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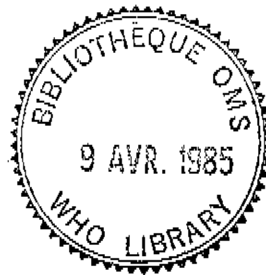

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ENVIRONMENTAL HEALTH IMPACT OF BIOTECHNOLOGY

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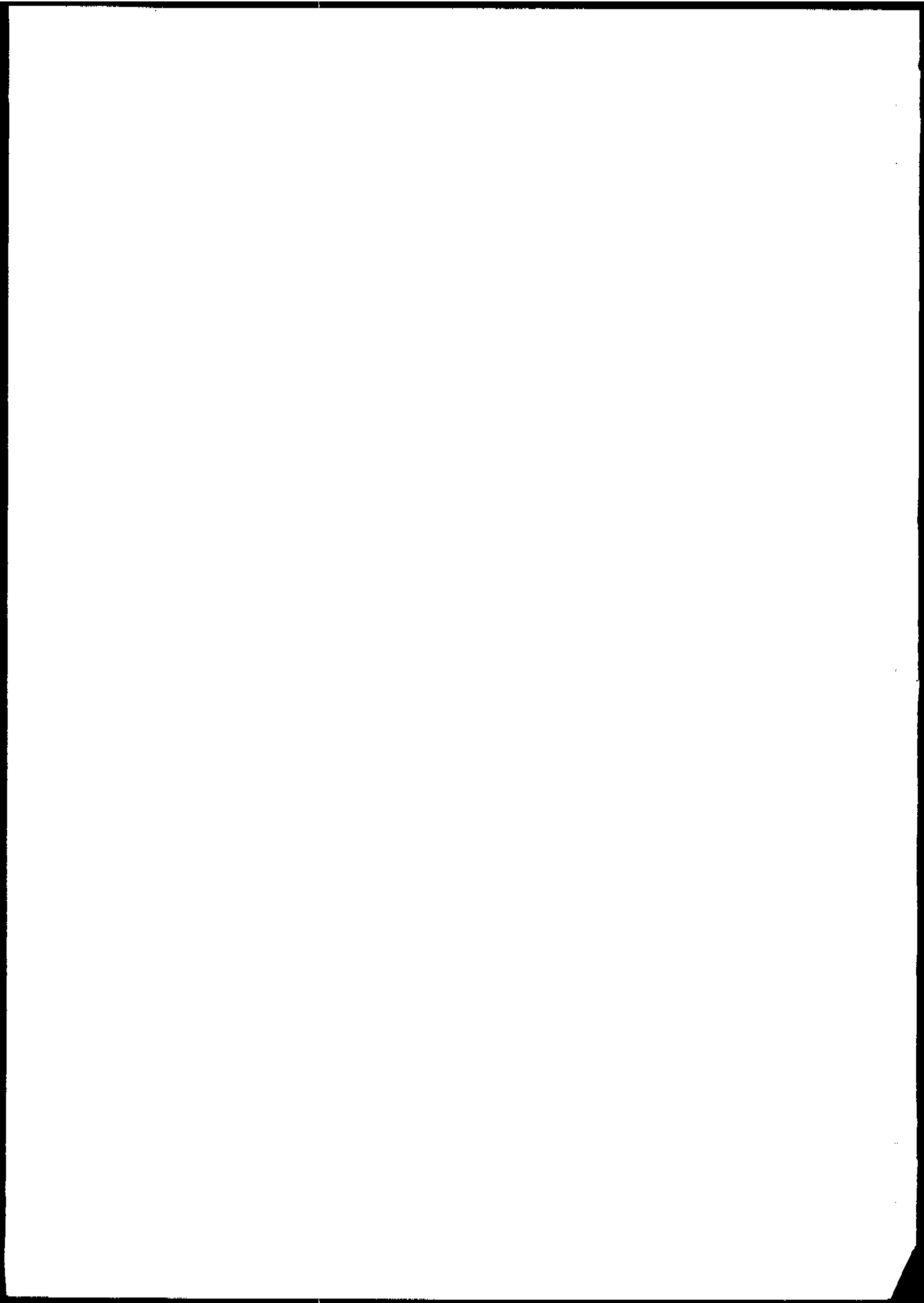

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CONCLUSIONS

1 Biotechnology is not a new discipline but the level of activity will rise considerably in the future. This increased activity will include an expansion in the use of present day processes together with an increasing number of new processes on an industrial scale.

2 There will be a wider range of microorganisms used on an industrial scale in future and these will include those modified by genetic engineering.

3 A new range of products including bulk chemicals and fine chemicals for the food and human/animal health care industries will become available.

4 The environmental health impact of biotechnology is fundamentally distinctive from that of most other industrial activities only in that it involves some potential for release of infective organisms which might cause disease and in the problems that may be associated with greatly increased production of biological wastes. However, it must be noted that one of the benefits of developing biotechnology will probably be more effective ways of treating such wastes with their conversion to useful products such as methane and ethanol.

5 Deliberate use of mammalian pathogens on an industrial scale is highly unlikely but some plant pathogens are now being used in large quantity production.

6 More needs to be known about airborne transmission of microorganisms, particularly in the form of aerosols.

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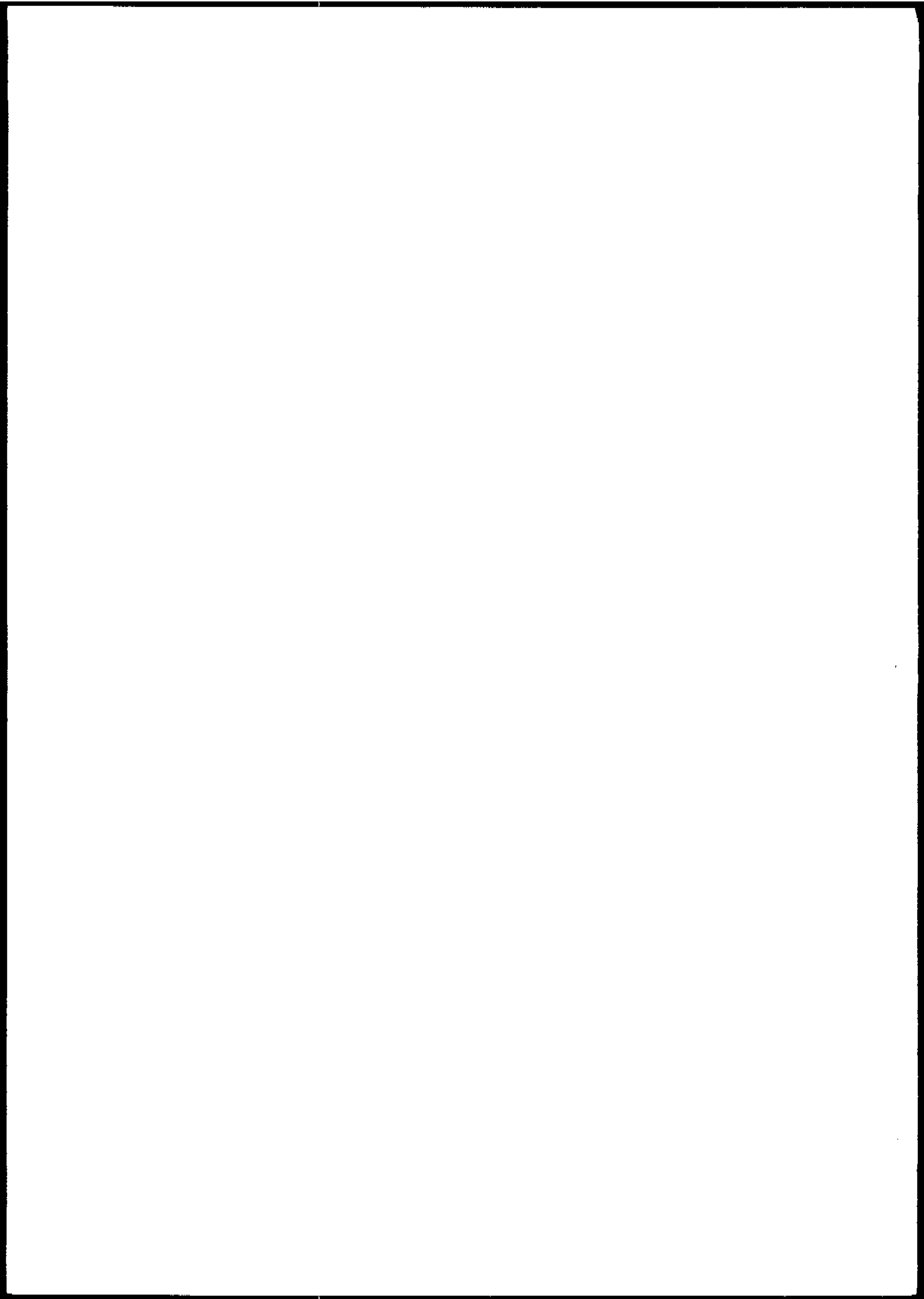

7 More needs to be known about the general spread of plant pathogens and the development of plant diseases.

8 There is a need for improved methods for the rapid detection of small numbers of contaminant microorganisms in the large populations grown for biotechnological production processes.

9 Genetic engineering of microorganisms with the safeguards proposed and implemented should not cause any significant new environmental hazards although the situation should be carefully monitored.

10 There is a need for improved methods of toxin detection at low concentrations so that production of materials to be used in food, such as single cell protein, can be aborted before contamination becomes dangerous.

11 It is possible that many of the materials used in or produced by biotechnological reactions may be allergenic. Although this hazard can often be minimized by containment of such materials, further research into methods of minimizing allergic responses by early detection and treatment should be carried out.

12 Problems of waste disposal associated with biotechnology are not likely to be qualitatively different from those which currently occur. Scaling up of present procedures should be sufficient in most circumstances.

13 With regard to workers in biotechnological industries, particular attention must be paid to inhalation and dermal exposure in toxicity testing of raw materials, intermediates and products.

RECOMMENDATIONS

1 Use of organisms pathogenic to any form of life should be permitted only after careful assessment of the consequences of any release of the organisms into the general environment.

2 Use of human and mammalian pathogens should be forbidden except under license and in premises regularly inspected and approved by the competent government authority.

3 Use of plant pathogens likely to attack essential crops should be discouraged and research to find less hazardous substitutes should be actively encouraged.

4 Research into the spread of pathogens, particularly airborne transmission in aerosols, should be increased.

5 Improved methods for rapid detection of small numbers of microorganisms must be developed, especially where they occur as contaminants in large populations of commercially useful organisms. Special attention should also be paid to rapid methods for monitoring viruses.

6 Improved methods for rapid detection of low concentrations of toxins must be developed.

7 Research into methods of minimizing allergic responses to components of biotechnological systems siould be initiated.

8 Improved methods for relating toxic effects to inhalation or dermal exposure should be developed.

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1 INTRODUCTION

Biotechnology has been defined as the application of biological organisms, systems or processes to the manufacturing and service industries.

The use of microorganisms in the production of beer, wines, vinegar, fermented milk products etc has been exploited for thousands of years but many applications have a more recent history. Solvents such as butanol and acetone and glycerol for the manufacture of explosives were produced in large scale by fermentation only after war time conditions earlier this century restricted the supply of the more established source materials. Large scale production of antibiotics began in the 1940s and modern fermentation technology essentially dates from that time. Currently biotechnological processes are involved in the production of a wide range of materials including enzymes, hormones, fuels, polysaccharide gums, vaccines, vitamins and flavour enhancers and form the basis of waste treatment technology.

The current interest in biotechnology originates from -

1 An awareness of the limited supply of fossil fuels and the need to supply alternative (and renewable) sources of energy and chemical feedstocks.

2 Advances in molecular biology and genetics which indicate the potential for increasing the efficiency of production of existing products and of manufacturing a wide range of new products using recombinant DNA technology (genetic engineering).

3/

3 The increasing ability to exploit animal and plant cell cultures to produce useful products.

The success of biotechnology will require the active collaboration of biological scientists and chemical and process engineers in order to produce the biological organisms, systems and processes required and to optimise the safe use of these for industrial and environmental exploitation.

2 PRESENT DAY APPLICATIONS OF BIOTECHNOLOGY AND THEIR FUTURE POTENTIAL

As was indicated above many biotechnological processes are of ancient origin although these may be made more efficient by more effective application of both science and engineering. Many improvements may be made by exploiting more advanced fermentation design and process control (especially with microprocessor - controlled systems). Some likely trends in this area are outlined in section 3.

Tables 1 - 6 and the notes below on metal extraction and recovery and animal cell culture outline some present day applications and suggest improvements which are likely in the future. This information is illustrative and no attempt has been made to provide a comprehensive treatise. For further information please refer to the articles listed in the references.

Metal extraction using micro-organisms. This is an old established process of draining dissolved metals from mine workings, waste dumps and coal heaps. The principal metals concerned are copper and uranium (recovered from low grade ore containing 0.01 to 0.05% U_3O_8) and the process involves the acid leaching of mineral sulphides. Organisms involved include a range of thiobacilli. 10-15% of the production of copper in USA involves microbial leaching. Very little is known about the biochemistry and genetics of the organisms involved and this will severely impede future progress. That these organisms are lithotrophs and do not require an organic carbon source is a major factor influencing process economics. New approaches include the screening of bacteria and fungi for metal extraction capabilities and studies of the mechanisms of resistance to high metal concentrations. High sulphur coals containing pyrites are not used commercially due to the potential environmental damage from SO_2 production on combustion. Large scale removal of the pyrites by leaching might lead to the use of this material of which large reserves are available. Metal ion resistance in the thiobacilli is known in some instances to be plasmid-borne. This provides an opportunity for genetically engineered organisms to be produced with higher resistance.

Metal recovery. Many microorganisms are able to take up and accumulate metals from dilute solutions; others accumulate metals by adsorption to their surface polymers, while the activity of sulphate reducing bacteria leads to the removal of metals from solution and precipitation as metal sulphides. Iron, copper, mercury, silver, cadmium, cobalt and nickel are some examples of the metals concerned. These properties are in part plasmid-borne. Advances in this area might include the use of mixed cultures to remove toxic metal ions from waste with the potential for their recovery. Heterotrophic organisms are involved and recovery processes will be economic only if low cost carbon and energy sources are/

are available. The potential of microbial cultures to accumulte very toxic metals such as uranium and plutonium and the potential to engineer organisms to possess abilities to accumulate a number of such metal species should not be overlooked.

Animal cell cultures. The use of such cultures for the production of vaccines, especially for veterinary applications is well established. Two basic methods of culture (1) in monolayers and (2) in suspension are in common practice. Monolayer cultures are employed in up to 1000L units and suspension cultures in 4,000L units. With good equipment and plant management a contamination level of about 2% should not be exceeded. Monolayer cultures are generally favoured since they contain fewer oncogenes, are in general susceptible to a wider range of virus types, and will produce fibroblastic interferon. Monolayers, however, require a solid support for growth, provide more problems for scale-up and sampling, and tend to require a higher concentration of better quality serum for successful growth. Scale-up involves either packed beds (eg using glass beads) or microcarriers (eg polystyrene, polypropylene or DEAE sephadex supports). These latter float and may be used in a stirred tank fermenter. Not all cell lines used commercially will grow on the microcarrier supports.

The increased used of microcarrier systems in conventional stirred fermenters (currently up to 1000L) together with continuous culture methods appears to be the way in which this field will progress.

TABLE 1

FOOD AND BEVERAGE INDUSTRIES - PRESENT PRACTICES AND FUTURE POTENTIAL

Industry	Comments
Brewing	Largest bulk fermentation industry (700 Mhl/a). Materials used include malted barley, roasted barley, maize and wheat as components for fermentation wort. Flavour with hops and fermentation with *Saccharomyces* sp. Extensive use of enzymes (starch and protein degrading) in production and storage. Some national industries maintain traditional methods. Some work on new yeast strains (produced by hybridisation and protoplast fusion) to utilise an increased range of substrates for fermentation (eg dextrins). Problems have been encountered with flavour components (eg 4 vinyl guaiacol). Scope for genetically engineered strains avoiding these flavour complications.
Milk Fermentation Industries	A wide variety of products including cheeses, yoghurt, buttermilk, kefir, koumis etc, some of local or national origin. Large scale production of cheeses and yoghurt involves specific starter cultures. The largest problem is the susceptibility of the organisms concerned (*Streptococcus* sp, *Lactobacillus* sp) to lysis by bacteriophage. Defined and single organism cultures used in USA and Australia but less favoured in Europe. Large scope for improvements in rates of fermentation and stability by genetic engineering. Microbial rennins have potential to replace animal products in production of curd (gelling of casein components of milk). Prospects of better process control and continuous processing.

Flavour/

Flavour Component Manufacture	Monosodium glutamate production is considered in Table 2. Other flavour enchancing components include guanosine 5' monophosphate, inosine 5' monophosphate and xanthosine 5' monophosphate which are produced commercially by *Corynebacterium glutamicium* and *Brevibacterium ammoniagenes*. Potential for strain improvement and alternative fermentation feedstocks.
	Most colouring flavour compounds are derived from plant sources. The potential exists for producing these (eg the 'hot' principle of *Capsicum frutescens*) in plant cell cultures (Section 5) or in genetically engineered bacteria (long term). As with other food industry applications, however, the safety regulatory procedures and the high cost of processing successful applications for the use of microorganisms will be a major disincentive.
Single Cell Protein	see Table 2.

TABLE 2

SINGLE CELL PROTEIN PRODUCTION - THE ORGANISMSAND SUBSTRATES USED WITH NOTES ON CURRENT AND FUTURE DEVELOPMENTS

Organism	Substrate	Comments
Methylophilus methylotrophus	Methanol - chosen rather than methane due to ease of use and safety factors	Large scale production (1,500 m^3 continuous fermenter). Protein used as animal food. Organisms genetically engineered to increase yield.
Saccharomyces cerevisiae	Molasses	Local use, partly for waste utilisation.
Candida tropicalis	Waxy hydrocarbons	'Toprina' process abandoned at production stage.
Kluyveromyces fragilis	Milk whey	Local use, partly for waste utilisation.
Endomycopsis fibuligira and *Candida utilis*	Starchy wastes (potato processing)	'Symba' process. Product used for animal food. Continuous two stage process.
Paecilomyces varioti	Sulphite liquor, molasses, whey etc, (waste materials from other processes)	'Pekilo' filamentous fungus with fibrous product. Continuous process for cattle food.

TABLE 2 (continued)

Organism	Substrate	Comments
Fusarium graminiarum	Starch and other polysaccharides	'Mycoprotein' approved in UK for human consumption. Fibrous material as potential new food product. Continuous process.
Algae including *Chlorella*, *Scenedesmus*, *Spirulina*	CO_2/sunlight	Large scale production in ponds and lagoons to remove organic pollution and provide animal foo

NOTE:

In the USSR some 86 SCP plants are thought to be operating with some 12 or so using alkanes as substrates. Few details are available.

Potential future developments in this area include:

Use of cellulose, hemicelluloses etc. as substrates including genetically engineered organisms with a number of degradative properties.

Use of H_2 / CO_2 utilising organisms including photosynthetic organisms and lithotrophic bacteria.

Upgrading of SCP by genetic engineering by cloning animal (or human) proteins eg ovalbumin into producer bacteria/yeasts.

TABLE 3

INDUSTRIAL FERMENTATIONS - PRESENT PRACTICE AND FUTURE POTENTIAL

Product	Process	Comments
Ethanol	Large number of potential substrates including sugar cane, cassava, straw, cellulose and hemicullulоses. Yeast fermentation most common.	Pre-1940-produced mainly by fermentation. In recent years production of non potable spirit predominantly from petrochemicals. In USA alone yearly production about 650 t/a (300M$ / a) based on the utilisation of maize starch. Ethanol is used extensively as a chemical feedstock. Change in price of crude oil has changed economic prospects of fermentation process. Conventional technology currently applied in Brazil, output now 4 x $10^6 M^3$ with projected 16 x $10^6 M^3$ by 1990 - intention to replace petrol entirely with green ethanol In Brazil sugar cane is used as a substrate but increasingly being replaced by starch from cassava - and babassu starch. In future fermentation ethanol is likely to become increasingl significant both as a fuel and as a chemical feedstock. Genetically engineered Saccharomyces cerevisiae able to utilise a wider variety of fermentation substrates (especially xylose/xylulose derived from the hemicellulose component xylan/

Product	Process	Comments
		xylan is a likely development. The use of other organisms such as *Zymomonas mobilis* which may be engineered to utilise cellulose, *Clostridium* spp and filamentous fungi such as *Fusarium* is also probable. Other likely future developments include the use of thermophilic microorganisms, novel substrates, mixed cultures and immobilised cells and enzymes. Continuous processing for both fermentation and distillation appears to be essential.
n-Butanol/		

TABLE 5 (continued)

Product	Process	Comments
n-Butanol	Conversion of sugars by *Clostridium* sp. Molasses as substrate in S Africa.	Pre-1940 produced mainly by fermentation, now from petrochemicals. Important solvent and chemical feedstock. Poorly understood metabolic pathways. High potential for increased yield and resistance of producing organism for product. Genetically engineered organisms likely.
Glycerol	*Saccaromyces cerevisiae* 'steered' fermentation.	Possibility of fermentation in future without requirement for steering agents.
Acetic acid	Oxidation of ethanol by acetic acid bacteria.	Biological system only for vinegar production. Commercial chemical production by methanol carbonylation estimated at 1.4M t/a (500M$). Acetic acid is an important chemical and fermentation feedstock. Future potential includes the conversion of CO_2/H_2 to acetic acid by *Acetobacterium woodii* and *Clostridium aceticum* and conversion of cellulose by thermophilic bacteria. Genetic of producer organisms poorly understood and restricting developments.

Citric/

TABLE 3 (continued)

Product	Process	Comments
Citric acid	Produced from molasses by *Aspergillus niger*	Widely used food ingredient. Surface and submerged batch cultures both used currently although continuous process more efficient. World sales 0.2M t/a (260M$). Molasses increasingly expensive as fermentation feedstoc and so starch (corn or wheat) under investigation as alternative. Possible use of genetic engineering to enable *A. niger* to degrade cellulose etc. Yeast process using alkanes not now economical.
Lactic acid	Produced from sugars by *Lactobacillus delbrueckii*	Used in a variety of industries. Production in West about 40K t/a (56M$). Mostly made by chemical synthesis in USA. Scope for use of less expensive substrates. Genetics of producer organism poorly understood.
Other organic acids	Sugars to propionic acid with *Propionobacterium* sp. Sugars/alkanes to fumaric acid with *Rhizopus* sp/*Candida* sp. Sugars to gluconic acid with *Aspergillus niger*.	In all cases there is considerable scope for improving economics with cheaper fermentation-feedstocks, perhaps with genetically engineered organisms. With the exception of *A. niger* the genetics of the producer organisms is poorly understood.

Amino/

TABLE 3 (continued)

Product	Process	Comments
Amino acids	Glutamic acid (mono sodium salt) and lysine produced from glucose and acetic acid.	Mono sodium glutamate production with Corynebacterium glutamicium and Brevibacterium flavum estimated at 300K t/a. High yield fermentation with product excretion. Scope for cheaper fermentation feedstock and better understanding of excretion process with probability of genetically engineered organisms. Lysine production by fermentation 40K t/a, outlook similar to monosodium glutamate. While MSG is employed as a flavour enhancer, lysine is used as a good supplement. Methionine (food supplement) is currently produced by chemical synthesis (100K t/a); genetically engineered organisms are expected to be used in future to produce methionine by fermentation. Scope for production by fermentation of other amino acids such as glycine, alanine, cysteine, glutamine, histidine and argini for use in the food and health care industries. There is scope for improvements in yields and the use of alternative substrates. The genetics of the producer organisms is poorly/

TABLE 3 (continued)

Product	Process	Comments
		poorly understood.
Vitamins	Carotene (vitamin A precursor) produced during growth of *Blakeslea trapora* on molasses etc.	
	Riboflavin by *Ashbya gossypii* grown on sugars etc.	
	Steps in vitamin C production (Sorbitol to sorbose) carried out by *Gluconobacter oxidans*.	
	Vitamin B_{12} production by a variety of organisms on molasses, sugars etc.	
Methane	Anaerobic fermentation of organic wastes.	Complex mixtures of anaerobic bacteria involved in most efficient systems. Microbiology poorly understood. Useful at local level to reduce biochemical oxygen demand of wastes and provide useful fuel. Expensive process relative to natural gas and coal gasification.
Antibiotics	Production of antimicrobial agents, at present by 6 genera of filamentous fungi (1000 compounds), 3 genera of actinomycetes (300 compounds) and 2 genera of non filamentous bacteria (500 compounds).	Secondary metabolites with world sales in excess of 5000M$ / a. 25% sales for penicillins, 10-15% for cephalosporins and 10-15% tetracyclines, all fungal products. Strain selection/mutagenesis screening traditional ways of increasing yields and finding new compounds.

The/

TABLE 3 (Continued)

Product	Process	Comments
		The genetics of the production of secondary metabolites is poorly understood. The present industry is likely to benefit from more advanced fermentor design and control together with the possibility of higher yielding strains being produced by protoplast fusion and genetic engineering. Future developments include the use of alternative fermentation feedstocks with the possibility of antibiotic producing strains being engineered to degrade these directly.

TABLE 4

PRODUCTION OF
MICROBIAL POLYSACCHARIDES - PRESENT PRACTICE AND FUTURE POTENTIAL

Product	Comments
Curdlan	A $\beta 1 \rightarrow 3$ glucan produced by Alcaligenes faecalis in high yield. Proposed use as gelling agent in the food industry.
Dextrans	α-linked glucans used as blood expanders, as adsorbents in biochemical and pharmaceutical industries for separation of materials, and as supports for immobilised enzymes. Mainly produced by Leuconostoc mesenteroides.
Alginates	Co-polymer of DL mannuronic and L-guluronic acids. Mainly produced from sea weeds but potential for development of bacterial product eg from Azotobacter vinelandii. Many applications in food industry.
Xanthan	Substituted cellulose polymer from Xanthomonas campestris. Widely accepted for food and non-food use e.g. forms stable gel in frozen and tinned foods, instant puddings, toppings etc together with oil drilling muds, paint stabiliser, flocculant for water clarification, gelled detergents etc.
Erwinia polysaccharide	Glucose, galactose, glucuronic acid and fucose containing polysaccharide of high viscosity with potential in paint industry.
Scleroglucan	β-glucan produced by Sclerotium sp. Forms viscous gel in water with similar properties and potential uses to xanthan gum.

NOTE:

Future developments in this expanding area will include the development of additional polysaccharides on an industrial scale, improved methods of production and strain improvements to increase production, produce modified products and possibly the use of a wider range of fermentation feedstocks to replace glucose or starch products [illegible].

TABLE 5

PREPARATION OF MEDICAL PRODUCTS - PRESENT PRACTICE AND FUTURE POTENTIAL

Product	Process	Comments
Steroids	Microbial transformation of plant steroids	Sources of steroids are the barbasco plant and soya bean leaves. Organisms involved include mycobacteria for sterol isolation and Rhizopus arrhizus for hydroxylation. Chemical steps have been reduced from 37 to 11 in cortisone synthesis. Products include cortisone, hydrocortisone, prednisolone dexamethasone, testosterone, estradiol and spironolactone. Well established methods with world sales of 300 M $.
Antibiotics	Considered in Table 3.	
Vaccines	Production of bacterial vaccines	Use of living attenuated strains eg. tuberculosis (BCG), Salmonella, Brucella abortus and anthrax. Use of killed organisms eg menigococci, Haemophilus influenzae, pneumonococci. There is scope for the further development of components of the cell envelopes of the organisms concerned as protective antigens, eliminating the use of whole cells with associated problems and side effects. Protective antigens may also be produced in genetically engineered organisms thus avoiding the need to grow the pathogenic organisms/

Product	Process	Comments
		organisms in large quantities; possibility also of multivalent vaccines.
	Production of viral vaccines	Clearly associated with animal cell culture (Table 7). Use of attenuated viruses eg for poliomyelitis, measles, mumps, rubella, influenza. Use of killed viruses for poliomyelitis, influenza. As with bacterial vaccines the identification and isolation of protective antigens (eg for adenovirus) avoids the requirement for using whole virus. There is a large potential for advances in this area by using genetically engineered bacteria to produce protective antigens. Advantages of such products include better storage characteristics and avoidance of dangers of handling live virus and the possibility of the presence of live virus in the final product. For example, rabies vaccine is currently produced from the brains of infected animals. This is a highly dangerous practice and there would be great value in a genetically engineered product. Problems also arise with viruses which cannot be produced by conventional methods due to poor or no-growth in tissue culture (eg hepatitis B). An alternative strategy is to engineer harmless virus to include protective antigen(s) - in both strategies multivalent vaccines are possible. The market size is high, for example, at present some 10^9 doses of foot and mouth vaccine are produced in a tissue culture each year.

TABLE 6

INDUSTRIAL AND MEDICAL APPLICATIONS OF ENZYMES

a) Present day large volume applications

Enzyme	Organism	Use	Comments
Proteases	Bacillus subtilis Bacillus licheniformis Aspergillus niger Aspergillus oryzae	Biological detergents, biscuit manufacture, brewing, tanning of leather	Bacterial enzyme production caused allergic response, overcome by encapsulation. World production bacterial 500 t/a (66 M$), fungal 10 t/a. Proteolytic activity from fungi used as rennin substitute in cheese manufacture. Animal rennins are currently worth 64M$).
Amyic glucosidase (glucamylase) α amylase	Bacillus spp Aspergillus niger Aspergillus oryzae Rhizopus spp	Degradation of starch to fermentable sugars in brewing, bread making, confectionery and textile industries. Corn syrups. Biological detergents.	World production of amyloglucosidase 300 t/a (36 M$), of amylase 300 t/a (12 M$).
Glucose isomerase (xylose isomerase)	Bacillus coagulans Arthrobacter spp	Production of high fructose syrups.	Uses immobilised in plus flow reactors. Bacillus system is a whole cell preparation. Use widespread in Japan and USA, not Western Europe due to the common agricultural poly of EEC. World production ~50 t/a (56M$).
Pectinase/			

TABLE 6 (continued)

Enzyme	Organism	Use	Comments
Pectinase	Aspergillus sp Penicillium sp	Clarification of fruit juices; Coffee manufacture.	World production 10 t/a. Mixture of pectin esterase, polygalacturonase, polymethylgalacturo[illegible] and pectin transeliminase.
β-galactosidase	Kluyveromyces fragilis Candida pseudotropicalis	Ice cream manufacture	Prevents lactose precipitation. Market potentia[illegible] high for immobilised enzyme.
Amino acylase	Aspergillus oryzae	Production of L-amino acids from racemic mixtures.	L-amino acids for human nutrition prepared by treating acylated racemic mixtures with immobilised enzyme.
Pencillin amidase (Penicillium, acylase, deacylase)	Escherichia coli	Production of semi synthetic penicillins	Immobilised enzyme/cell systems used in production of 6-amino penicillanic acid, the substrate for semi synthetic penicillin manufacture.
	Escherichia coli cells immobilised in polyacrylamide or carrageenan	Conversion of fumaric to aspartic acid	Current production 600 t/a.

Immobilised/

TABLE 6 (continued)

Enzyme	Organism	Use	Comments
	Immobilised *Brevibacterium ammoneagenes*	Production of L-malic acid	Current production 180 t/a
	Immobilised *Curvalaria lunata* and *Corynebacterium simplex*	Production of cortisol	Both organisms entrapped in polyacrylamide. Annual production 250 Kg worth 20 M$.
L-asparaginase	*Escherichia coli* *Erwinia carotovora*	Treatment of asparaginase-sensitive leukemia	*E. coli* and *Erwinia* enzymes antigenically distinct allowing prolonged treatment by intravenous injection.
Various	Various	Clinical analysis	Examples include cholesterol oxidase for cholesterol assay and uricase for assay of uric acid.
b) Potential uses			
Heat stable α amylase	B. subtilis following the insertion of amylase gene from a thermophile	Higher temperature enzymic degradation of starch.	Faster process rates
Pyranose/			

TABLE 8 (continued)

Enzyme	Organism	Use	Comments
Pyranose 2-oxidase Haloperoxidase Epoxidase	*Oudemansiella mucida* *Calderiomyces* *Flavobacterium*	Conversion of alkenes to alkene oxides	Economic advantages claimed over chemical synthesis. Estimated market of about 2000M$ if proved to be a suitable alternative method for feedstock production for the plastics industry.
Pyranose 2-oxidase Epoxidase	*Oudemansiella mucida* *Flavobacterium*	Conversion of glucose to fructose.	100% conversion possible compared with 50% for glucose isomerase. Vast potential market as sweetener.
Urokinase	Genetically engineered bacteria	Blood clot dispersion	Human fibrinolytic enzyme cloned and expressed in *E. coli*.
Immobilised yeast	*Saccharomyces cereviseae*	Ethanol production	Presently at laboratory scale; promise of high yields and rates of production.
	Immobilised Pseudomonas denitrificans	Removal of nitrate from drinking water	
	Immobilised plant cells	Production of alkaloids	

3 FERMENTATION TECHNOLOGY

Industrial applications of fermentationare clearly highly diverse and this is reflected in the many different ways in which biologists and engineers have combined their skills in order to optimise productivity, throughput, recovery etc. Some processes such as conventional waste water treatment make use of the indigenous microflora in non aseptic conditions of fermentaion. Others, however, require highly sophisticated fermenters working aseptically and in a manner such as to contain the specific organisms employed. This latter type of application is particularly likely in the health care industries. Some examples of a range of applications are included in Table 7. More details of the processes concerned are included in other sections of this paper. In many instances fermentation equipment is designed with the performance characteristics of a particular organism in mind and increasingly requires highly sophisticated control equipment. Batch processing is most common although continuous fermentation finds large scale applications in the waste treatment and single cell protein manufacturing industries. It seems likely that continuous processes will be used more in the future, especially with immobilised films and cells.

TABLE 7 SOME EXAMPLES OF APPLIED FERMENTATIONS

Type of Process	Application	Comments
1 Non aseptic fermentation indigenous flora	Waste water treatment	Largest contained application, continuous activated sludge processe of 27,000m^3 capacity. Rapid aeration and mixing required.
	Biogas	Anaerobic fermentation, static or intermittently stirred tank.
	Traditional wine production	Favoured in France, requires natural 'must' on grapes.
	Metal recovery	Naturally occurring *Thiobacillus* spp. Very large scale spoil heaps used.
2 Non aseptic fermentation using specific microbial inocula	Beer	Wort essentially sterile when yeast added. Rapid fermentation ensures minimum of contamination problems. Fermenter size up to 320m^3.
	Bulk wine production	Widespread use of specific inocula in Europe, USA, Australia.
	Bread - bakers yeast	Highly mechanised aerobic yeast growth traditionally using molasses
	Milk fermentations	Milk usually pasteurised to eliminate pathogens. 20m^3 scale.
	Alcohol	Large scale fermentation currently based on yeast.
3 Aseptic fermentation using specific microbial inocula	Antibiotics	Typical fermenter size 200m^3 with batch stirred tanks using complex media and high yielding strains. Highly controlled fermentation.
	Single cell protein	Some processes based on wastes using non aseptic fermentation. Mostl continuous fermentations with one 1500m^3 fermenter installed for methanol process.
	Cultured/	

TABLE 7 (Continued)

Type of Process	Application	Comments
	Cultured plant cells	At development stage but continuous flow and immobilised cell systems likely.
4 Aseptic and specially contained fermentation	Cultured animal cells	At 1 to $4m^3$ capacity with microcarrier systems.
	Plant pathogens	Large scale use for asparaginase and xanthan production.
	Animal pathogens	Unlikely to be used on large scale but may be required for vaccine production.
	Genetically engineered organisms	Processes designed to suit specific organism and product requirements.

4 IMPROVEMENTS IN MICROBIAL PERFORMANCE BY GENETIC MANIPULATIONS

Microorganisms in general have well controlled metabolic sequences designed for optimal growth in their natural growth environment. The development of organisms for commercial exploitation usually involves modifying such organisms by eliminating those properties which in an industrial context are undesirable and amplifying other properties which are considered useful and beneficial.

The traditional mechanisms for changing these properties are by mutation and selection. The natural mutation frequency of DNA is about 1 in 10^8 but this may be increased to about 1 in 10^5 by chemical or physical mutagenesis. Since this is a random process, however, careful selection is required to isolate those organisms with the enhanced properties required. This type of approach has served the antibiotic industry well with, for example, the yield of penicillin production improved some 10^3 times by mutation and selection to procure the high producing strains used today.

The production of primary metabolites (ie those synthesised during active growth) is often well understood at a metabolic level. An example is the biosynthesis of the amino acids mono sodium glutamate and lysine by Brevibacterium and Corynebacterium (Table 3). In wild type organisms the synthesis of these amino acids is controlled so that production is maintained only at a level sufficient for growth. By producing mutants with altered control characteristics, however, a vast overproduction is possible with yields of up to 75 g/L growth medium being achieved.

A further way in which the genetics of an organism may be altered is by recombination. This may be carried out by mating for example between closely related bacteria/

bacteria (conjugation) or between the different mating types of yeasts such as *Saccharomyces cerevisiae.* The production of improved yeasts for the baking industry by such hybridisation procedures is a particular example of success in this area. When direct mating is not or occurs at very low frequency, eg between organisms of widely differing types, then protoplast fusion offers a further method of hybridisation. Protoplast fusion in *Streptomyces* spp increases recombination frequency from 1 in 10^6 to 1 in 5. Examples of protoplast finish include increased anitbiotic yields from *Streptomyces* sp and improved brewing strains of yeasts.

In addition to the genetic information encoded in nuclear DNA, many bacteria and some yeasts also contain small DNA molecules (plasmids) which are inherited after cell division and which are able to replicate autonomously within the cell. Bacteria contain 2-60 plasmid copies per cell (the copy number) and each plasmid contains between 2 and 25 genes. Plasmid DNA may code for a number of important properties in bacteria including antibiotic resistance (R plasmids), colicin production (col plasmids), properties associated with pathogenicity (virulence plasmids) and enzymes concerned with the breakdown of a wide range of molecules (degradative plasmids). In addition conjugative plasmids (sex factors) code for sex pili through which DNA molecules pass from donor to a recipient cell in the process of conjugation. This is a mechanism whereby plasmid encoded properties such as antibiotic resistance may be transferred between members of a bacterial population. Plasmid encoded and nuclear encoded properties may also be transferred by the involvement of a bacteriophage in the process of transduction. Bacterial cells may often contain more than one type of plasmid although most plasmids code for more than one property. Plasmids which co-exist within one cell are called compatible and those that cannot, incompatible.

The/

The transfer of naturally occurring plasmids has been used to construct a strain of *Pseudonomas putida* able to degrade xylene and toluene (XYL plasmid) napthalene (NAH plasmid) and camphor, octane, hexane and decane (parts of the CAM and OCT plasmids (usually incompatible) as a hybrid plasmid). This organism is able to grow well on crude oil and a role for it is suggested in cleaning up oil spills and oil tanks.

Plasmids have another use as vehicles (vectors) for the cloning of DNA into a recipient organism - a process called recombinant DNA technology or genetic engineering. Plasmid DNA may be separated from cell lysates and may be added back to a further cell (suitably prepared) in the process of transformation. If an additional gene(s) is added to the plasmid before introduction into the new host, then the plasmid has served as a vector of that gene into that new host. If the plasmid now replicates in that host the new genes will be inherited through successive generations.

Much of the current excitement and concern surrounding biotechnology involves this ability to produce in bacteria products coded by genes from a wide variety of sources including other bacteria, eukaryotic microorganisms and higher plants and animals. While such bacteria provide an ability to produce by fermentation materials such as animal hormones and enzymes there is also the possibility of producing an organism with undesirable qualities such as a pathogen with unknown properties.

The basic procedures involved in genetic engineering are as follows and are illustrated in figure 2.

1 Preparation of plasmid vectors. The most commonly used vectors are derived from *Escherichia coli* (an organism present normally in the human intestine) and include plasmid pBR322 (figure 1) and others derived from plasmid pMB1. The host organism/

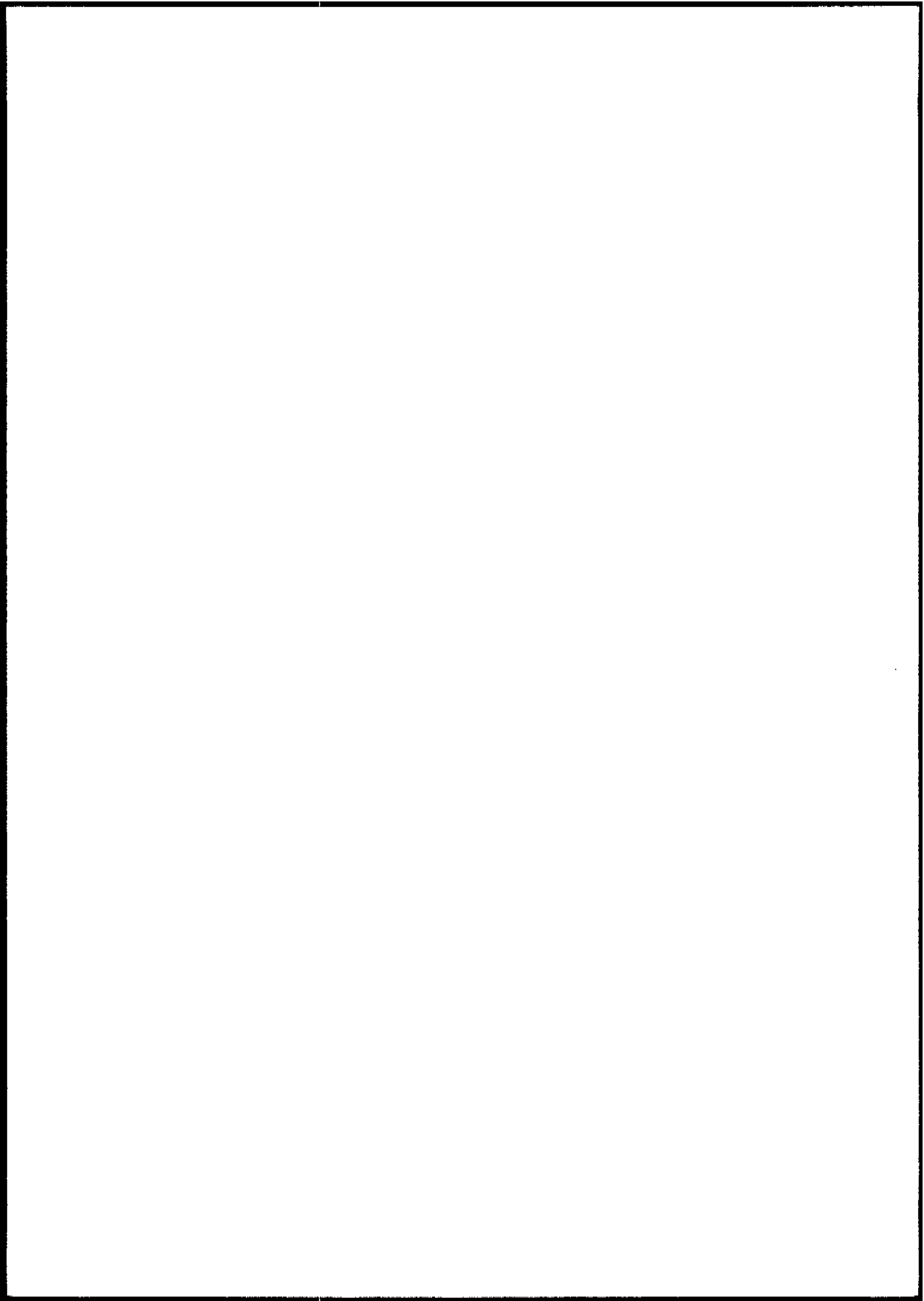
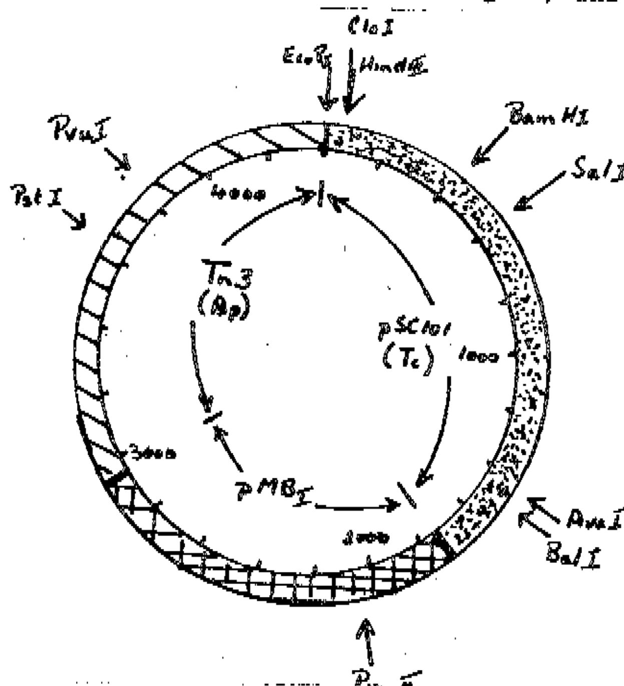


FIGURE 1

THE PLASMID pBR322

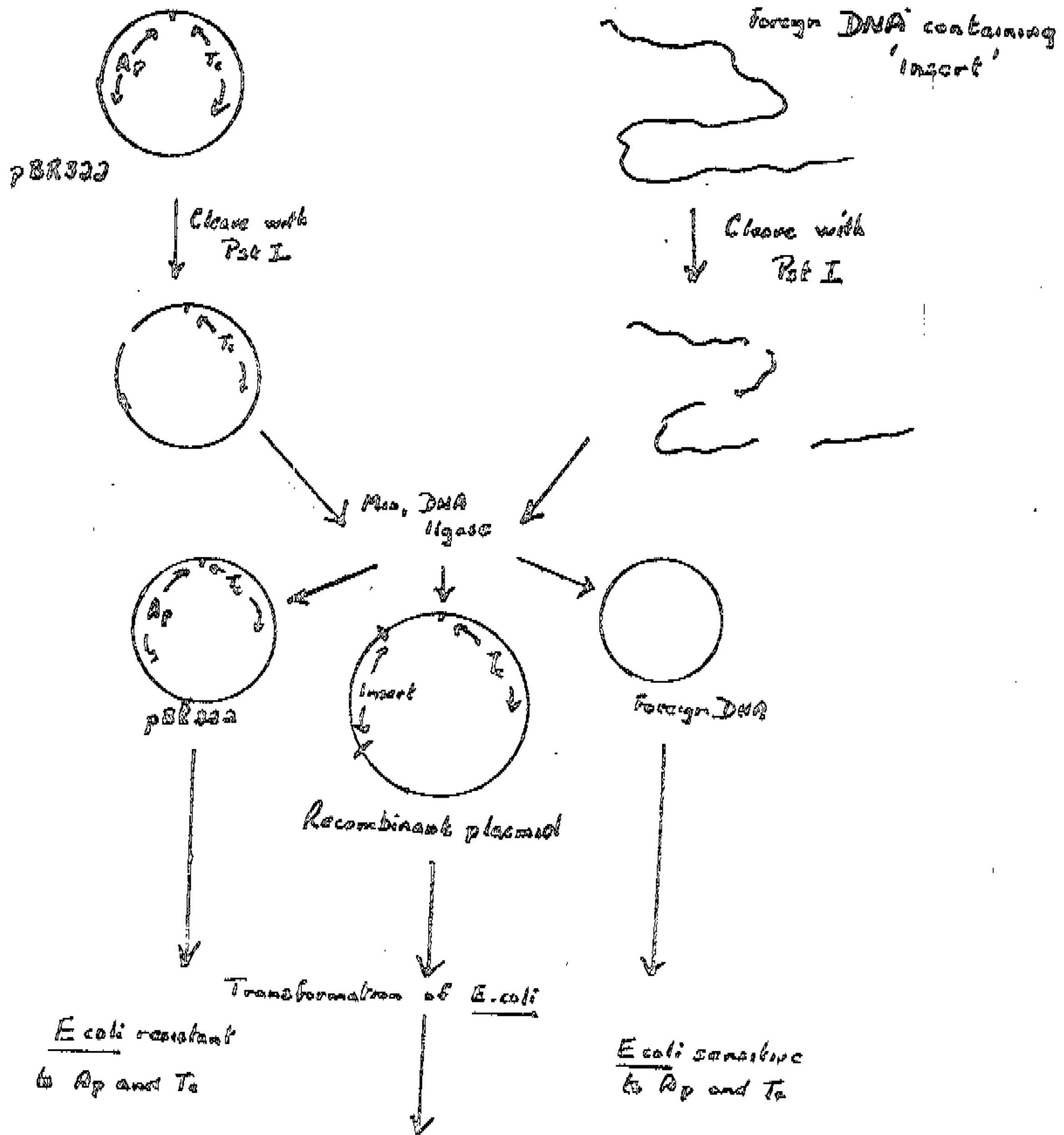
This plasmid is employed extensively as a vector in genetic engineering and was constructed from three other plasmids (as shown below). The composite plasmid pBR322 contains 4361 base pairs (see numbering) and confers both ampicillin (Ap) and



tetracycline (Tc) resistance on the recipient organism. pBR322 is usually present at a copy number of 60. This plasmid is cleaved by a number of restriction endonucleases at specific sites as indicated by external arrows. As outlined in section 4 and Figure 2 these endonucleases together with DNA ligase may be used to clone insert DNA into the vector. The use of the enzymes PvuI and PstI results in loss of ampicillin resistance while resistance to tetracycline is lost on the use of BAM HI, SalI and HindIII.

Figure 2

Basic Procedures Involved in Genetic Engineering



Resistant to Tc, sensitive to Ap.

Tc = Tetracycline Ap = Ampicillin

For details see section 4.

organism for these vectors is usually a strain of E. coli K[12] suitably disabled by mutation so that it has specific growth requirements met only in specially prepared growth media. Vectors for use in other organisms are mentioned later. pBR322 was constructed by combining DNA from palsmids Tn3, pSC101 and pMB1. pBR322 codes for ampicillin (from Tn3) and tetracycline (from pSC10L) resistance and may be cleaved at known loci by a number of restriction endonucleases (see 2 below). pBR322 also contains the essential replication region of pMB1. This confers an ability to continue replication when chromosome replication is inhibited by chloramphenicol, a property important in plasmid amplication, and also to exist as multiple copies within the host organism. The plasmid DNA may be isolated by lysing the cells and then separating it from chromosomal DNA in a caesium chloride density gradient containing ethidium bromide.

2 Preparation of restriction endonucleases. Restriction endonucleases are enzymes derived from a wide range of bacteria and which have the ability to cleave DNA at or near specific sites specified by particular base sequences. More than 140 types of type II endonucleases (those of particular use in genetic engineering) are known and these have been given symbols describing their origin (Eco R1 is derived from E.coli containing plasmid R1 which codes for the enzyme and Hind III is one of 3 enzymes produced by Haemophilus influenzae serotype d. Thus specific endonucleases may be used to cleave plasmids at specific sites, for example in figure 1 it can be seen that 10 enzymes cleave the plasmid in this way.

3 Preparation of DNA for insertion. DNA to be inserted (cloned) into the plasmid vector may come directly from another microorganism or from a virus by 'shotgun cloning' or may be synthesised in vitro either from messenger RNA using the enzyme reverse transriptase (to produce complementary or copy - C DNA) or by direct chemical synthesis which is possible if the amino acid sequence of the product protein is known. Examples are given in Table 8. The DNA inserts are then treated with the same/

same endonuclease used to cleavethe vector DNA. This ensures base complementarity between the free ends of the vector and insert which are then jointed using the enzyme DNA ligase.

4 Transformation. If recipient E. coli cells are treated with 0.1M Ca Cl_2 they become competent to receive DNA in transformation. In general closed loop DNA molecules (as in plasmids) are transformedmore readily than linear molecules and the higher the molecular weight of the DNA the lower the frequency of transformation. For plasmid pBR322 which contains 4361 base pairs the transformation frequency is approximately 10^6 transformants per DNA.

5 Identification of clones. Identification of clones may be difficult without specific marker properties. With pBR322 such markers are ampicillin and tetracycline resistance outlined above. Thus transformants into a recipient E. coli (lacking resistance) will acquire resistance to both antibiotics. If, for example, the Pst 1 (from Providencia stuartii 164) endonuclease has been used, then tranformants containing cloned DNA will acquire tetracycline but not ampicillin resistance.

There are many variations possible in the schemes outlined above both with respect to the vectors and endonucleases used and the origin of the DNA inserts. Bacteriophages may also be used as vectors. For example, the temperate phage has been developed for use in this way. The modified virus may be introduced into hosts by transfection at a higher frequency than is possible by transforamtion.

E. coli is the natural choice for these processes due to the level

of/

of understanding of the physiology and genetics of this organism. For industrial use, however, the use of other host organisms, especially Bacillus spp. Streptomyces spp and the yeast Saccharomyces cerevisiae (all of which are widely used and accepted in the fermentation industry) is desirable (see below for additional comments). For Bacillus spp. vectors based on Staphylococcus aureus antibiotic resistant plasmids are available as are those from thermophilic bacilli which may be expressed at high temperatures. Bacteriophage vectors (using phages such as p11) may also be employed. In addition plasmids constructed from both E. coli and B. subtilis replicons have been shown to replicate in both host organisms.

The construction of vectors for use in Streptomyces spp began with the isolation of plasmid SCP2 from Streptomyces coelicolor and has continued with others from Streptomyces lividans and the construction of a plasmid with an antibiotic resistance marker.

Amongst eukaryotic cells, the yeast Saccharomyces cerevisiae is most suitable for genetic engineering. Some organisms contain a 2 μ plasmid which replicates autonomously and is suitable as a vector. Bifunctional plasmids using E. coli pBR322 and the yeast 2 μ DNA have been constructed and these replicate in both organisms.

Some progress has been made in animal cells using animal viruses as vectors. The Simian Virus (SV) 40 has been used to clone β-globin genes which have been expressed in infected monkey kidney cells.

Cloning in plant cells is also possible with vectors including the cauliflower mosaic virus/

virus (CMV) and the tumour inducing (Ti) plasmid of Agrobacterium tumefaciens. Examples of successful cloning procedures are contained in Table 8.

Genetic engineering is especially useful in cloning specific information into organisms in which the control of gene expression is understood. The success of the plasmid pPR 322 vector and E. coli as host especially when the insert DNA is spliced to the lac promotor is a good example of this. The production of secondary metabolites is poorly understood and high yielding strains (eg for antibiotic production) have been produced by mutation and selection, with strain stability especially during scale up the chief problem encountered. There is little prospect in the immediate future of significant advances in this area being made by the genetic engineering route. The use of new fermentation substrates (eg lignocellulose) and the synthesis of complex molecules (eg antibiotics) require a number of metablic steps. The aim of many researchers is the construction of plasmids coding for all the properties required and this is an exciting prospect.

Problems assocaited with E. coli as a host include the fact that this organism is a commensal in the human intestine. The use of nontransmissible vectors and disabled hosts (not able to survive outside specific laboratory media) diminishes these risks substantially. Acceptance of products produced by E. coli is another problem; for example, is natural human insulin equivalent to chemically modified pig insulin and are these equivalent to that produced in E. coli from C-DNA and then oxidised chemically to form the 2 chain molecule (Table 8). All three molecules may be identical chemically, but clearly the route of production is/

is of prime concern in determining acceptability. A related problem is that of extraction of materials from fermentation broths. A product which is excreted is much easier to extract and purify than one synthesised intracellularly and not excreted. In E. coli a useful approach to this problem is to clone by fusing to a periplasmic system ie one in which synthesis and excretion are linked. This should (1) lead to a lower level of product degradation by the intracellular enzymes, (2) increase the extraction efficiency and (3) ensure a lower level of contamination from host components and hence an increased likelihood of consumer acceptability. A clear alternative is the use of Bacillus spp. in which several proteins such as α -amylase are actively excreted. This is due to the presence of a sequence of 15-30 amino acids in a signal or leader sequence preceeding the N terminus of the excreted protein which ensures the excretion. A strategy for excretion is therefore to clone the insert DNA to C-DNA cloning for the leader sequence and in turn splice this to a powerful promotor involved in protein excretion (eg α-amylase).

A different problem is that of expression in the host organism. In the case of the nitrogen fixation (nif) genes cloned into eukaryotes this has not been solved (see section 5).

Many proteins of eukaryotic origin are modified after synthesis (for example by glycosylation). Polypeptide sequences synthesised in bacterial hosts are not modified in this way and therefore may not be biologically active. The alternative is to use eukaryotic host organisms for these vectors and that of choice is the yeast Saccharomyces cerevisiae.

TABLE 8

SOME EXAMPLES OF SUCCESSFUL CLONING PROCEDURES

Product	Vector	Insert	Comments
Human insulin	pBR322 in E. coli	Chemically synthesised separate DNA chains (amino acid sequences known)	Protein with 2 polypeptide chains, A (21 amino acids) and B (30 amino acids). Each DNA fragment fused to the β-galactosidase gene from E. coli to utilise lac promotor in expression. Fused A and B chains separated purified and joined. Commercial competition with modified pig insulin - chemically identical.
Somatostatin	pBR322 in E. coli	Chemically synthesised DNA	First human polypeptide (14 amino acids) cloned into E. coli DNA insert added to lac promotor plus part of β-galactosidase gene. Fused protein treated with cyanog bromide to release polypeptide - identical to natural product.
Human growth hormone	pBR322 in E. coli	Hybrid chemically synthesised DNA and enzymically copied DNA from m RNA (obtained from human pituitary)	Single polypeptide chain (191 amino acids); lac promotor used in insert. Production of immunologically similar protein to natural product. Yield of 100 mg/L culture reported.

Penicillin/

TABLE 8 (continued)

Product	Vector	Insert	Comments
Penicillin acylase	pBR322 in E. coli	Cellular DNA of E. coli inserted into cosmid vector (pBR322 plus phage cos site) cloned fragment containing acylase DNA obtained after screening and subcloned into pBR322	Screening of 10,000 colonies carried out before positive clone identified. Although pBR322 amplified to about 50 copies per cell, enzyme activity increase only 6 fold.
Hepatitis B virus antigens	pBR322/ PACYC184 in E. coli	Hepatitis B virus DNA of 3 different sterotypes isolated from blood of infected patients	10% Western Europe and USA and up to 70% Africa and South East Asia population carry serological markers for HBV. Limited host range and not grown in tissue culture. Cloning in E. coli enables HBV antigen production on large scale and vaccine development. Radioimmunoassay used for detection of colonies carrying recombinant plasmids.

Human/

TABLE 2 (continued)

Product	Vector	Insert	Comments
Human leukocyte interferon	pBR322 in E. coli	C DNA prepared from human leukocytes producing interferon	Product a polypeptide with interferon activity as assayed by immunological and biological methods.
Nitrogen fixation	pWK120 in E. coli	Plasmid PRD1 containing nitrogen fixation genes (nif) of Klebsiella pneumoniae	Of research use in developing understanding of nif control.
Ammonia assimilation in Methylophilus Methylotrophus AS1	pBR322 in E. coli	E. coli glutamate dehydrogenase (GDH) gene cloned and selected in GDH - (and GS/GOGM) deficient mutant	Organism for large scale production using methanol contained only an energy consuming (GS/GOGAT) route of ammonia assimilation. To a mutant lacking these enzymes was cloned the E. coli plasmid containing an alternative enzyme (GDH) without the energy requirement. Resulting increase in yield of 5% (reported) produced.

5 FUTURE POTENTIAL FOR BIOTECHNOLOGY

No attempt has been made to list all potential applications of genetic engineering and biotechnology to agriculture, the chemical industry, medicine etc and the following comments are intended for general information and to encourage discussion. A number of specific examples are also included in other sections of this report.

5.1 ANIMAL AND HUMAN HEALTH CARE

Much of the present interest in biotechnology stems from health care applications and examples of these applications are given in section 2. Many advances involving genetically engineered organisms are necessarily medium to long term ventures owing to the need for careful development and regulatory control procedures. Examples of such advances include -

1 Vaccine development, for example for influenza, foot and mouth virus, the antigen genes for hepatitis B (all of which have been cloned) and rabies. The cloning of the genes for the protective antigens of viral, bacterial and parasite pathogens is necessary for the production of sub-unit and mutli-valent vaccines and diagnostic reagents. See also Table 8.

2 Peptide Hormone production. Insulin and human growth hormone production is being developed; other hormones which may be produced in future include the α and β sub-units of gonadotrophin, neuropeptides and parturition hormones. See Table 8.

3 Production of therapeutic enzymes and proenzymes. The genes for the blood clot removing (fibrinolytic) enzyme urokinase and the blood coagulation factors VIII and IX have been cloned. Other genes which are being cloned are those for enzymes for the treatment of genetic deficiencies and for disease diagnosis.

4 Production of other proteins including the interferons, acetyl choline receptor protein, components of the complement system, and blood carrier proteins such as serum albumin. Some problems associated with these advances are outlined in section 4. In most instances these are examples where production would be on a relatively small scale so that manufacturing safety problems would be minimised. Some uses are in clinical analytical and diagnostic schemes ie, they do not involve the products coming in direct contact with human patients and safety clearance requirements are clearly less stringent.

5 Production/

5 Production of monoclonal antibodies. Antibodies are usually obtained from animals immunised with specific antigens. After extraction and purification the yields and activities are usually relatively low. The culture of natural antibody producing cells is not a solution since these usually grow for only a limited number of generations, produce only small amounts of antibodies, and produce mixtures of antibodies which then have to be purified as before.

A new development of vast potential is the ability to produce monoclonal antibodies from hybridomas - hybrids produced by the fusion of antibody producing cells with myelomas. The hybridoma retains properties from both parents ie the ability to produce a specific antibody and the ability to proliferate rapidly and (in principle) indefinitely. If an animal (such as a mouse) is injected with an antigen then antibodies are produced against the antigen. An antigen may contain a number of antigenic determinants and specific antibodies are produced against each, lymphocytes separately synthesising and secreting antibodies against specific antigenic determinants. Antisera prepared from whole blood therefore contain a mixture of antibodies. Lymphocytes removed from the spleen of an immunised animal are mixed with the malignant myeloma cells and fusion hybridomas are produced. These must then be screened for specific antibodies after cloning on agar. While success has been obtained with animal cell lines much further research is required on human cell lines since few produced so far actively secrete specific antibodies. Propagation of the hybridoma may be carried out in a host animal or in a culture. The latter will involve the methods outlined in Section 2 with these cells able either to grow in suspension culture or possibly fixed onto solid support/

support materials as immobilised cell 'reactors'. There is scope for scale up developments.

Opportunities for developments in this field include general immunotherapy, the diagnosis and treatment of specific disease (e.g. anti-cancer therapy by linking cytotoxic reagents to specific antibodies i.e. 'targetting' of cytotoxic agents), in organ transplants, and in a wide range of medical and veterinary diagnostic and laboratory testing schemes. Many applications may require a combination of the use of monoclonal antibodies and genetic manipulation. A specific example is the use of a monoclonal antibody (MCA) with affinity towards a specific interferon, the attachment of the MCA to a solid support (eg dextran beads) provides a highly specific immuno - affinity chromatography system for the separation and purification of the interferon.

5.2 AGRICULTURE

Plant cell culture for the production of the high priced materials such as opiates, anti-tumour and anti-leukemic agents, alkaloids, perfumes, flavours etc. is outlined below. The cloning of new properties into plants is at an early stage of development but the use of the Ti plasmid from Agrobacterium tumefaciens (which unfortunately infects only dicotyledeonous plants) and the cauliflower mosaic virus (Ca MV) as vectors offers a number of possibilities for the future. These include the transfer and expression of the nif genes (nitrogen fixation) which is discussed below. Another possibility is that of increasing the content of amino acids essential for human and animal nutrition (eg lysine and methionine) in strategic crop plants and therefore upgrading the value of vegetable proteins. These transfers would be carried out in cell culture, the recipient cells cloned and regenerated into entire plants. Transfer of properties between plants using plasmid or viral vectors is another interesting possibility/

possibility. For economic reasons however, some products may in future be expressed in microorganisms rather than in plants and plant cell cultures.

There are clearly many significant advances being made in the animal health care area with vaccines, monoclonal antibody development etc (see above). Fish farming and other aspects of aquaculture are also fast developing areas with little selectivebreeding of the animals so far and the industry facing all the classical problems of farming 'wild' animals. There is scope for the development of effective vaccines for preventative treatment against such diseases as furunculosis of salmonids, bacterial kidney disease, inefective pancreatic narosis etc. Long term, single cell protein and fat production may become more attractive economically so realising the potential for the investment already made in this area. The need for fermentation feedstock for single cell protein, energy and chemicals production on a large scale clearly requires close co-ordination of agricultural policies and the development of these biotechnological processes.

Another area of large potential is the development of microorganisms and viruses as control agents for insect pests so reducing the dependency of agriculture on chemical pesticides which are often harmful environmentally. Some of these entomopathogens may be grown in conventional fermentation equipment while others such as some protozoa and viruses at present require either live insects or tissue culture methods for their propogation. Possible environmental side effects of the increased use of these organisms by dusting and spraying on to crops should not be overlooked. The potential of developments in nitrogen fixation with the long term aim of cloning the nitrogen fixing capability into crop plants attracts much interest. The _nif_ genes have been cloned and transferred between bacteria and much is known of the details of

the/

the genetics and control of this process. Transfer to yeast has also been successful but no gene expression was possible in that organism. Other strategies are at the planning stage including whole organism transfer imitating the Rhizobium/legume symbiosis. Work is also proceeding on strain improvements of Rhizobium which potentially could increase the efficiency of legume nitrogen fixation. Fixation of nitrogen into ammonia for fertiliser manufacture is achieved chemically by the energy intensive Haber process. The potential exists for a biological process and work is proceeding on the fixation and excretion of nitrogen (as ammonia) by cyanobacteria. These photosynthetic organisms utilise sunlight as energy source.

Intensive cattle, pig and poultry rearing is common practice in mechanised agriculture and creates problems of waste disposal. Significant advances have been made in designing anaerobic fermentation plants to produce methane (biogas) on site. While capital costs are high this process produces a valuable fuel for heating and running farm machinery and leaves a manure with little removal of nutrients (but much lower BOD). This practice is likely to increase in future.

5.3 PLANT CELL CULTURE

At the present time a large range of materials can be formed in plant cell culture but relatively few can be produced in a commercially viable way relative to the use of whole plants and/or synthetic chemistry. Plant cell culture offers advantages of control of supply and quality and avoids problems of microbial contamination found in whole plant products. Most cell lines will grow in defined media and mass culture. Particular applications of cell culture include -

1/

1 Production of compounds currently prepared from whole plants eg. quinine, pyrethroids.

2 Production of chemical intermediates eg. thebaine (from *Papaver*) in the synthesis of morphine and codeine.

3 Direct production of new products eg. rutaculin from cultures of *Ruta*.

4 Use as biotransformation systems either singly or as a specific step in a chemical process eg. digoxin synthesis.

A lack of knowledge of the basic biochemical processes involved in the biosynthesis of many plant components hinders the application of modern genetic methods. In addition the production of some materials occurs only after some cell differentation about which little is known at a molecular level. Products closest to commercialisation include diagnostic enzymes (eg tobacco phosphodiesterase), digoxin, opiates, anti-leukemic and anti-tumour agents, steroids, tobacco biomass and some alkaloids. There is also scope for developments in process technology and part of the future potential must be in the transfer of the plant DNA concerned to microorganisms and expoitation by fermentation methods.

5.4 CHEMICAL INDUSTRY

Applications include the production of chemical feedstocks by fermentation of plant materials (renewable resources) and wastes from other industries. These are outlined below. Conventional fermentations often produce effluents of high organic content owing to incomplete utilisation of substrates, eg spent grains and stillage in brewing/distilling/alcohol production and whey from the milk processing industries. Biotechnological advances have much to gain from adopting the co-ordinated utilisation of materials approach in common practice in the chemical industry.

Of/

Of the 100 organic chemicals of greatest significance for chemical syntheses only 6 have been produced in commercial quantities by fermentation. These are acetic acid, acetone, n-butanol, ethanol, glycerol and isopropanol. In general all are currently produced by chemical synthesis for reasons of economy. (Local conditions eg trade embargos, aims for self-sufficiency and local taxation make fermentations more successful in some instances). The continuing rise in oil prices and problems of availability of this material should make the fermentation processes more economically attractive in future. Some new products will then almost certainly be added to those listed above including alkene oxides (produced by biological oxidation) for polymerisation into plastics and possibly poly β hydroxy butrate, also for plastics production.

In contrast to chemical processing, biological catalysis is carried out at low temperatures and pressures and this should become more attractive economically with increases in energy costs. Biological catalysts, however, are inefficient in terms of their stability and the concentration of enzyme produced per unit of organism. The former should be improved with better immobilisation methods and process engineering and the latter by genetic engineering.

5.5 ENERGY

Power alcohol is the subject of extensive development at the present time with the Proalcool programme in Brazil producing 4×10^9 L/a with a target of 11×10^9 L/a by 1985. This programme is based on fermenting sugar cane juice alongside some cassava and molasses. In the USA the Gasohol programme is based on the fermentation of maize starch. Both processes are based on conventional technology. Some likely developments are listed in Table 3.

Biogas/

Biogas is produced from sewage and agricultural wastes and on a local basis can be a valuable energy source. At present large scale production from biomass is not attractive commercially where there is a source of natural gas or coal (which can yield methane on gasification).

Hydrogen may be produced by fermentation or in reactions involving photosynthetic organisms. It has long term potential as an energy source.

Fuel cells are at present mainly of academic interest although these are claimed to be 70% efficient in conversion of chemical to electrical energy. In the near future, a likely use of fuel cells is as sensors; for example, the use of methanol dehyrogenase in such a system allows the detection of 10^{-12}M methanol.

5.6 WASTE TREATMENT AND RECOVERY; RENEWABLE RESOURCES

The treatment of domestic and industrial liquid wastes is the basis of a well developed and engineered biotechnology industry. The general aim is that of detoxification and reducing biochemical oxygen demand, however, rather than recovery and re-use. Waste treatment requires mixture of organisms to degrade the diverse range of materials present. Many advances have been made in engineering to improve these processes; these include the use of O_2, the deep shaft process and the development of microbial film reactors.

A change in emphasis towards recovery rather than removal is indicated for reasons of economy and social demands. Organic wastes (see Table 9) are potentially valuable feedstocks whether upgraded for human consumption, for fish or cattle food, for biogas formation, as a fuel or as a chemical feedstock/fuel after pyrolisis. Some organic wastes may be in the form of dilute solutions or suspensions which may be treated using continuous flow microbial film reactors. 'Clean' wastes such as whey, sulphite liquor and starch residues are all suitable for/

for single cell protein production (Table 2) and potentially for the production of ethanol and other fermentation products. With the exception of lignocellulose all other waste components may be converted to methane although this process may be slow and uneconomic. It is difficult to generalise on the economics since these are highly site dependent.

Some conventional biotechnological process are creators of polluting wastes, for example, one brewery could produce an effluent of BOD equivalent to a population of 200,000. The design of new processes should take this fully into account.

Not all synthetic chemicalsare readily biodegradable. Genetically engineered organisms may be produced against specific waste materials. An example is the Pseudomonas putida constructed to degrade a number of components of crude oil and therefore of potential use in cleaning up after an oil spill. Biodegradable co-polymers including starch or sucrose have been developed for use as plastics and surfactants and there is further potential here.

Many wastes contain high levels of metals which are toxic preventing the use of the organic content for single cell protein production etc. The microbial removal of such metals (see Section 2) could result both in their recovery and allow more extensive use of the wastes for other purposes.

The increased cost of fossil fuels for energy and chemical feedstocks has reversed the steady trend from biological to chemical synthesis and has renewed interest in the use of natural plant materials (renewable resources) and wastes as substrates for the/

the production of fermentationfeedstocks and as potential sources of energy. The latter, principally ethanol and biogas (methane) are included in Table 3. The ultimate source of energy is sunlight 'trapped' in the process of photosynthesis. Plant photosynthesis fixes approximately 2 x 10 t/a carbon with an energy content of 2 x 10^{21} J which is approximately 10 times the global annual energy use. The most efficient photosynthesisers involved are the C_4 plants such as maize, sorghum and sugar cane. The structural and storage components of plants (plant biomass) are a renewable source of energy and fermentation/chamical feedstocks and these include sugars, starch, cellulose, hemicelluloses and lignins. Plant biomass may be utilised directly as crop plants grown specifically as fermentation/chemical feedstocks or as natural vegetation used for the same purpose or may be utilised indirectly as wastes (by products) from other industries. Smith (1981) has produced a useful list of such waste materials from the agriculture, forestry and process industries and domestic waste and this forms the basis of Table 9. Raw materials with a high or easily extracted content of sugars serve readily as substrates for fermentation processes and will be used increasingly. New markets for these are in any case required with the advent of the high fructose corn syrup products and the potential for the total conversion of glucose to fructose outlined in Table 3. The most abundant potential substrates, however, are cellulose, hemicellulose and lignins which are most often closely associated as 'lignocellulose'. Ofthese cellulose isreadily degraded either chemically or enzymically to yield glucose (a preferred feedstock). The short term future for lignin appears to be as a fuel. Of the hemicellulose fractions, xylan (which can account for 30% polysaccharide) may be degraded chemically or enzymically to yeild xylose. Xylose (converted to xylulose) is a potential substrate for enthanol production (Table 3) and other fermentations.

TABLE 9

WASTE PRODUCTS USABLE AS CHEMICAL/FERMENTATION FEEDSTOCKS AND FOR ENERGY PRODUCTION

Process Industry and Domestic Wastes	Agriculture	Forestry
Molasses	Straw, bagasse	Sulphite liquor
Distillery wastes	Coffee, cocoa and	Bark, sawdust,
Milk whey	coconut ~~waste~~	cellulose fibre
Waste and wash water from	Fruit peels and waste	
food industries	Tea wastes	
Fishery wastes	Oilseed cake	
Meat by-products and	Cotton waste	
abattoir wastes	Bran	
Sewage and garbage	Animal effluents	
Paper		

5.7 POTENTIAL USE OF MICROBES FOR OIL RECOVERY

The use of microbial polysaccharidesin drilling muds and for tertiary oil recovery is mentioned in Table 4. Primary and secondary oil recovery removes only up to 25% of the reserves present. Non-recoverable 'reserves' in the USA alone are estimated as about 3 x 10 brls (10^{14} - 10^{15}\$). Non-recoverable 'reserves' in UK waters are estimated at about 10^{12}\$. Secondary recovery (flooding) requires high pressure due to interfacial pressure in the water/oil/rock system. Materials which maintain viscosity under such pressure and are miscible in water/oil are required to prevent 'fingering'. Such materials (involved in tertiary recovery) include the xanthans and sufactants.

At present the cost of tertiary recovery is often greater than the value of the oil if recovered. The possibility exists, however, to use microorganisms in situ to increase recovery by

(1) physical change, eg in viscosity of the oil,

(2) increase in reservoir size (especially in limestone) by acid formation during metabolism,

(3) production of gas to increase reservoir pressure,

(4) generation of extracellular materials, some being viscous polymers, others being surfactants.

Such organisms would have to be engineered to withstand high pressure/temperature regimes (eg 200-400 at. at 90-120° - such conditions exist in the deep sea with active microbial flora) and should ideally be lithotrophic/autotrophic or able to grow in very cheap carbon and energy sources. A potential problem is that of 'clogging' the reservoirs during growth. This is an area requiring high investment but with vast potential if only a fraction of oil present can be recovered.

6 SOME SPECIFIC ASPECTS OF THE ENVIRONMENTAL HEALTH IMPACT OF BIOTECHNOLOGY

6.1 Direct impact on man. A large number of microorganisms are pathogenic. Their adverse effects on human health include the production of toxins within the body (eg tetanus) or outside the body (eg mycotoxins) and the production of allergic responses. Contact with harmful organisms may occur in a variety of ways but in biotechnological processes the formation of aerosols from centrifugation, filtration, exit air filters and the stirring of cultures appears to be the most significant problem (for a detailed review see Evans et al 1981). Clearly for harmful effects to occur the organisms used must be pathogenic and it is assumed that no large scale use of known human pathogens is likely to be undertaken. Small scale use, however, should not be ruled out for special product and vaccine manufacture. Cultures of non pathogenic 'acceptable' microorganisms, however, may become infected with pathogens during use and, while it is unlikely that such an infection would remain undetected, the need for vigilance is clear. A further possibility is that an acceptable organism may through mutation or phenotypic variation (due to altered growth conditions such as variations in growth method, growth rate, substrate limitation, temperature, pH etc) show altered behaviour. The careful choice of organism and the precise control of growth conditions appear to be essential. As outlined in section 4 Escherichia coli has been used in most cloning experiments and is the organism of choice for the production of the first generation of products by genetic engineering. The use of genetically engineered organisms is the subject of statutory controls in Western Europe and the USA. In the UK, for example, in addition to controls on earlier stages of work specific permission from the Genetic Manipulation Advisory Group (GMAG) is required for each scale up operation. For production, however, E. coli is not an ideal organism since it is found as a commensal in the human gut and other hosts such as Bacillus subtilis and Saccharomyces cerevesiae are clearly to be preferred (see section 4). The use of disabled strains of E. coli unable to grow outside the laboratory/factory environment minimises the risks associated with use of the organism.

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6.2 Impact on food, animals and plants. It is unlikely that organisms pathogenic to domestic and food animals will be used on a large scale and comments made above refer equally to man and animals.

The possible consequences of the large scale use of plant pathogens has been summarised admirably in a recent report by Evans et al (1981). They conclude that while there is no evidence that the industrial use of plant pathogens has so far caused the spread of plant disease there may be some hazards associated with the steady increase in the industrial scale use of such organisms. These authors suggest that the potential problems be eliminated by the use of non pathogenic and saprophytic organisms whenever possible but that if a pathogen must be used then appropriate containment procedures must be adopted. There is a need for basic research on the nature of the pathogenic behaviour of microorganisms towards plants and possibly a requirement for modifying the existing control regulations for the industrial use of such organisms.

6.3 Impact on the environment. The reduction of biological oxygen demand and the removal of toxic wastes by waste treatment procedures eliminate many of the hazards of life in a crowded urban environment (see Section 8). The truth of this is evident whenever a man-made or natural disaster upsets the smooth running of the waste - and water - treatment procedures with the concomitant dangers of cholera, typoid, dysentery, etc. Many current applications of biotechnology lead to the incomplete utilisation of substrate materials and the production of wastes. There are obvious examples in the alcoholic fermentation and food industries and in timber utilisation as outlined in Section 5. In many instances these wastes are a clear embarrassment and economic alternatives to their discharge are sought. As indicated in Section 5 many wastes are the potential substrate materials for other processes and their use in this way will increase in the future. The construction of specific organisms to deal with particular pollution/

pollution problems (such as the Ps. putida mentioned in Section 4 for dealing with oil components) is likely to increase in future. The fact that in the USA such organisms may be protected by patents is a clear indication that industrial interest in this area is high. The potential also exists to produce immobilised organisms for reactions to deal with difficult wastes.

There is alwasy the possibility of accidental discharge of live organisms, for example from a fermenter into the environment. Most organisms used and likely to be used in industrial processes however are either 'hot house plants' which are most unlikely to compete with those in the natural environment or are disabled so that they cannot survive outside the controlled and favourable environment of the industrial fermenter.

The leakage of low concentrations of antibiotics into the environment from production units is another potential problem. There is a clear possibility of selecting organisms which are resistant and the added danger of this resistance being plasmid-borne and hence transmissible.

6.4 Future applications. As indicated in sections 2, 4 and 5 the positive impact of biotechnology on human and animal health care is already substantial and will increase with the availability of new products from genetically engineered organisms. The formation of new products from new organisms is clearly associated with potential hazard although the existing procedures of control and licencing appear to be able to cope with these problems. Indeed the exhaustive testing required before a new product reaches the market and the associated time delay and costs are major disincentives for the developments in many instances.

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As biotechnology develops it is clear that the scale of use of many organisms will increase. The pressure to use the yeast Saccharomyces cerevisiae as the host for genetically engineered vectors originates from the use of this organism in a number of large scale fermentations over a long period of time without associated health hazards. Workers within factories dealing with new processes will of necessity run the highest risk of contact. For example, workers producing bacterial proteases suffered allergy problems before the organisms and products were contained with improved work practices. The manufacture of a new range of highly purified protein products by fermentation with genetically engineered organisms may bring about additional problems of this nature although the relevant health authorities should be aware of this potential. In addition to genetically engineered organisms carrying 'foreign' genes for product expression the scale of use of other microorganisms is certain to increase. For example, a large number of different organisms are used for single cell protein production. Licencing of Fusarium gramimarum for human consumption took over ten years in the UK and this organism was grown on substrates (sucrose, starch) which are readily acceptable in other fermentations. In contrast the use of more novel substrates such as methanol may appear more difficult. Methanol is toxic to man and animals and the product must not contain methanol at toxic concentrations. The possibility of long term exposure to low levels of toxic material should not be overlooked. This is of prime significance since methanol is a fermentation feedstock of very high potential. The possible parallel with the reported presence of alkanes in the fat of pigs reared on a part diet of alkane-grown yeast is clear.

The large scale use of an increasing number of organisms is a special challenge to the chemical and process engineer. The increased use of continuous fermentations with associated increases in process efficiency should improve both product and effluent quality/

quality. The ideal fermentation is a process which produces cells (and other products) plus water ie the nutrient balance and concentration being sufficient only for the process and without excess. While this may be approached there is the inherent danger of inadvertently altering growth conditions (eg limiting substrate) and hence the properties of the organisms concerned. If complex substrates, eg molasses or distillers wastes, are used then the known variability of their composition compounds this problem.

7 GENERAL PRINCIPLES FOR ASSESSMENT AND MINIMIZATION OF THE ENVIRONMENTAL HEALTH IMPACT OF BIOTECHNOLOGY

7.1 Introduction

Although the upsurge in biotechnology is a new phenomenon, triggered by the realisation that mineral oil as a chemical feedstock is a limited resource likely to become increasingly costly and by the potential of genetic engineering to produce micro-organisms of almost unlimited potential to synthesize chemicals of all kinds, the likely environmental health impacts are not inherently new. They can readily be predicted from existing industries employing biotechnological techniques, notably the fermentation industries such as brewing, those producing industrial enzymes and those producing antibiotics.

With regard to environmental health, there are three subdivisions which can be distinguished - the industrial environment, the general environment outside the industrial premises (including the problems of waste disposal), and the products of the industrial processes which may be widely dispersed by way of trade. The hazards which affect these subdivisions may themselves be divided into those which are essentially microbiological (involving living micro-organisms) and those which are essentially chemical (involving constituents or products of the micro-organisms). The distinction may not always be clear-cut but it is a useful one as the methods of dealing with microbiological and chemical hazards are different.

7.2 Environmental Health Impacts in the Industrial Environment

In most cases, the first health impact of a new technology will occur in the industrial environment. Physical hazards should be no serious problem in relation to biotechnology as they will be of a kind which has

already been covered by appropriate design and control procedures as a result of long experience in the chemical industry. Microbiological and chemical hazards, on the other hand, will vary from process to process. This means that for each biotechnological process the raw materials, intermediates, end products and byproducts must be carefully assessed. However, this is not enough as the main health impacts may very well turn out to be associated with contaminants, especially microbiological contaminants which flourish under the culture conditions used for fermentations or in the nutrient media used to maintain and grow organisms essential for the biotechnological processes. Even if all materials used, reactor vessels, pipelines and other surfaces are sterilized, it is very difficult to ensure the integrity of entry and exit points in the system to prevent contamination completely. Further, microbial contamination may occur prior to sterilisation and may produce toxins which survive the sterilization procedure. Consequently, continuous monitoring of the process materials will be required to check for contaminant micro-organisms and associated toxins.

Whether contaminated or not, many of the process materials used in biotechnology may be allergenic and hence cause skin rashes, hay fever, asthma etc. Susceptibility to such effects is highly variable throughout the population and must be carefully checked in any pre-employment medical examination. It must also be checked routinely during employment.

Having determined the existence of a chemical or microbiological hazard, the next important thing is to examine the design and function of the equipment containing that hazard with a view to detecting fugitive or planned leaks and emissions which might release the hazard into the surrounding environment. Consideration should also be given to possible accidents. If the risk of human exposure to the hazard is serious, steps must be taken to reduce this risk. These steps will probably involve

continuous monitoring of the equipment and its environment and the issue of protective clothing to the work force. Modification of equipment to give greater control of emissions and leaks may be required. New work hygiene practices may have to be developed and training in these practices instituted.

Once new biotechnological processes are functioning, it should be possible to establish a health impact data base for use in epidemiological studies. Such a base can be used to initiate prospective morbidity investigations and, in due course, for retrospective assessment of mortality. These investigations should relate not only to the workers in biotechnological factories but also to members of the general public living in the vicinity of these factories. Where possible, a retrospective data base should be assembled for assessment of the health impact of existing biotechnological industries.

7.3 Assessment of Microbiological Hazards

Reference has already been made to the risk of microbiological contamination. The first step in assessing this is to implement a reliable sampling scheme for routine monitoring of all materials which may become contaminated. Such a scheme must involve detailed documentation of all samples and procedures for the preservation and storage of samples to minimize post-sampling changes. Monitoring must extend to stored products and to wastes.

Contamination may involve any kind of micro-organism and so the whole range of techniques for microbiological analysis must be employed. However, many

micro-organisms are not health hazards and may be tolerated as contaminants. On the other hand, some are well known pathogens and must be carefully eliminated. Others may have been regarded previously as harmless because human exposure has been on too small a scale to have an effect. These last may become a problem for people exposed to the large quantities that may be produced by industrial scale processes. Particular attention must be paid to the possibility of hypersensitivity reactions (allergies). These are immunological responses which produce tissue damage in reacting individuals. There are four main types. Type I leads to asthma, hayfever, eczema and urticaria and less commonly to anaphylactic shock. Anaphylactic shock is characterized by general circulatory collapse, pulmonary oedema and death. Type II may lead to haemolytic anaemia, bleeding and purpura. Type III may lead to glomerulonephritis and other tissue damage as a result of deposition of protein complexes in vulnerable parts of the circulation. Type IV reactions differ from the other three in that they appear much more slowly (24-48h) after exposure to antigenic material. They produce skin rashes, inflammation and tissue destruction and develop readily in response to microbiological antigens.

Once microbiological contaminants have been identified, they must be carefully characterized. Their properties may already be known but, if not, a full investigation must be undertaken. This would involve determination of growth requirements, of pathogenicity, of ability to produce toxins, and of immunological properties which may lead to hypersensitivity reactions (see above). The investigation will also involve describing any diseases in experimental animals induced by exposure to the contaminating micro-organisms and determining the quantitative relationship of these diseases to degree of exposure. All of this is an essential pre-requisite to risk assessment. An extrapolation must be made from these fundamental studies to predict human health risk, taking into account the risk of

exposure inherent in the biotechnological processes. An assessment of the risk of general environmental damage should also be made.

7.4 Minimization of Microbiological Hazards

The first step in minimizing microbiological hazard is the identification of raw materials likely to be contaminated with micro-organisms and the recommendation of decontamination procedures. Contamination of materials during processing and contamination of the final products must also be avoided. Waste materials may have to be disinfected in some way and suitable methods adopted to prevent contamination of equipment, buildings etc. and, in emergency, for decontamination. Contamination may be prevented by sterilizing all raw materials as far as possible and designing equipment to maintain sterile conditions. Sterilization is most likely to involve heat treatment (including steam sterilization of equipment), treatment with ionizing radiation (at least 5K Gy or equivalent), or treatment with chemical agents. Similar methods may be used for decontamination and for disinfection of waste though in this case extended storage before discharge may be all that is necessary (see Section 8). Whatever method of treatment is used, account must be taken of the hazards associated with that method and these may in some cases be considered to outweigh the likely hazards of the microbiological contamination.

7.5 Assessment of Chemical Hazards

The first step in assessment of chemical hazards is the identification of the hazards and the determination of their physical and chemical properties and especially those likely to contribute significantly to foreseen dangers. From this basis, good methods for monitoring the hazardous substances may be derived.

Once methods of monitoring have been chosen, a fully documented

sampling scheme may be implemented for all foreseen chemical hazards. Techniques for preservation and storage of samples must minimize post-sampling change. Sampling must extend to monitoring cleanup procedures, raw materials and products in storage, permitted and fugitive emissions, and waste disposal. Particular care must be taken over any change in processing. It may be necessary to devise or improve methods of fractionation of samples to isolate biologically active components.

For many of the chemicals associated with biotechnological processes no toxicological data will be available. Thus, toxicity tests must be carried out. Potential for mutagenicity and carcinogenicity must be assessed using both short term and long term tests. Since inhalation and dermal exposure are the main routes by which exposure may occur, they must receive particular attention. This may require the development of methods to permit controlled inhalation exposure of animals to dusts, aerosols and gases and of methods to determine the resultant dose. Thereafter, lung diseases and non-neoplastic skin diseases will require special attention in addition to the assessment of systemic effects, effects on the reproductive system, teratogenicity, and effects of combined exposures. The relationship of significant effects to dose and time of exposure must be determined. In addition, the fate of inhaled toxicants must be established. Once the fundamental toxicity tests have been carried out, an attempt must be made to examine the influence of pre-existing disease and to discover whether there are any inherited characteristics which increase susceptibility. Simple screening tests for the detection of susceptible individuals and of incipient ill effects should be developed if at all possible.

Apart from inhalation and dermal exposure, other possibilities in normal circumstances are ocular and oral exposure. Hence, effects resulting from these must also be assessed. In addition, certain

toxicity tests require the application of the toxicant in ways which are unlikely to occur naturally, eg. intraperitoneally. The results of these tests give valuable information but care must be taken to allow for the unusual route of exposure in any extrapolation to predict risk to human beings.

While the hazards associated with direct human exposure must be our first concern, indirect exposure following general environmental release may turn out to be just as important. In this context, three possibilities must be given special attention. Firstly, there is the possibility of environmental interactions and transformations leading to the appearance of derivatives more toxic than the original chemicals. Secondly, there is the possibility of food web accumulation leading to high levels of toxicants in human foodstuffs. Thirdly, there is the possibility of harmful environmental effects other than direct toxicity, eg. eutrophication leading to the elimination of aerobic life in enclosed waters and, in particular, the death of commercially important fish stocks.

7.6 Assessment of Risk from Chemicals and its Minimization

Extrapolation from the toxicological studies above permits an assessment of human health risk, taking into account process related hazard and the risk of general environmental damage. Minimization of this risk depends upon using all the information available to recommend and implement measures to remove chemical hazards. This will require identification of all materials likely to be contaminated, from raw materials to waste, and development of methods for their decontamination. Such methods must take into account both economic and safety requirements, i.e. they must be inexpensive to operate while ensuring that safe levels of toxicants are attained. If at all possible, contamination should be prevented by good design of equipment and buildings and good factory practice. Plans for

dealing with accidents must be formulated. Ultimately, the responsibility for risk minimization rests with management and it must be their task to ensure full implementation of risk avoiding procedures.

TABLE 10 TASKS IN ASSESSMENT AND CONTROL OF EXPOSURE HAZARD ASSOCIATED WITH BIOTECHNOLOGICAL PROCESSES

Group I Manufacture

1 Characterization of raw materials, intermediates, end products, by-products and possible microbiological and chemical contaminants in the manufacturing processes.

2 Determination of fugitive and planned leaks and emissions which might occur and lead to dermal or inhalation exposure of people and/or environmental contamination.

3 Evaluation of likely accidents and their potential health effects.

4 Assessment of risk of human exposure to microbiological or chemical hazards.

5 Development of surveillance methods to prevent or minimize accidents.

6 Development of predictive models for assessment of future expansion or innovation.

7 Development of methods for monitoring potential hazards in environmental samples.

8 Development of methods for monitoring human contamination with hazardous micro-organisms or materials.

9 Development of methods for data handling, storage and retrieval for results of monitoring.

Group II. Protection and Decontamination of People at Risk

1 Evaluation of control systems as effective means of reducing health hazards.

2 Development of the best practicable control systems to minimize health hazards.

3 Recommendation of protective clothing and respirators for use where required.

4 Development of medical screening procedures to identify people likely to be particularly sensitive to probable hazards.

5 Development of work hygiene practices to minimize risk.

6 Development of training programmes for employees in biotechnology on work hygiene practice, possible health effects of hazards, and early warning signs of serious exposure.

7 Development of criteria for routine medical examinations which will identify physiological and psychological changes which are precursors of disease.

Group III. Epidemiology

1 Assembly of a retrospective data base containing results of epidemiological studies relating to existing biotechnological industries, eg brewing and others based on fermentation.

2 Initiation of prospective morbidity studies on workers in biotechnology.

3 When possible, initiation of retrospective mortality studies on workers in biotechnology.

4 Development of a programme to assess whether the general public living in the vicinity of biotechnology based factories are at higher health risk than the public at large.

11 TASKS IN ASSESSMENT OF MICROBIOLOGICAL HAZARDS OF BIOTECHNOLOGICAL PROCESSES

Group I. Sampling for Identification of Hazardous Micro-organisms

1 Design of a sampling scheme to permit routine monitoring of all possible microbial hazards.

2 Collection of samples of process materials, products and emissions and documentation of sampling.

3 Preservation and storage of samples to minimize post-sampling changes.

4 Monitoring of cleanup procedures and changes in processes.

5 Monitoring of stored process materials and products.

6 Monitoring of waste disposal.

Group II. Isolation and Identification of Hazardous Micro-organisms

1 Isolation and identification of viruses.

2 Isolation and identification of mycoplasmas.

3 Isolation and identification of bacteria.

4 Isolation and identification of yeasts and fungi.

Group III. Characterization of Properties of Hazardous Micro-organisms

1 Determination of growth requirements.

2 Determination of pathogenicity.

3 Determination of ability to produce toxins.

4 Determination of immunological properties.

5 Description of disease induced by exposure to hazardous micro-organisms.

6 Determination of the quantitative relationship of disease to exposure to hazardous micro-organisms.

Group IV. Risk Assessment

1 Extrapolation from fundamental microbiological studies to predict human health risk taking into account the assessment of process related hazard.

2 Assessment of risk of general environmental damage.

Group V. Minimization of Risk Associated with Micro-organisms

1 Identification of microbial hazards and recommendation of decontamination procedures.

2 Recommendation of methods to prevent contamination of materials during processing.

3 Recommendation of methods to prevent contamination of products.

4 Recommendation of methods for disinfection of waste materials.

5 Recommendation of methods to prevent contamination of industrial equipment, buildings and the general environment.

6 Recommendation of methods for decontamination in the event of accidents.

12 TASKS IN TOXICOLOGICAL ASSESSMENT OF CHEMICAL HAZARDS OF BIOTECHNOLOGICAL PROCESSES

Group I. Physicochemical Studies

1 Determination of physical and chemical properties of potential hazards.

2 Identification of physical and chemical properties likely to promote hazard.

3 Development of methods for monitoring environmental levels of hazardous substances.

Group II. Sampling Hazardous Substances for Screening and Toxicity Testing

1 Design of a sampling scheme to permit routine monitoring of all foreseen hazards.

2 Collection of samples of process materials, products and emissions and documentation of sampling.

3 Preservation and storage of samples to minimize post-sampling changes.

4 Monitoring of cleanup procedures and changes in processes.

5 Monitoring of chemical and microbiological modification of process materials, products and emissions.

6 Monitoring of the effects of storage on process materials and products.

7 Monitoring of waste disposal.

8 Fractionation of samples to obtain biologically active constituents.

Group III. Screening Tests of Potential Hazards for Mutagenicity and Carcinogenicity

1 Determination of mutagenic potential using micro-organisms eg the Ames test.

2 Determination of potential for cell transformation using mammalian cells in culture.

3 Determination of potential to act as initiators, promoters or co-carcinogens.

Group IV. Exposure and Toxicity Assessment Following Inhalation of Toxicants

1 Development of methods to permit controlled inhalation exposure of animals to hazardous materials as dusts, aerosols or gases.

2 Development of methods to determine dose of hazardous materials following inhalation.

3 Determination of potential for causation of lung disease.

4 Determination of systemic effects by study of histopathology, haematology and functional changes, especially in the cardiovascular, renal, endocrine, nervous and immune systems.

5 Determination of effects on the reproductive system.

6 Determination of teratogenic effects.

7 Determination of carcinogenicity.

8 Determination of effects of exposure to other substances combined with inhalation exposure (looking especially for co-carcinogenicity).

9 Determination of the fate of inhaled substances.

10 Determination of the relationship of all significant effects to dose and time of exposure.

11 Determination of the influence of pre-existing disease and inherent characteristics of people at risk.

12 Design of screening methods for the detection of susceptible individuals.

13 Design of screening methods for early detection of ill effects.

Group V. <u>Exposure and Toxicity Assessment Following Dermal Exposure to Toxicants</u>

1 Determination of the potential for causing non-neoplastic skin diseases.

2 Determination of systemic effects by study of histopathology, haematology and functional changes, especially in the cardiovascular, renal, endocrine, nervous and immune systems.

3 Determination of effects on the reproductive system.

4 Determination of teratogenic effects.

5 Determination of carcinogenicity.

6 Determination of effects of exposure to other substances combined with dermal exposure (looking especially for co-carcinogenicity).

7 Determination of the effects of light (phototoxicity, photo-allergy, and photosensitization).

8 Determination of the fate of dermally absorbed substances.

9 Determination of the relationship of all significant effects to dose and time of exposure.

10 Determination of the influence of pre-existing disease and the inherent characteristics of people at risk.

11 Design of screening methods for the detection of susceptible individuals.

12 Design of screening methods for early detection of ill effects.

Group VI. Exposure and Toxicity Assessment Following Exposure by Other Routes

1 Determination of toxicity as a result of ocular exposure.

2 Determination of toxicity as a result of oral exposure.

3 Determination of toxicity associated with exposures used in toxicological tests eg. intraperitoneal exposure.

Group VII. Exposure and Toxicity Assessment Relating to General Environmental Release

1 Characterization of environmental interactions and transformations.

2 Determination of possible food chain effects.

3 Determination of environmental effects other than direct toxicity eg eutrophication.

Group VIII. Risk Assessment

1 Extrapolation from toxicological studies to predict human health risk taking into account the assessment of process related hazard.

2 Assessment of risk of general environmental damage.

Group IX. Minimization of Risk Associated with Toxic Substances

1 Identification of raw materials likely to be hazardous and recommendation of detoxication procedures.

2 Recommendation of methods to prevent contamination of materials during processing.

3 Recommendation of methods to prevent contamination of products.

4 Recommendation of methods for detoxication of waste materials.

5 Recommendation of methods to prevent contamination of industrial equipment, buildings and the general environment.

6 Recommendation of methods for decontamination in the event of accidents.

8 DISPOSAL OF WASTE FROM BIOTECHNOLOGICAL PROCESSES

The growth of biotechnological industry will result in a great increase in the production of biological waste. However, disposal of this waste is in principle fairly straightforward. The processes involved are substantially those currently applied to sewage treatment (Figure 3). In sewage treatment, mixed microbial populations are used to reduce the amount of oxidizable organic material and to alleviate health hazards by producing conditions which destroy many, though not all, pathogens. The resultant effluent can then be discharged into the surrounding environment with a minimum of adverse effects.

While the methods of sewage treatment might be applied to almost any biological waste with little change, it is likely that the cost of treatment will increasingly be defrayed by the sale of products which can be generated from the waste by adapting the microbial population used. Such products may include methane, ethanol and animal feedstuffs. Where production of feedstuffs is planned, this may be dependent on the development by techniques of genetic engineering of micro-organisms which can degrade potentially toxic chemicals.

Even after treatment, wastes will still remain and these will eventually enter the surrounding environment. Predicting the fate of these wastes will require an exact knowledge of their composition. The main chemical components will be nitrogen, phosphorus and total organic carbon. There will also be minor components some of which may be toxic, and residual pathogens. The possible fates of these components are discussed below.

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8.1 THE MAIN CHEMICAL COMPONENTS

The main chemical components of biological waste which must be accounted for are nitrogen, phosphorus and total organic carbon. Nitrogen and phosphorus facilitate eutrophication of natural waters while organic carbon can create an oxygen demand which may lead to localized deoxygenation and the elimination of aerobic life forms. Nitrogen is present in biological waste in both organic and inorganic forms in varying proportions. Organic nitrogen is largely converted to ammonium ions while inorganic nitrogen tends to take the form of nitrate. Nitrate may be formed from ammonium ions by the process of nitrification. Since nitrate is an anion it is not adsorbed on to clay particles; it does not form insoluble precipitates and it is easily moved in the soil solution and in surface water. Availability of nitrogen from wastes depends on the interactions of a number of processes which are shown diagrammatically in Figure 4.

Phosphorus, like nitrogen, occurs in biological waste in both organic and inorganic forms. About 33% of the total phosphorus in animal wastes is in the organic form but most biological wastes have not been adequately characterized for organic and inorganic phosphorus fractions. Where biological waste has been dumped on land, availability of phosphorus derivatives in the soil solution is determined by adsorption and precipitation reactions, by mineralization of organic phosphorus and by plant uptake. The relationship between these processes is shown in Figure 5.

Any disposal of biological wastes on land increases the carbon content of the soil. This improves the infiltration capacity of the soil and consequently reduces the amount of runoff water. However, there is also an increased potential for runoff of carbon compounds/

compounds and hence for deoxygenation of receiving waters. This potential pollution, as measured by biochemical oxygen demand (BOD), chemical oxygen demand (COD) or total organic carbon (TOC) may be reduced by microbial activity. Such microbial activity must be carefully assessed in predicting the environmental impact of biological wastes. Before carrying out the assessment, the organic compounds present must be identified and quantified. Attention should also be paid to chemicals which may inhibit microbial activity. While each group of organic compounds may have a different fate, the general outline of the processes which organic compounds undergo is shown in Figure 6.

8.2 THE MINOR CHEMICAL COMPONENTS

Minor chemical components may include antibiotics, auxins, vitamins, enzymes, pigments, heavy metals and other toxic susbtances. Though they may be present in small amounts, their effects may be quite considerable. Growth of micro-organisms and plants may be inhibited or stimulated, breakdown and transformation of other wastes prevented or facilitated and toxic chemicals may pass through food webs and accumulate until human health is threatened. Unfortunately it is impossible to generalize. Each minor component must be assessed on its own merits and then in relation to all the other components and to the environment at risk. This brings into play the whole range of analytic and toxicological procedures and is probably the most difficult part of evaluation of the fate of any waste material.

8.3 PATHOGENS

Possibly the worst problem posed by increased amounts of biological waste will be the potential for increased levels of environmental pathogens. If wastes are not specially treated to remove pathogens or if pathogens survive treatments, they will enter the environment and either become hazards or disappear as a result of environmental conditions causing their death or destruction. The possible environmental fates of pathogens are shown in Figure 7.

There/

There are various processes commonly used to eliminate pathogens from wastes. Extended storage may be sufficient but it may be supplemented by heat treatment, exposure to ionizing radiation or treatment with chemical agents. Halogenation is the most widely used of the chemical treatments and is very effective but can have harmful effects on other organisms. In aquatic environments halogens can cause a reduction in the numbers of phytoplankton, fish kills, and impaired reproduction and growth of these and other aquatic animals. Further, halogenated hydrocarbons may be formed and these may be carcinogenic and otherwise toxic to human beings. Halogens may be removed from treated wastes with sulphur dioxide or activated carbon but this adds about 30% extra cost to the treatment process. An alternative to halogenation is ozonation. Ozone is a good bacteriocide and virucide. Its residual levels following treatment are low. This means it poses little threat to other organisms but, on the other hand, it does not prevent pathogen contamination subsequent to treatment. Another possible treatment to remove pathogens is subjection to ultra-violet radiation. However, this treatment is only completely effective on clear solutions as turbidity and colour absorb or deflect the ultra-violet rays.

Whether such treatments as those described above are necessary in a given case is difficult to ascertain. While it is relatively easy to monitor wastes for bacterial pathogen contamination, it is still difficult to monitor for viral pathogens. Further, viral pathogens may be resistant to procedures which readily eliminate bacteria and thus the absence of viable bacteria is no indication of the absence of infective viruses. For example, chlorination which kills bacteria appears not to inactivate hepatitis A virus. Similarly, ether or chloroform will kill bacteria and fungi without harming enteric viruses. Thus, it may be necessary to seek improved methods of waste treatment where virus pathogens are likely to be present.

FIGURE 3

Sewage Treatment

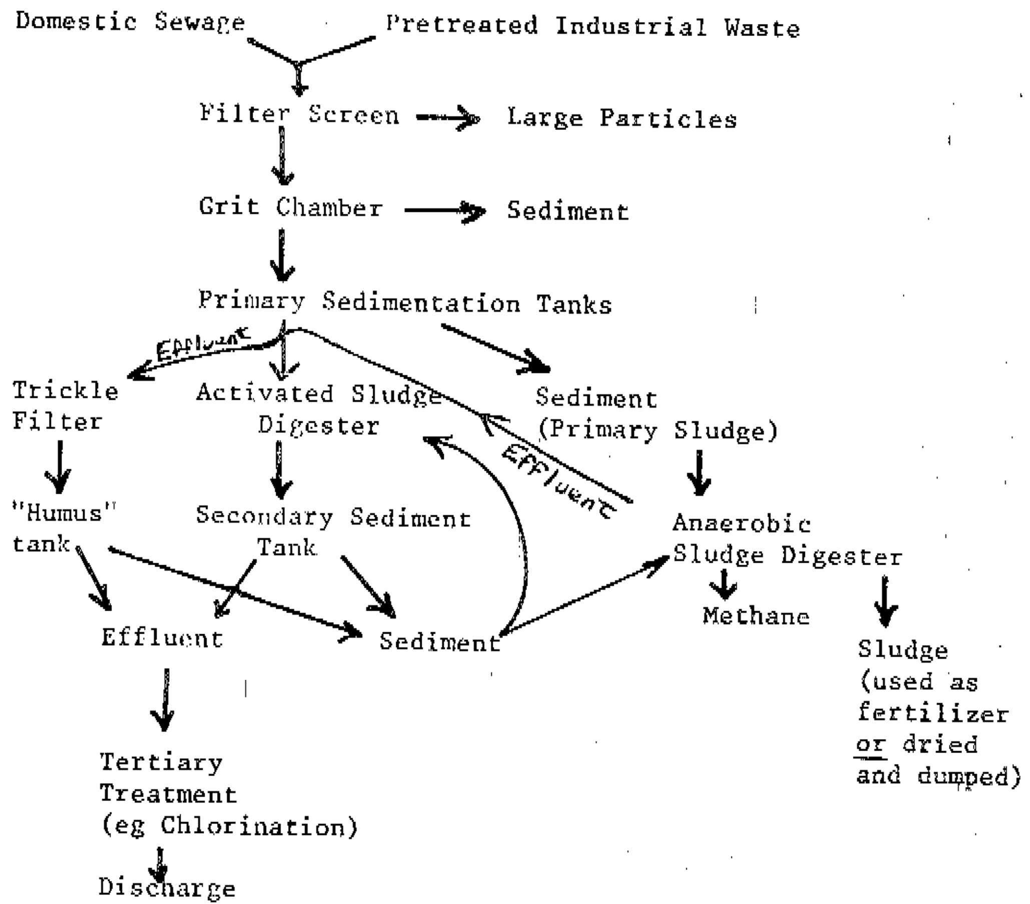


FIGURE 4

The Fate of Biological Waste Nitrogen

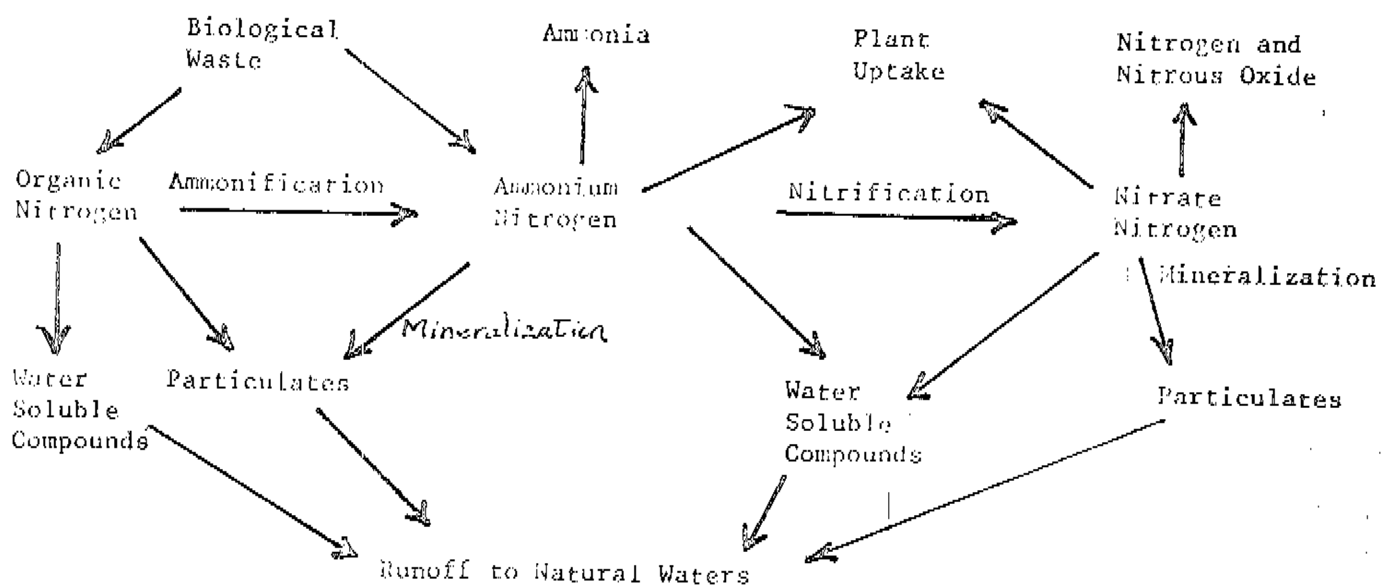


FIGURE 5

The Fate of Biological Waste Phosphorus

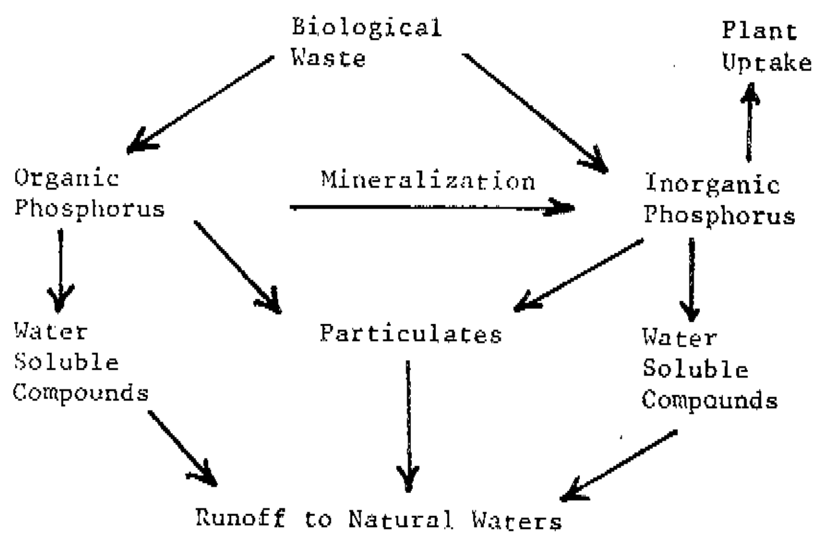


FIGURE 6

The Fate of Biological Waste Carbon

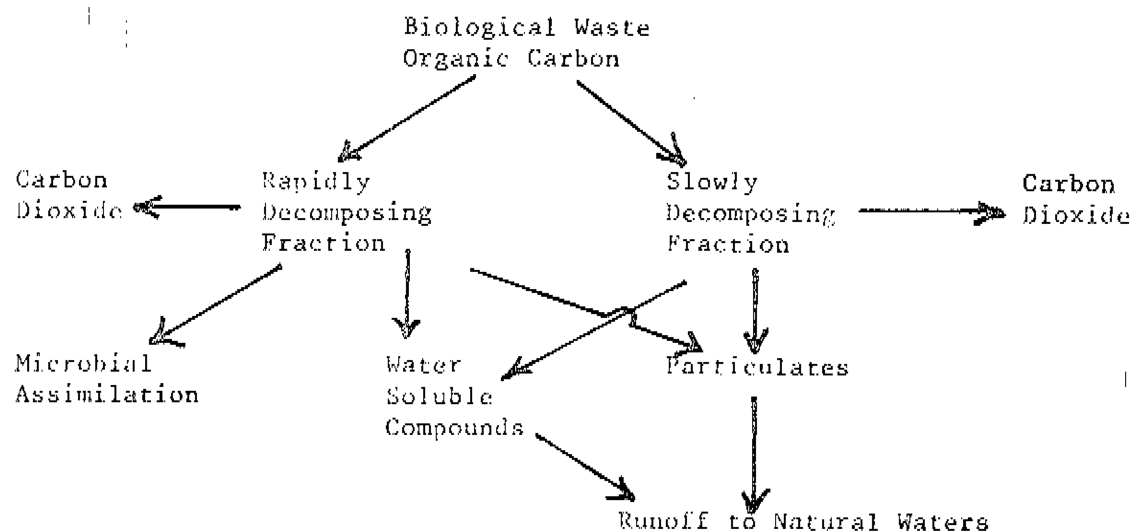
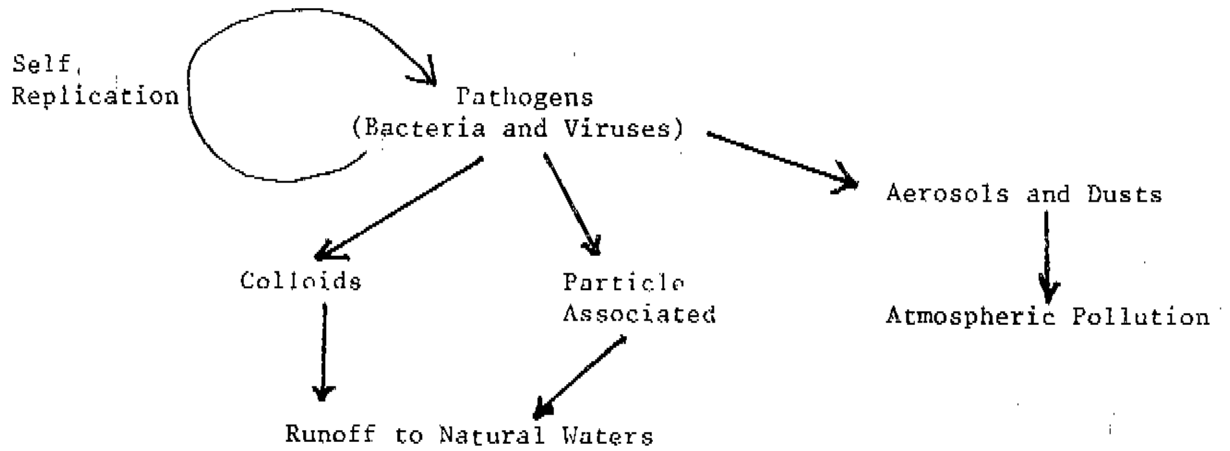


FIGURE 7

The Fate of Pathogens Associated with Biological Waste



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