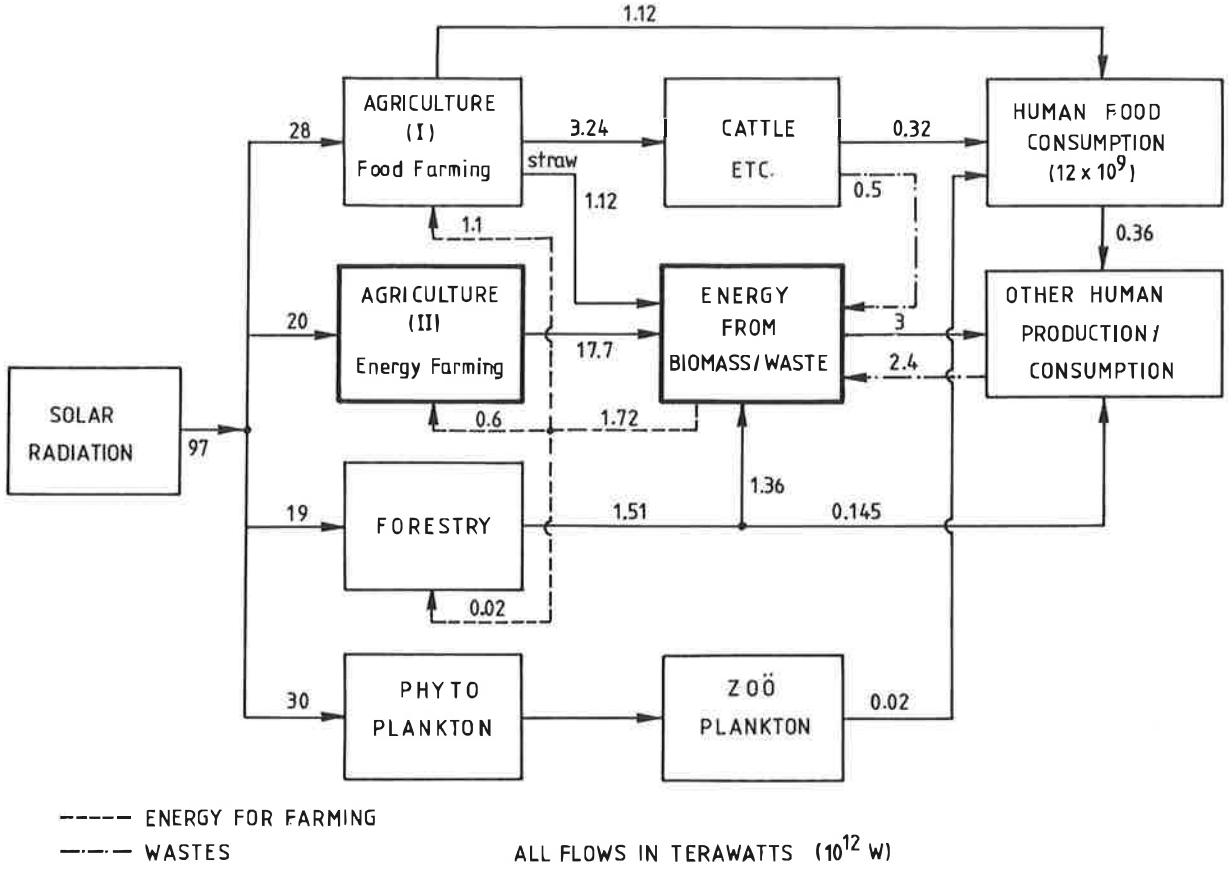


Fig. 7. Overview of hypothetical global energy relations for a world population of 12×10^9 people using maximum agricultural potential.

ADVANTAGES and DISADVANTAGES of AGRICULTURAL WASTES as ENERGY FEEDSTOCK.

1. ADVANTAGES :
   - 1.1 Renewable raw material
   - 1.2 Low cost of raw material before collection (if no alternative use exist)

2. DISADVANTAGES :
   - 2.1 Bulky raw material creates storage/transportation cost
   - 2.2 Poor consumer acceptance unaltered wastes
   - 2.3 Consequent need for conversion technology: costly, loss energy content
   - 2.4 Low energy content/kg compared with fossil fuels
   - 2.5 Availability subject to farmers judgement/competing industrial users
   - 2.6 Depletion soil humus level/N/minerals ?

Fig. 8. Advantages and disadvantages of agricultural wastes as energy feedstocks.

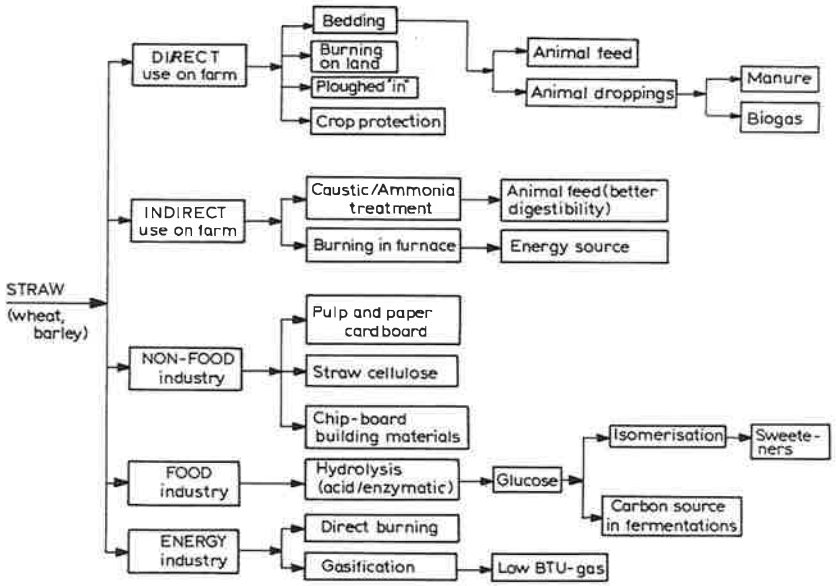


Fig. 9. Alternative uses for the straw in the U.K. (12).

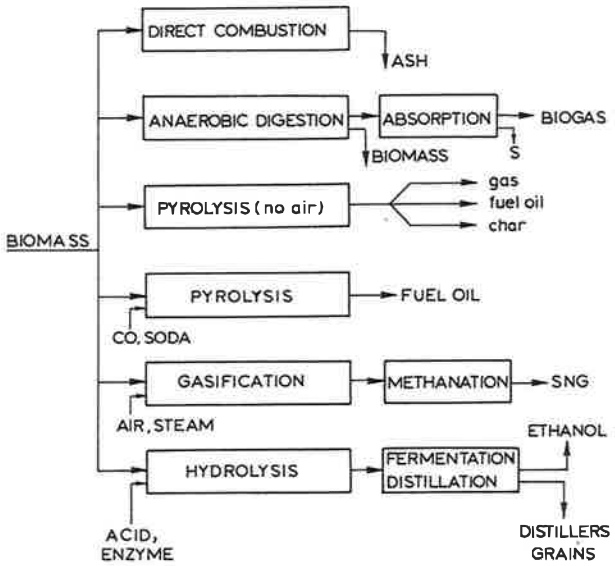


Fig. 10. Alternative processes for the utilization of biomass as energy resource.

ANAEROBIC DIGESTION:

$$C_n H_a O_b + \frac{4n-a-2b}{4} H_2O \longrightarrow \frac{4n-a+2b}{8} CO_2 + \frac{4n+a-2b}{8} CH_4$$

carbon source — carbondioxide — methane

e.g. Cellulose:

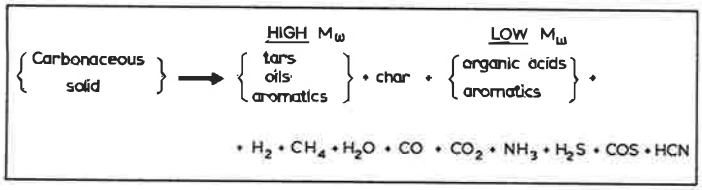
$$C_6 H_{10} O_5 + H_2O \longrightarrow 3\,CO_2 + 3\,CH_4$$

Mono saccharide:

$$C_6 H_{12} O_6 \longrightarrow 3\,CO_2 + 3\,CH_4$$

Fig. 11. Generalized reaction scheme for anaerobic digestion of wastes.

PYROLYSIS:



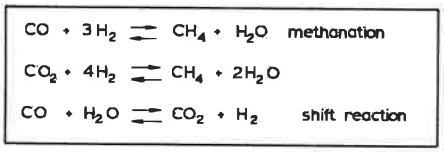


Fig. 12. Reactions occurring in pyrolysis.

GASIFICATION:

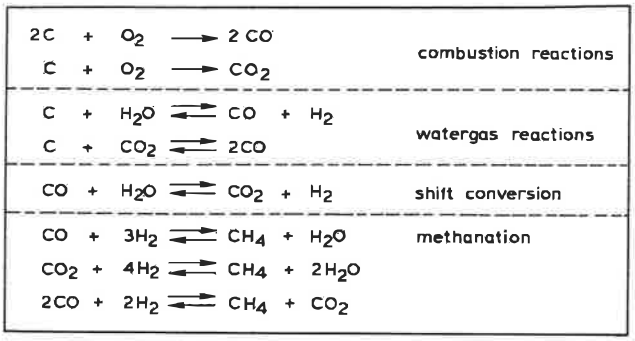


Fig. 13. Gasification reactions.

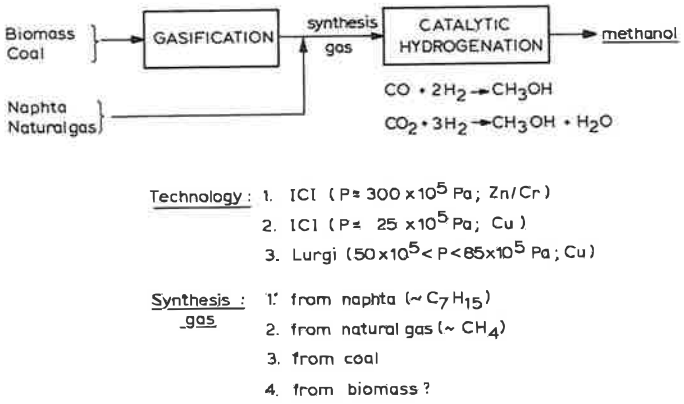


Fig. 14. Production of methanol from naphta, natural gas, coal or biomass.

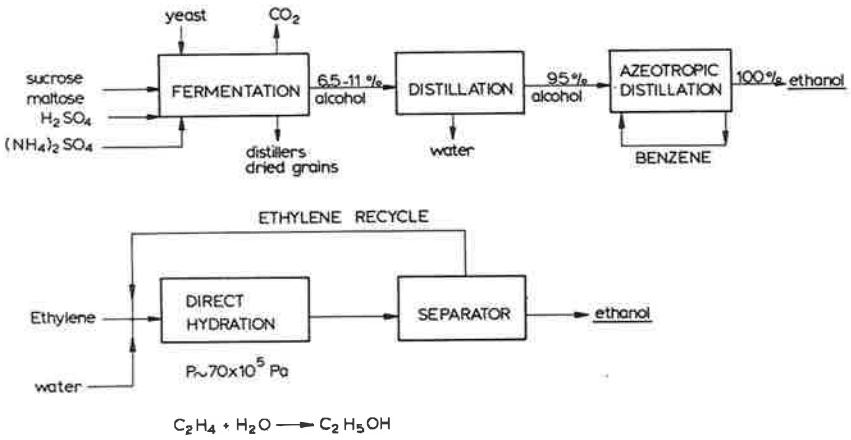


Fig. 15. Production of ethanol from sugar containing raw materials and from ethylene.

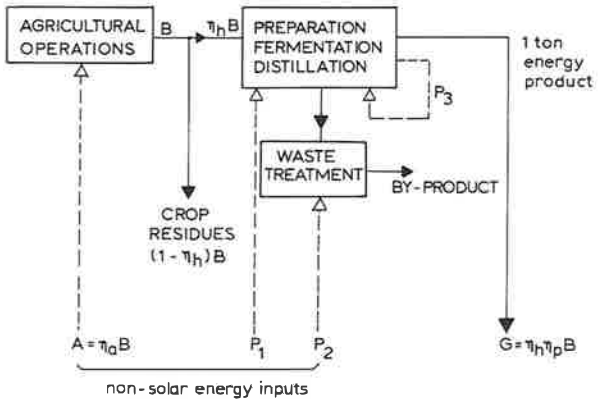


Fig. 16. Definition sketch of an agriculture based energy production process.

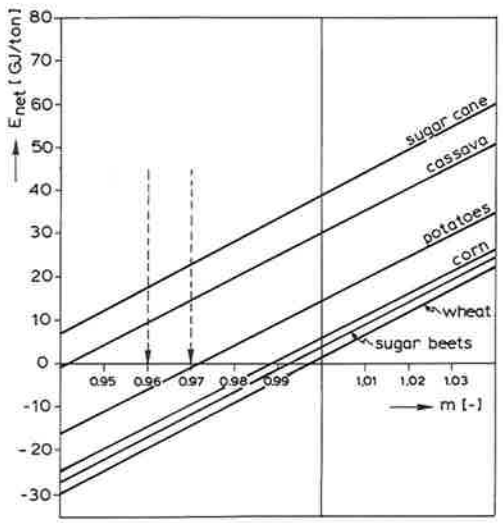


Fig. 17. Net energy production, E_{net}, per ton of ethanol produced for several crops plotted against the mileage factor, m.

WORLD ENERGY RESOURCES (non renewable)
EXPRESSED IN YEARS OF 1975 LEVEL CONSUMPTION.

SOURCE	YEARS
COAL *)	90 (1350)
OIL	45
GAS	45
URANIUM - LWR	11
URANIUM - FBR	1040
FUSION	$\simeq 8 \times 10^9$

*) PROVEN: 90; PROVEN + EXPECTED: 1350

Table I. World energy resources (non renewable) expressed in years of 1975 consumption level.

ENERGY CONSUMPTION RATES PER CAPITA.
(1976 UN Statistical Handbook)

COUNTRY	ENERGY CONSUMPTION PER CAPITA (W/cap.)	
	TOTAL	ELECTRICITY
USSR	5200	1266
USA	10000	3100
Japan	3400	1320
BRD	4900	1510
France	3700	1100
Netherlands	6200	1900
China	520	45
India	210	44
Brazil	620	225
Nigeria	80	16
Bangladesh	30	5
Indonesia	170	8
WORLD	2250	

Table II. Energy consumption rates per capita in some countries.

SOURCE	GLOBAL (TW)	RECOVERABLE FLOW (TW)
Solar radiation	10^5	50 - 100
Wind	350	15
Waves	3	0.1 - 1.0
Ocean thermal gradients	150	<10
Tidal movements	2.7	0.06
Geothermal	3.0	0.1

Reference: "World Energy Resources" 1985 - 2020,
Latzko, "Energievoorziening in Nederland"

Table III. Order of magnitude of non-conventional energy resources and recoverable fractions.

MEASURED MAXIMUM PHOTOSYNTHESIS EFFICIENCIES.

Plant	Efficiency (%)
1. C_4 - Plants	
Sugarcane	2.4
Napier grass	2.4
Sorghum	3.2
Corn	3.2
2. C_3 - Plants	
Sugar beet	1.9
Alfalfa	1.4
Chlorella	1.7
Wheat	1.7

Table IV. Measured maximum photosynthesis efficiencies for C_4 and C_3 plants during growth period.

SYSTEM	NET PRODUCTION LEVEL (T/HA/YEAR)
NATURAL SYSTEMS	
• Spruce forest, Germany	15
• Grassland, New Zealand	30
• Tropical forest, West Indies	~60
• Algae on coralreef Marshall Islands	50
• Seaweed, Novo Scotia	20-25

Table V. Some productivities of natural ecosystems.

SYSTEM	NET PRODUCTION LEVEL (T/HA/YEAR)
AGRICULTURAL SYSTEMS	
• Sugar beets, Netherlands	7 - 10
• Potatoes Netherlands	8 - 12
• Wheat, Netherlands	
straw	3.7 - 4.2
grains	4.3 - 6.2
• Corn (maize), USA	5.6 - 14
• Corn silage, USA	~27
• Sugar cane, Java	70 - 94
• Cassava	14 - 50
• Oil palm plantation Malaysia	6 - 8.3
• Soybeans	2.5 - 4.5

Table VI. Some productivities of agricultural crops.

CROP	K_m (kg N/ha)^2	$r_{B,max}$ (t/ha/year)
. Summerwheat (15% moist. grains)	495.	7.6
. Winterwheat (15% moist. grains)	440.	7.9
. Potatoes (24% dry solids)	81.3	80
. Sugar Beets (sugar)	371	11

Rate Equation :

$$r_B = r_{B,max} \frac{[S]^2}{K_m + [S]^2} \quad \text{(t/ha/year)}$$

$[S]$ = Nitrogen uptake (kgN/ha)

Table VII. Rate equation constants for some crops in Dutch agriculture.

MAXIMUM AGRICUTURAL PRODUCTION POTENTIALS.

CONTINENT	POTENTIAL AGRICULTURAL LAND [10^6 ha]	NON IRRIGATED MAX. PROD. DRY MATTER [10^6 ton/year]	IRRIGATED MAX. PROD. DRY MATTER [10^6 ton/year]
ASIA	890	25000	33000
SOUTH AMERICA	590	25200	25700
AFRICA	710	24100	25100
NORTH AMERICA	630	15400	16300
EUROPE	400	8300	9600
AUSTRALIA	200	5300	5400
TOTALS	3420	103300	115100

Source: Buringh, Van Heemst, Staring [ ]
Buringh [ ]

Tabel VIII. Maximum agricultural production potentials according to Buringh et al in the world.

LIMITS OF AGRICULTURAL PRODUCTION POTENTIALS (NETHERLANDS)

CROP	LIMIT OF PRODUCTION (10^6 t/year)	TOTAL AREA (10^6 HA)	PERCENTAGE TOTAL AGR. AREA
• GRAINS (starch)	8.25	1.46	69
• POTATOES (starch)	24.5	1.34	64
• SUGAR BEET (sugar)	12.3	1.26	60

Table IX. Maximum agricultural production potentials in the Netherlands (not feasible)

ENERGY BALANCES FOR SOME CROPS.

RAW MATERIAL	ENERGY REQUIREMENT (GJ/t)	NET ENERGY *) (GJ/t)
Corn (USA)	70	- 43
Wheat (USA)	71	- 44
Straw	~140	~ - 113
Sugar beets	48	- 21
Potatoes	38	- 11
Wood (acid)	98	- 71
Wood (enzymatic)	240	- 213
Sugar cane	40→47	-13→-20
Cassava (Brasil)	33	-6
Cassava (Surinam)	35	-8

*) Heating value of 27 GJ/t ethanol

Table X. Energy balances for production of ethanol for some crops.

ENERGY INPUTS PER TON ETHANOL PRODUCED.

CROP	$(1-\eta_h)B$ (GJ/t)	P_1 (GJ/t)	P_2 (GJ/t)	P_3 (GJ/t)	A (GJ/t)
• Corn	23	29	23	0	17 – 19
• Wheat	20	30	23	0	18 - 20
• Potatoes	0	28	-1 → -6	0	11
• Cassava	11.4	29	-1 → -6	0	5.3
• Sugar beets	0	16	-1 → -6	0	3.3
• Sugar cane	0	9.5	-1 → -6	32	5.5 - 6

Table XI. Breakdown into various energy inputs and by product energy contents for ethanol production from some crops.

MATERIAL	PRICE OF SOURCE ($/ton)	ENERGY COST ($/GJ)
1. CRUDE OIL		
- naphta	~110	~3.0
- gasoil		~2.4
- fuel oil		~2.0
2. NATURAL GAS	100	~2.4
3. COAL		
- as is	40 - 50	1.5 - 1.8
- Fischer/Tropsch		9 - 10
- fuel oil		4.2 - 4.6
- methanol		3 - 8
- low BTU-gas		3 - 3.5
- SNG (Lurgi)		5.5 - 6
- gasoline		8 - 9

Tabel XII. Estimated energy costs for fuels from fossil energy resources.

MATERIAL	PRICE OF SOURCE ($/ton)	ENERGY COST ($/GJ)
1. MUNICPAL TRASH		
- methanol	+ 5	9 - 10
	- 5	4.3
2. STRAW		
- as is	50 - 65	3.2 - 4.5
- SNG	50	~6
5. CORN		
- ethanol	20	15 - 55
6. WOOD		
- as is	25 - 35	1.3 - 2.0
- low BTU-gas	25 - 30	5 - 10
- methane	20	6
- methanol		15
- ethanol		15 - 35

Tabel XIII. Estimated energy costs for fuels from biomass and wastes.

APPENDIX A:

Sensitivity Analysis of the Bio-Energy Model System

In this appendix the Bio-energy model of Fig. 7 will be explained and a sensitivity analysis for some of the parameters done. We will presume that a steady state exists, mainly controlled by the stable population level, P.

1. Areas required for various activities.

The food supply consists of two components: the grain supply of 0.481 kg grains/capita/day and a meat/milk supply of 0.75 kg milk/capita/day and a 0.08 kg meat/capita/day. The agricultural areas for grain production, A_{fg}, milk production, A_{fm}, and meat production, A_{fv}, are related to yields by:

$$A_{fg} = P \times \varphi_g / Y_g \tag{A.1}$$

$$A_{fm} = P \times (\varphi_m . \alpha_m) / Y_m \tag{A.2}$$

$$A_{fv} = P \times (\varphi_v . \alpha_v) / Y_v \tag{A.3}$$

where φ_g, φ_m and φ_v are grains, milk and meat consumption rates per capita, Y_g, Y_m and Y_v the productivities of agriculture for grains (t grains/ha/year) and milk and meat (t forage/ha/year) respectively. The factors α_m and α_v are conversion factors for milk and meat production systems. Alberda (13) gives α_v = 11 kg dry herbage/kg meat and α_m = 1.25 kg herbage/kg milk. The total agricultural land required for food production thus is:

$$A_{ft} = P \left(\frac{\varphi_g}{Y_g} + \alpha_m \frac{\varphi_m}{Y_m} + \alpha_v \frac{\varphi_v}{Y_v} \right) \tag{A.4}$$

The area needed to live (houses, roads, schools, etc.) is estimated at 0.1 ha/capita (12). At present this living area is not counted as agricultural land. When we assume that a fraction f_a of the population growth will settle on potential agricultural land the requirement of land to live, A_l is:

$$A_l = (P - P_{ref}) \times 0.1 \times f_a \tag{A.5}$$

The area available for non-food agricultural purposes, A_{nf}, then becomes:

$$A_{nf} = A_{max} - A_{ft} - A_l \tag{A.6}$$

Part of this area is in use for cash crops, A_c, such as coffee, tea, cocoa, cotton. This area is estimated at $A_{c,ref} = 500 \times 10^6$ ha (12) at present. We assume that the increasing world population still will need these products either as an agricultural income, or to enjoy it or both. We further take it that the area required is roughly:

$$(P > P_{ref}) : A_c = A_{c,ref} + (P - P_{ref}) \times 62.5 \times 10^{-3} \tag{A.7}$$

Essentially a 50% more efficient agricultural production in future agricultural practices in this activity is assumed.

The remaining agricultural land is free for energy farming; A_e equals:

$$A_e = A_{max} - A_{ft} - A_l - A_c = A_{nf} - A_c \quad (A.8)$$

The forestry system also can supply bio-energy. The area of forests that can be harvested is two third of the present area of 3.8×10^9 ha, the remainder has to be kept in its present state (shielding). Another 2.00×10^9 ha of forest has been converted to agricultural land in our efforts to maximize agricultural land. Also we assume that 0.6×10^9 ha of waste lands have been turned back into forests (15). So a total of 1.13×10^9 ha is available. We take a maximum wood production of 4 m^3/ha/year of these forests because this is a reasonable rate at which new biomass is formed and can be logged without permanent damage to the forests (15). This forest system has to supply 0.0363 m^3/capita/year wood for industrial purposes. This figure is obtained by extrapolation of present-day data (15). The forestry area, A_b, is taken as:

$$A_b = \frac{2}{3} \times 3.8 \times 10^9 - (A_{max} - 1.4 \times 10^9) + 0.6 \times 10^9 \quad (A.9)$$

2. Energy flows to the biomass/waste conversion system

The total energy consumption consists of food energy, E_f, and non-food energy, E_{nf}. When the world population is P individuals:

$$A_f = P \times e_f \quad (A.10)$$

$$E_{nf} = P \times e_{nf} \quad (A.11)$$

where e_f is the food consumption rate per capita, taken as 120 W/capita, and e_{nf} is the non-food energy consumption rate, taken as 4000 W/capita in the base case. The food energy flow consists of the grains, milk and meat components:

$$E_f = A_{fg} + E_{fm} + E_{fv} \quad (A.12)$$

where:

$$E_{fg} = A_{fg} \cdot Y_g \cdot H_g \cdot 31.7 \times 10^{-9} \cdot R_A \quad (A.13)$$

$$A_{fm} = A_{fm} \cdot Y_m \cdot H_m \cdot 31.7 \times 10^{-9} \cdot R_A \quad (A.14)$$

$$E_{fv} = A_{fv} \cdot Y_v \cdot H_v \cdot 31.7 \times 10^{-9} \cdot R_A \quad (A.15)$$

R_A is the fraction of productivity of agriculture relative to the base case. H_g, H_m and H_v are the energy contents of grain, milk and meat (in GJ/t). The factor 31.7×10^{-9} converts from J/year to Watts. Values for energy contents are:

$$H_g = 16.8 \quad GJ/t \quad (A.16)$$

$$H_m = 2.26 \quad GJ/t \quad (A.17)$$

$$H_v = 7.96 \quad GJ/t \quad (A.18)$$

The energy production in the energy farming system, E_e, is:

$$E_e = A_e \cdot Y_e \cdot H_e \cdot 31.7 \times 10^{-9} \cdot R_E \quad (A.19)$$

Where R_E is the fraction of productivity of energy farming relative to the base case situation. The agricultural food system supplies two additional waste streams that can be used for energy conversion: straw and animal droppings E_s and E_c respectively.

$$E_s = A_{fg} \cdot Y_g \cdot H_s \cdot 31.7 \times 10^{-9} \cdot R_A \qquad (A.20)$$

and

$$E_c = 0.154\,(E_{fv} + E_{fm}) \qquad (A.21)$$

The forestry system supplies energy for conversion purposes, E_{be}, is:

$$E_{be} = (A_b \cdot Y_b \cdot R_B - 0.0363\ _w \cdot P) \cdot H_b \cdot 31.7 \times 10^{-9} \qquad (A.22)$$

where R_B is the fraction of productivity of forestry relative to the base case situation, and ρ_w the density of wood as used in forestry ($\rho_w \sim 660$ kg/m^3) and H_b the lower heating value of wood: $H_b = 15.9$ GJ/t. Finally human refuse gives an energy contribution to the conversion system, E_w:

$$E_w = 200.P \qquad (A.23)$$

because the waste production is about 200 W/capita. The total gross energy supply to the biomass/waste conversion system, E_{tot}, thus is:

$$E_{tot} = \underbrace{(E_s + E_c + E_w)}_{\text{wastes}} + \underbrace{(E_e + E_{be})}_{\text{energy farming + forestry}} \qquad (A.24)$$

These streams can be converted into energy products that can substitute natural gas, crude oil, coal, electricity, motor fuels. Some typical conversion efficiencies are: 50-60 nm^3 gas/ton manure; 200-250 nm^3 gas/ton straw or wood; 0.07 m^3 ethanol/ton sugar cane; all raw materials delivered at factory gate. Taking an average conversion efficiency η_e we have for the net energy that can be supplied for energy consumption:

$$E_{net} = E_{tot} \cdot \eta_e - E_{mf} - E_{me} - E_{mb} \qquad (A.25)$$

where E_{mf}, E_{me}, E_{mb} are the energy consumptions of the food farming, energy farming and forestry subsystems. The fraction of total energy demand that can be supplied by bioconversion of solar energy, η_b, thus is:

$$\eta_b = \frac{E_{net}}{P \cdot e_{nf}} = \frac{E_{net}}{E_{nf}} \qquad (A.26)$$

3. Definition of the base case

The base case refers to an estimated world population of 12 billion people. We give a list of values given to the parameters in eqns (A.1) - (A.26):

$A_{c,ref} = 0.5 \times 10^9$ (ha)

$A_{max} = 3.4 \times 10^9$ (ha)

A_b	$= 1.13 \times 10^9$		(ha)
P	$= 12 \times 10^9$		(-)
P_{ref}	$= 4 \times 10^9$		(-)
φ_g	= 0.176	grains	(t/cap/year)
φ_m	= 0.274	milk	(t/cap/year)
φ_v	= 0.0292	meat	(t/cap/year)
Y_g	= 13.4	grain	(t/ha/year)
$Y_m = Y_v$	= 13.3	herbage	(t/ha/year)
α_m	= 1.25		(-)
α_v	= 11		(-)
f_a	= 0.75		(-)
e_f	= 120		(W)
e_{nf}	= 4000		(W)
H_g	= 16.8		(GJ/t)
H_m	= 2.26		(GJ/t)
H_v	= 7.96		(GJ/t)
R_A	= 0.75		(-)
R_E	= 0.75		(-)
Y_e	= 66.6		(t/ha/year)
H_e	= 14		(GJ/t)
H_s	= 15.1		(GJ/t)
ρ_w	= 660		(kg/m^3)
Y_b	= 2.64		(t/ha/year)
H_b	= 15.9		(GJ/t)
R_B	= 1.0		(-)
η_e	= 0.20		(-)

4. Sensitivity analysis around the base case

The base case defined in section 3 gives as output of the biomass/waste conversion subsystem an energy flow of 2.81 TW, available for human consumption, which covers 5.8% of the required energy.

The sensitivity of our results for some critical assumptions have been checked. We analysed sensitivity for:

- size of world population (P)
- non-food energy consumption per capita (e_{nf})
- yield of agricultural system (R_A)
- yield of forestry system (R_B)
- yield of energy farming system (R_E)
- available maximum agricultural land (A_{max})

The results are summarized in Fig. A1 in the form of a "spider". On the vertical axis the % of world energy need that is covered by bio-energy is plotted. The horizontal

axis gives % changes (plus or minus) that a variable is given relative to the base case-value of that variable. The curves are representing computed percentage contributions obtained for changes in one variable. Large slopes mean that the model is very sensitive to a variable.

The maximum amount of arable land is a very sensitive data, as is world population. When the maximum agricultural land is less than 2.6×10^9 ha no energy farming with a net output is possible! If mankind were successful in stabilizing the world population at 6 billion people 7.9 TW could be supplied by bio-energy, or about 30% of the energy needed. For the present population of 4 billion 9.6 TW could be supplied, or 2400 W/capita, or 60% of the energy need at 4000 W/capita.

The sensitivity for agricultural yields and forestry yields is also tested. We see that sensitivity to the yields in the energy farming system (R_E) is the highest. We take 50 t/ha/year as the base case, for world-wide average yields. When 25 t/ha/year is taken about 3% of the energy needed can be produced, when 100 t/ha/year is taken 17% would be obtained. Sensitivity to the agricultural productivity (R_A) is also strong. In the base case productivity of agriculture is 10 t/ha/year. When this productivity drops below 6 t/ha (- 40%) no net energy can be gained from bio-energy in the far future.

It is also fascinating to calculate the present theoretically possible bio-energy supply. This means that A_{max} is set at the area now in cultivation: $A_{max} = 1.4 \times 10^9$ (9), and P is the present world population: $P = 4 \times 10^9$. When we take the base case asumptions for productivities, that is agriculture gives 10/t/ha year, energy farming 50 t/ha/year, forestry 2.64 t/ha/year the maximum of 2.76 TW can be squeezed out of the biomass/waste conversion system or 17% of world consumption on 4000 W/capita, but 30% of our actual world energy consumption.

Panel discussion II

Panel:

Prof. Dr. G.J.M. van der Kerk, chairman
Th.M. van Bellegem
Prof. Dr. S. Bruin
Dr. R.E. Cripps
Prof.Dr. K.Kieslich
Dr. K.J.Parker
Dr. P.J. Senior
Dr. J. Windass

(Van der Kerk) We have just three quarters of an hour to answer the questions that have been put. It seems optimistic to try and answer all in so short a time, but let us have a go at it.
The first question I have here is for Drs. Senior and Windass and it reads: "How much extra work has to be done before the authorities will allow larger scale - pilot plants, for instance - experiments or production units where recombinant DNA-organisms are used?". Dr. Senior?

(Senior) Throughout this project and throughout all our projects, be they biotechnology, a new polymer, a new fertilizer or whatever, we keep close contact with Government, regulatory bodies - and we are at the moment in contact with organizations like GMAG and we will be talking to a number of regulatory authorities - about the kinds of checks and balances that are required for putting a product of this kind into the market place. Those checks and balances will naturally consist of toxicological testing in a variety of models, and, no doubt, also testing in target species. There will be some extra work and we will incur the cost of that. Does that answer the question?

(Comment) No, it does not.

(Van der Kerk) Use the microphone, please.

(Comment) It is not just a new strain you are using, it is a strain with new genetic information in it. So the heart of the matter is: Do you think that in this case you will have to do the same amount of work as when you introduce a new strain, or do you expect it will be more? Do you think that the authorities will allow you to do the normal range of toxicological studies that are done for a new strain, or do you expect a far larger and heavier testing programme?

(Senior) We are not at the stage yet where we can say exactly what is going to be required of us. But for our own purposes we want to assure ourselves that this micro-organism is as safe as its parent strain, because we have a responsibility to our work-force and our shareholders, and in addition we don't want to work with something of which we don't know how safe it is. So we will investigate and do the necessary tests, to satisfy ourselves as well as other people.

(Van der Kerk) If I may make a comment, I would be inclined to be much less in fear about the actions of companies than about the actions of certain governments. It seams to be wrong to put the blame of this so-called danger - we really don't know for certain - entirely on industry. Would you like to comment on that?

(Senior) No, I don't think I should comment on the activities of governments.

(Van der Kerk) Then the next question is for Dr. Cripps: "Do you see any scope for the development of improved strains by, for example, genetic engineering for seeding of metal leaching systems?".

(Cripps) Well, yes, I do, I see a great deal of scope. But it depends on what you mean by 'genetic engineering'. For every system that one wants to devise, you have to have the most appropriate organism, particularly with regard to the toxic effects of trace metals you have in your system, and you have to develop a strain that fits the job you are

trying to do. Every mineral is going to be different and that argues that you'll need a different organism for each system. This can probably be achieved by simple mutation selection procedures or adaptation, slowly developing a strain with the required characteristics. It is not only the metals that might be toxic to your organism if, for example, you are using a solvent extraction step to recover your desired metal. These usually involve biochemicals and they too may be toxic to the organism, so you may have to develop a strain resistant in that respect as well.
If you go further and talk about what, I think, most people think of in terms of genetic engineering, there may be a role to play, but I am not sure how far research has gone towards the cotton-stitch techniques with the organisms that will be involved in microbial leaching. But I suspect that in the longer term that too could be important.

(Van der Kerk) Thank you. The next question is for Professor Bruin and it runs: "The production of ethanol from biomass is performed in a very old-fashioned way (Van der Kerk: and usually it tastes good, but that is an aside not in the question).
By biotechnology production may be highly improved: higher yields, less distillation costs and shorter retention times. Has this been taken into account in the calculation of net energy balances?".

(Bruin) No, it has not. The balances that I have given refer to standard technology, and I think it might be very well possible to improve on these balances by renewed attention, using advanced chemical engineering and biotechnological principles. But the main message I tried to give you this morning is that making athanol from biomass does not solve the energy problem. Producing ethanol costs so much energy that the energy gain is rather minimal. So I think that the research effort in yield improvement would be worthwhile only if applications other than fuel would be developed for the ethanol produced.

(Van der Kerk) Thank you. The next question is for Prof. Kieslich. It came in so late that there was no time to write it down and it was given to me verbally. I hope I'll be able to repeat it correctly. It is: "Professor Kieslich has given a very imposing example of how an extremely sophisticated chemical synthesis can be simplified by biotechnological means. He described a synthesis of 26 steps which was reduced to no more than 12 or 13 steps by using biotechnology. Could Prof. Kieslich indicate whether this is general or an exception? If it is an exception, it would have been a little sensational".

(Kieslich) I think it is a bit of an exception, as it is a rather strong example. But in general I think that you can steer the direction of the attack on a given steroid if you have different possibilies for hydroxylation. Then you can change the structure in such a way that the attack of the enzyme goes into the desired direction. And you can make a tailor-made substrate.
If you have the 12-$\beta$and the 15-αhydroxylation you can change the direction of the attack to the one side or the other.
Then you can change the molecule to get an attack upon another position that would not be attacked normally. In addition you have different micro-organisms that will attack the steroid from different sides. So I think the direction of attack on the molecule can be influenced by a number of techniques.
A rather recent technique is to change the position of the polar group, and Prof. Jones in Great Britain has given some examples of that. His results indicate that a change in the position of the polar group may result in a change in the direction of the attack on the molecule. I hope that answers the question.

(Van der Kerk) Is there a comment from the audience? Professor Kieslich, you can rest assured, there is not. Thank you.
Then I have a question for Mr. Van Bellegem: "The BOD of the effluent of the anaerobic system is relatively high. Is this due to the presence of ammonia and sulphur? What happens to these compounds in the effluent?"

(Van Bellegem) Ammonia is not included in the BOD analysis, because we have used the BOD-5 analysis technique. It is only in BOD-20 analyses that ammonia is included. Anaerobic systems give reduced compounds - methane, ammonia and hydrogen sulphide. The amount of the compounds you get depends on the amount of sulphur and nitrogen in

the waste water you have to treat.
We think we have solved the sulphur problem and we will try it out on a technical scale. Nitrogen (ammonia) is still a major problem.
The effluent of an anaerobic plant can be treated by aerobic systems, for example the activated sludge system. As I showed in one of my slides, there is a relation between the BOD of the effluent of an aerobic plant and the load. If you want to have a BOD of lower than 20 in the effluent of an aerobic system, you can find out the load which will lead to that BOD. Does that answer the question?

(Van der Kerk) No, I think not as there seems to be a comment from the floor.

(Comment) I want to make a few points. The first is that I think your BOD a bit flattered because you did not include the ammonia. My second point is: If you have to add an aerobic plant after the anaerobic one, are not the advantages of the anaerobic treatment lost to a large extent?

(Van Bellegem) No, I don't think so. Why do you treat the waste water in the first place? Only to conform to Dutch laws about environmental pollution. If you would use aerobic treatment with an influent of 10,000 BOD, you would need a two-step system too. Just think about the sludge problem you would get from that. So the advantages of the anaerobic system are not lost because you have to top it up with aerobic step.

(Van der Kerk) Thank you, I hope this answers the question.
The next question is for Dr. Senior. "Replacement of the GOGHTGS-system - this is very difficult, it is this terrible custom of people who know everything in one particular field of knowledge and think that everyone else knows it too.
Could the questioner explain what GOGHTGS stands for?

(Comment) It is the ammonia assimilating system which was removed from the production strain and was replaced by the glutamate hydrogenate system in the newer strain. But I don't think it is essential to tell what the letters stand for.

(Van der Kerk) Thank you. Here we go again. "Replacement of the GOGHTGS-system by the GDH-system can give, on the basis of calculations about the energy costs of cell synthesis, only an increase in yield of three to five per cent. It that sufficient for a new toxicological survey of the new strain, as will be required by GMAG?"

(Senior) If the increase in yield was three to five per cent, it would be worthwhile to do the toxicology.

(Van der Kerk) Long question, short answer. I have another question, which is addressed to Dr. Windass. It is also a long question, and perhaps Dr. Windass would be so kind as to read or summarize it, as I feel myself unable to do it without holding up the audience.

(Windass) Of course I'll do so gladly. It is a very long question and I am certainly not competent to answer all parts of it. If someone in the audience can provide an answer I would be very grateful. It reads: "Concerning the advances in biotechnology and the solutions of problems I described, would I care to speculate on the long-term future bringing together firstly data-banks of functional characteristics of organisms, plasmids and genes, secondly mathematical models of molecular structure, reaction dynamics and metabolic pathways, and thirdly artificial intelligence software as a means of designing or improving biological systems".
Well, I can only comment on the first one. I believe that such data banks are already being set up. As Dr. Cape said yesterday, we now have a fair idea how to persuade a whole variety of genes to be expressed in different places. We can only learn by trial and error and I believe that people are already setting up effectively data-banks relevant to their own particular problems.
Mathematical models of molecular structure, reaction dynamics and metabolic pathways; I know that we in ICI use such models. Artificial intelligence software as a means of designing or improving biotechnological systems; I really have no comment.

(Van der Kerk) Thank you. Then I have a personal question for Dr. Parker. It intrigued me that he gave an example of the use of sucrose and in general of products from carbohydrates made by biotechnological means. Now, I would like to ask Dr. Parker what his

outlook is on the future of biotechnological aromatics based on lignin. Lignin is a considerable part of wood and I don't know whether you or anyone else hase considered this possibility, and whether it is worth considering.

(Parker) Professor Van der Kerk, this is in fact a very interesting question. One feature of existing processes for producing chemical intermediates from regenerable raw materials is that they lead to aliphatic products rather than to aromatics.
Lignin itself is an aromatic substance and if it were possible to degrade it, one has a source of benzene rings or aromatic compounds. Unfortunately lignin is extremely resistant to biological degradation. Some fungi and moulds can degrade it, but it takes years. The enzyme system present in these fungi and moulds has never, to my knowledge, been studied or exploited from the point of view of producing low molecular weight products from lignin.
Some time ago we did a brief search for other lignases or sources of lignin hydrolizing enzymes, but we were not successful in finding anything which would have had any practical value.
At the moment lignin or its derevatives are used as an extender of resins and as an alkylating agent. The only aromatic compound that is produced from it, is vanillin.
And apart from that lignin is very good source of carbon.
But it would be very nice if someone could come along with an enzyme system of a micro-organism which could degrade lignin quite rapidly.
The other point is that total utilization of wood could be extremely valuable if one is looking at wood as a regenerable resource. There are enormous reserves of wood in the world, particularly in the Soviet Union and North America which represent an untapped potential. But at the moment, apart from burning wood - and I mentioned that half of the population relies for their energy requirements on as much as 90% on wood - apart from that we have no effective way of making use of this particular resource. Even cellulose is in short supply and commands a relatively high price. So there is room for a system that separates lignin and cellulose from wood effectively, but to my knowledge there is no such system in existence. But we are looking for means of degrading wood in a useful manner.
Professor Van der Kerk, does that answer your quenstion?

(Van der Kerk) Yes it does, you did point out the difficulties and the possibilities quite nicely. May I go on to the next question, which is for Dr. Cripps. It is in two parts. "As solvent extraction and electrolysis is gaining in importance, does the lower iron content and the organic contamination reduce leaching efficiency?". The second part runs: "Could Dr. Cripps say a little more about the current state of the art of coal desulphurisation by microbial means?".

(Cripps) I think I have already answered the first question when I was asked to answer my first question as a member of the panel. Solvent extraction materials certainly do pose a problem, but I think that can be overcome by correct selection of strains. The other part of the first question refers to the lower iron content: yes, you have to remove iron before you can use electrolysis to recover your desired metal.
Therefore the iron that you require for the leaching process has to be supplied from the ore itself. So it boils down into the fact that you must have an ore with sufficient iron in it in the first place. I think that is really all I can say on that part of the question.
And to the current state of the art of coal desulphurisation by microbial means, the answer is no, I am afraid I can't.

(Van der Kerk) Then I have a question for Professor Bruin: "I would be interested to know how the quoted expected average of 4000 W energy consumption has been arrived at".

(Bruin) It is just an amount that I thought might be reasonable, 4 kW of non-food energy requirement. It is partly based on the energy consumption figures for France. I looked at the table for per capita energy consumption and saw that a country like France had about that amount of per capita energy consumption, and I realised that it is far less than in the USA and even about two thirds of the energy consumption per capita in this country. Yet France is reasonable well developed and I thought I could take that figure as a reference point.

(Van der Kerk) Thank you. The next question is for Mr. Van Bellegem. It runs: "Paper factory Gennep uses a microbial process for the de-inking of its waste paper. In the process the cellulose fibres are separated from the starch, casein, fatty acids, coatings, binders and printing inks, so that the fibres can be re-used. The process produces about 50,000 cubic metres of sludge per year - 12,000 tonnes - that have an ash content of 40% and consist of the compounds just mentioned. Do you think the sludge could be converted into biogass?"

(Van Bellegem) It is a bit difficult to answer that one form behind this table. In reality the thing we would do is to get quite a number of samples and carry out some analyses. But still I can try to answer it in general. Starch and fatty acids pose no problem in anaerobic treatment, they can be degraded quite easily. Casein, we did not work with casein but with other proteins, which were degraded too, so I think casein would not give rise to serious problems. Whether coatings and binders will be degraded depends on their composition; I really can't say anything about that from here.

(Van der Kerk) Thank you. Then Dr. Senior is asked: "Can you enlarge upon air sterilization techniques in SCP production?"

(Senior) Sterilization of air in our plant is via filtration, although there is the advantage of the air stream being heated by the active compression, as we are compressing against quite a hydrodynamic head in the fermenter. Total sterilization by filtration is the method we employ, and I don't think there is an alternative just around the corner at the moment.

(Van der Kerk) Thank you. The next question is for Dr. Cripps and then I have a last, general question for the Panel as a whole up my sleeve. The question for Dr. Cripps is quite short: "Is there a microbial leaching technique for silver?". I happen to know that the short answer is 'no', but would you like to go into it somewhat further?

(Cripps) Certainly, Mr. Chairman, because actually it is a very interesting question. I am not really aware whether there is a microbial leaching technique for silver - and I am quite prepared to accept your word that there is not - as I don't know really what form silver ores are in. But silver occurs as a trace element in many ores, and it could be a problem or an economic advantage in other metal recovery systems.
There are some problems, as I mentioned in my paper silver is one of the metals that is toxic to leaching organisms, but perhaps you might be able to surmount that through adaptation and selection. From this moment I am going to speculate rather heavily, so don't accept anything I am saying as gospel truth.
In any metal leaching system before you can recover the metal you want, you have to remove the contaminating metals, principally iron. You do that by precipitation and with some chemicals you can precipitate out the potassium and sodium too. I speculate that in that stage you would remove your silver also. So I think that if you have an effective and economic leaching system for silver, you would have to adapt your processing system. But really, I am more thinking aloud than giving solid information. Does that answer the question?

(Van der Kerk) I think it did, thank you. Now I come to the last question, which is addressed to the Panel as a whole. "We went BTN (Back To Nature) for the last two days. Could we go BTR (Back To Reality) by indentifying the areas where biotechnology and its commercial exploitation is foreseen in the short, medium and long term?"
We have only a short time to discuss this last question, which is a pity as it is quite provocative. I think we could easily talk about it for half an hour or more, but that will not be possible. Which member of the Panel will have a go at it?
Dr. Senior?

(Senior) The question is about biotechnology and its reality. Now I think that a pile of ore, 1200 feet high, in Utah is very real, a 200 feet high, 600 tonne fermenter in England is pretty real, and a bottle of anabolic steroids is pretty real too. For the short, medium and long term, we will be pleased to receive this question as an order for a thousand tonnes of protein either in the short, the medium or the long term. And I think the copper and steroid producing companies will see the question in the same light. We are talking about a real world; there is a lot of money, time and effort going into it. And I think that some of the flights of phantasy we have just started, may take our minds

off the rather chilly economic situation that exists in the world at the moment.

(Van der Kerk) It seems that the question was not as unexpected as I thought. Thank you, Dr. Senior. Who has a further comment? Professor Kieslich? Professor Bruin? Yes, Dr. Parker.

(Parker) We think we are realists at Tate & Lyle, and the idea of going back to reality does appeal to us for that reason. One of the realities in industry is that one wants to have some return at the end of any investment. So the first requirement is that any investment, any development should meet a real need, it should be something which someone is willing to pay for and which will provide a return on the original investment. We have been talking about ethanol as a fuel additive, derived from regenerable resources, and that will be a development if someone is prepared to pay for it and for the plant that will produce it. But if nobody is, then, I'am afraid, nothing will happen.
We have a very good example of that in what we did for some years on microbial single cell protein production from agricultural waste. We developed a fermentation system which would deal with any type of agricultural waste, pineapple waste, banana waste, dates, olive oil and so on. The process is available to anyone who wishes to negotiate the installation of this. However, the only real application we found, was in the UK, where legislation demands the cleaning-up of waste water streams before discharge to the main drains. We are able to apply the principles of our fermentation process to these effluent streams, producing a single cell protein which is of value for animal feed. But we know that there vast amounts of utilizable agricultural wastes around the world, but without investment money no one is actually going to do anything about it. This is what I mean by being realistic.
We are interested in the utilization of carbohydrates too, but again we are necessarily looking at profitable investment. One process I touched on this morning was the production of biodegradable sucrose esters. This process uses sugar, which is a regenerable raw material, and triglycerides, such as coco-nut oil, which is a regenerable agricultural material. These two substances could be combined to produce a product which can be used for the production of detergents. If you apply this in a country which has no indigenous resources of oil, it becomes extremely attractive, particularly if there is sugar and vegetable oil. We are building a 25,000 tonne plant in the Philippines which will supply all their needs from their own indigenous materials. But we could not do this unless it was being paid for, and this seems me to be the realistic element in this type of development.

(Van der Kerk) Thank you. Mr. van Bellegem?

(Van Bellegem) Well, we had BTN and then BTR, but the best way of going Back To Reality (BTR) is by BTB: Back To Biotechnology. These two days we have heard quite a number of examples about biotechnological processes that are being applied now. I think that biotechnology is a reality and will stay so in the future.

(Van der Kerk) I think these positive words are a good ending to the panel discussion and the 13th International TNO Conference. Before saying a few words on the Conference as a whole, I wish to express my gratitude to the members of the panel.
Within the last two days we certainly did cover a lot of ground. The subtitle of the Conference is 'a hidden past, a shining future', and these two days have taught us that the future of biotechnology is shining, be it not equally strongly in every imaginable direction. Even when applying biotechnology as widely and intensively as possible, it would be wrong to consider it as a substitute for the present forms of process technology. But the imaginative pursuit of biotechnology and the full exploitation of its possibilities will eventually add a new dimension to technology as a whole, in particular if means are found to incorporate in a responsible way the anticipated developments in biomolecular and genetic engineering.
In my opinion the greatest challenge to biotechnology, which it shares with physical and chemical technologies, is its potential contribution to the harnessing of the solar energy which is shed on earth continuously. Let us hope that in the not too distant future another Conference will be necessary to report on the significant progress towards this goal.

Before closing the Conference, I would like to thank the Information Department TNO for their invaluable assistance in the preparation and organisation of the Conference, and the staff and management of the Hilton Hotel for their hospitality and care. A special word of gratitude is due to Ms. Van Giersbergen and her assistants from the Information Department TNO, who so ably, kindly and helpfully saw to the smooth running of the Conference.
And now I say to all of you: Have a good return journey, and au revoir.

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